Leveraging Contemporary Technology in Pharmacogenomics Research to Optimize Pharmacotherapy in Substance Abuse

Steven Tung[1*], Joseph Kitzmiller [2,3]

1Department of Anesthesiology, The Ohio State University, College of Medicine, Columbus, OH, USA

2Department of Biological Chemistry and Pharmacology, The Ohio State University, College of Medicine, Columbus, OH, USA

3Center for Pharmacogenomics, The Ohio State University, College of Medicine, Columbus, OH, USA

*Corresponding author: Steven R. Tung, MD, JD; Department of Anesthesiology, The Ohio State University College of Medicine, 410 West Tenth Avenue, Columbus, Ohio 43210, USA. Tel: +16142938487; Fax: +16142938153; Email: steven.tung@osumc.edu

Abstract

Substance abuse is a healthcare epidemic with substantial costs to society, both financial and quality of life. Effective pharmacotherapy options for treating substance abuse are critical, and approaches employing precision/personalized medicine hold promise for optimization, not only for improving drug- and dose-selection strategies, but also for addressing reversible, substance abuse-environment-coupled epigenetic changes. By presenting some of the more commonly studied genetic biomarkers relevant to opioid pharmacotherapies, we provide a brief introduction to pharmacogenomics research. Also presented are comprehensive descriptions and examples of contemporary and emerging methodologies and technologies being leveraged to advance pharmacogenomics research.

Although the US comprises only about five percent of the world's population, it consumes nearly seventy percent of the world's opioid production – including ninety-nine percent of the world's hydrocodone [1]. As the uptick in opioid-related deaths continues, the urgency to halt (or at least substantially alleviate) this epidemic becomes increasingly paramount [2]. In 2015 there were 52,404 drug overdose deaths in the United States, and more than sixty percent involved an opioid [3]. In the subsequent year the age-adjusted death rate attributed to drug overdose increased by more than twenty percent to 63,600, representing a 3-fold increase from 1999 to 2016 [1]. Much of the opioid epidemic has been attributed to the consistent rise in use and subsequent abuse of nonmedical pain relievers (NMPR) in past decades. Although NMPR abuse is the most common pathway to abuse of stronger opioids [4], other risk factors include various environmental and genetic factors. Genetic predisposition is thought to be one of the larger contributors to risk (*e.g.*, up to fifty percent in some studies) [5], and both drug abuse and alcoholism share genetic influences including some that are both developmental stage dependent [6].

Pharmacogenomics research involves the study of the effects of genetic variation (*i.e.,* gene mutations or polymorphisms that alter structure, function or expression of gene-encoded proteins) on patient response to pharmaceuticals. As risk of substance abuse has a strong genetic component, pharmacogenomics is poised to provide meaningful guidance for tailoring approaches for risk determination and treatment of substance abuse as well as pain treatment strategies that minimize risk of substance abuse. For most pharmaceuticals genetic variation accounts for twenty-five to fifty percent of interindividual variation in drug response, and contemporary scientific literature suggests its contribution may be even larger for illicit drugs [7]. A few of the more commonly described functional genetic variants pertinent to substance abuse include enzymes, receptors and transporters involving dopamine, glutamate, serotonin and morphine [8-9].

One of the more well-studied genes influencing opioid response is the mu opioid receptor gene (*OPRM1*). Crist *et al.*

recently reported that a single nucleotide polymorphism (SNP pronounce "snip") in the mu opioid receptor gene (*OPRM1*) was associated with clinical outcomes in a 582-patient cohort of European-Americans of a 24-week, randomized, open-label trial of methadone or buprenorphine/naloxone (Suboxone) for the treatment of opioid dependence [10]. The authors report the SNP (rs10485058), characterized by a guanin to adenine substitution resulting in variant mRNA and reduced expression of mu-opioid receptors, was associated with decreased risk (relative risk=0.76, 95% confidence intervals=0.73–0.80, P=0.0064) of opioid abuse relapse as determined by urine screen. They also reported supportive findings from analysis of self-reported data in the Comorbidity and Trauma Study (CATS) of 1215 Australian opioid dependent individuals of European descent. In the CATS patient cohort, rs10485058 predicted abstinence of relapse (measured during the final 30 days of the 24-week study) after achieving abstinence (p=0.003) [10]. Importantly, Oslin *et al.* had previously reported rs10485058 was associated with efficacy of naltrexone for the treatment of alcohol dependence. Homozygous rs10485058-Carriers had lower rates of relapse (0.26 vs. 0.47, OR=2.27, p=0.044) compared to homozygous wild-type (normal) and heterozygous carriers of rs10485058 [11]. Polymorphisms in *OPRM1*, including rs10485058 and others (*e.g.*, rs1799971, rs2075572, rs558025, rs9384179 and rs62638690), demonstrates promise potential for aiding prescribers in opioid and dose selection as well as opioid-abuse treatment strategies to achieve optimal clinical outcomes.

Another important gene influencing opioid response is *CYP2D6*, encoding the metabolism enzyme cytochrome P450 family 2 subfamily D member 6 cytochrome. Evidence demonstrating its utility in opioid and dose selection is sufficient that official recommendations have been established by the Clinical Pharmacogenetics Implementation Consortium (CPIC) [12] and in FDA-approved drug labeling for certain opioids [13]. Comprehensive information including references to additional clinical and translational studies as well as population-specific polymorphism frequencies for polymorphisms in a myriad of genes affecting the pharmacology of opioids and/or a variety of other pharmaceuticals is maintained by the NIH-sponsored website, www.pharmgkb.org [14]. Recent technologic developments improving our capacity to examine the human genome have greatly improved our understanding of the interplay between genetic structure, regulation and the subsequent downstream effects on clinical response to pharmacotherapies. The clinical relevance of gene-medication associations continues to expand as does the list of FDA-approved drug labels containing pharmacogenomic

guidance. In fact, more than 150 medications are now included in the FDA's Table of Pharmacogenomic Biomarkers in Drug Labeling table. [13].

Contemporary Technology in Pharmacogenomic Research

Bioinformatics

Bioinformatics applications include computational tools to organize, analyze, visualize and store information associated with biological macromolecules. Advances in bioinformatics allow for efficient processing and storage of large multivariate data sets of molecular structures, genetic interactions, high-throughput genotyping data and differential gene expression. With the aid of contemporary bioinformatics methodologies, gene expression studies can now be conducted without necessitating implementation of *in vitro* experiments. In addition, *in silico* gene expression analysis allows for quantitative analysis of the genes comprising the entire genome (rather than only a subset of genes fitted to a microchip array) [15].

Paramount to the recent transition of national health policy towards the principles of precision medicine, bioinformatics has merged with genomic medicine by (1) linking biobanks with electronic health records (EHR) for genomics analysis, (2) initiating patient genomics testing, (3) assimilating pharmacogenomics into routine medical care, and (4) utilizing genomics in drug development. [16].

Electronic Health Records and Biobanks

Electronic health records (EHR) are routinely utilized for storage and retrieval of patient health data, and inclusion of patient genomic data is becoming more common. Optimal EHR systems are user-friendly, scalable, and capable of storing large amounts of data for annotation, search and retrieval. As most patients are likely to change healthcare systems several times during their life, efficient, and accurate, transfer of data across various EHR platforms is another essential attribute. Common limitations in utilizing EHR for pharmacogenomics research include (1) inaccurate, incomplete or incompatible data, (2) inability to accurately identify and define phenotypes from available clinical data, (3) biased health records, and (4) difficulty in generating phenotype algorithms from complex data [17]. Biobanks are repositories of patient tissue specimens, often including genetic material. They are commonly linked to EHRs, creating a vast resource of genomic and health related data, enabling researchers to reclassify diseases based on molecular pathways. Schemes

linking EHRs to biobanks provide a powerful, unprecedented opportunity for investigating the relationship between heritability and drug response. Compared to traditional clinical trials, these methods involving the linkage of longitudinal phenotype data (captured via EHR queries of prescriptions, procedures and socioeconomic status) [18] to large patient repositories of either genetic data or access to biological samples for genomic analysis are superior for cost savings, efficiency and cohort size [19-20].

Genome-Wide and Phenome-Wide Association Studies

A Genome-wide Association Study (GWAS) is a statistical analysis that simultaneously measures association between hundreds of thousands of genetic polymorphisms and select phenotypes (traits). Without bias regarding location or plausibility of the genes and polymorphisms investigated, GWAS are particularly useful for *discovery* of association(s). A primary disadvantage of GWAS is that the p-value thresholds must account for multiple hypothesis testing (*e.g.,* p-values often need be $< 5x10^{-6}$ to be considered statistically significant rather than the customary p-value cut-off of $5x10^{-2}$), resulting in potentially important associations (those with biological plausibility) going undetected. Additional limitations include the inability to determine causality and linkage-dependent under- or over-estimation of association(s).

Coverage of most gene loci is commonly adequate to allow reliable utilization of proxies in GWAS. When the polymorphism of interest is not specifically included on a genetic assay, a proxy with known linkage to the polymorphism of interest can be substituted. The use of a proxy, however, introduces additional probability estimate(s) when linkage is not absolute. Haplotype maps (HapMap) are constructs of several proxy polymorphisms that can substitute (accurately predict) large genomic segments when several genotypes occur with near perfect linkage [21-23]. While GWAS have collectively made profound contributions to genetic discovery, GWAS findings alone are insufficient for properly informing clinical decision strategies. Replication studies utilizing a candidate-gene approach, limited to fewer select genes and polymorphisms, are superior for better characterizing association(s) and potential clinical importance. The utility of GWAS may likely be enhanced when coupled with emerging methodologies and technologies, providing greater clinical relevance and deeper understanding of the complex relationships constituting the heritability of response [24].

Measuring association(s) between genetic polymorphism(s) and a variety of phenotypes, Phenome-Wide Association Studies (PheWAS) are another complementary approach to GWAS. Phenotypic data, often retrieved from EHR(s), can be aggregated (*e.g.,* similarly-defined phenotypes can be combined assuming reasonable scale homogeneity) to drastically increase statistical power. As PheWAS-GWAS replication rates near seventy percent were reported as earlier as 2013 (near the infancy of PheWAS) [25], the potential for improved replication and PheWAS-GWAS symbiosis remains promising as PheWAS continue to integrate emerging methodologies and technologies [26].

Sequencing

ꟼ□□□□□□ꟼ□□□□□□□ꟼ□□□□□□□□□ dideoxy nucleotide triphosphates (DDNTPs) as DNA-chain terminators, fragments large DNA polynucleotides into smaller components and utilizes computer-based assembly of the □□□□□□□ꟼ□□□□□□ꟼ□□□□□□□□□□□□□□□□ꟼꟼ□ □□□□□□□ꟼ□□□□□□□□□□□□□□□□□□□□□□□□□ ꟼꟼꟼꟼ□□successful in detecting monogenetic pathologic traits in well-ꟼ□□□□□□□□□ꟼ□□□□□□□ꟼ□□□□□□□□□□ꟼ□□ꟼꟼ□□□□□ ꟼ□□□□□□□ꟼinclude inability to identify copy number variants and limited success for studies involving complex, heterogeneous genetic diseases. As most diseases are not monogenic (i.e., their etiology □□□ꟼ□□□□□□□□□□□□□□□□□□□ꟼ□ □□□□□□□□ꟼ□□□□□□□□□□□□has substantial limitations [27].

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NR2B gene was found after chronic administration of ethanol and was associated with demethylation [49] and histone acetylation [50]. Fetal Alcohol Spectrum Disorders (FASD), characterized by irreversible cognitive and behavioral disability, results from significant ethanol exposure *in utero*. Demethylation of normally hypermethylated imprinted regions in sperm DNA is associated with chronic alcohol use, suggesting a potential molecular mechanism for paternal transgenerational transmission of FASD [51]. **Opioids** Epigenetic activation and silencing of mu opioid receptor (MOR) expression can be achieved through coordination at both the histone and DNA levels. DNA methylation and histone deacetylation at the *OPRM1* promoter decrease/silence *MOR* expression. The *in vivo* interaction between the histones and MeCP2, a methyl-CpG binding protein, which binds preferentially to methylated DNA and directly represses transcription, was reduced in the *MOR* promoter region upon differentiation, and *MOR* expression increased. When siRNA was used to disrupt the *MeCP2*, *MOR* expression increased [52]. The *MOR* gene in blood and sperm DNA was significantly increased in opioid addicts, suggesting evidence of a mechanism for transgenerational continuation of the opioid dependence phenotype [53]. **Cocaine** Acute and chronic administration of cocaine produces immediate and lasting gene expression changes through epigenetic modifications mirroring the behavior adaptations seen in human cocaine addiction. Acute cocaine treatment induced hypermethylation of the promoter region of the DNA methyltransferase gene (*DNMT*) and resulted in transcriptional downregulation (transcriptional silencing) in the nucleus accumbens. Repeat cocaine administration resulted in hypomethylation and upregulation of *fosB* (immediate early transcription factor) in the nucleus accumbens [54].

Conclusion

Substance dependency and addiction result largely from environment-gene interactions occurring in specific areas of the brain. Pharmacogenomics focuses on heritable variations in the genome leading to individual differences in drug response and interfaces with bioinformatics, molecular genetics, neuroscience, clinical pharmacology, EHR/Biobanking, genome and phenome-wide association studies, proteomics, transcriptomics, and metabolomics. Interdisciplinary systems-biology approaches are increasingly needed to harness these contemporary and emerging technologies and methodologies to adequately characterize this complex disease at the molecular level. The linking of EHR and biobank systems and the application of genomic science and technologies to substance abuse are highlighting the potential of personalized-medicine approaches related to substance abuse.

Declaration of Interest

The authors report no conflict of interest. The authors alone are responsible for the content of this article.

References

1. Owen GT, Burton AW, Schade CM, Passik S (2012) Urine Drug Testing: Current Recommendations and Best Practices. Pain Physician 15: ES119-ES133.

2. Bonnie RJ, Kesselheim AS, Clark DJ (2017) Both Urgency and Balance Needed in Addressing Opioid Epidemic: A Report from the National Academies of Sciences, Engineering, and Medicine. JAMA 318: 423-424.

3. Rudd RA, Seth P, David F, Scholl L (2016) Increases in Drug and Opioid-Involved Overdose Deaths - United States, 2010-2015. MMWR Morb Mortal Wkly Rep 65: 1445-1452.

4. Muhuri PK, Gfroerer JC, Davies MC (2013) Associations of Nonmedical Pain Reliever Use and Initiation of Heroin Use in the United States. CBHSQ Data Review.

5. National Council on Alcoholism and Drug Dependence I. Family History and Genetics. 2015.

6. Grant JD, Scherrer JF, Lynskey MT, Lyons MJ, Eisen SA, et al. (2006) Adolescent alcohol use is a risk factor for adult alcohol and drug dependence: evidence from a twin design. Psychol Med 36: 109-118.

7. Lauschke VM, Barragan I, Ingelman-Sundberg M (2017) Pharmacoepigenetics and Toxicoepigenetics: Novel Mechanistic Insights and Therapeutic Opportunities. Annu Rev Pharmacol Toxicol 58: 161-185.

8. Haile CN, Kosten TR (2009) The potential of pharmacogenomics to treat drug addiction. Pharmacogenomics 10: 1883-1886.

9. Mroziewicz M, Tyndale RF (2010) Pharmacogenetics: a tool for identifying genetic factors in drug dependence and response to treatment. Addict Sci Clin Pract 5: 17-29.

10. Crist RC, Doyle GA, Nelson EC, Degenhardt L, Martin NG, et al. (2016) A polymorphism in the OPRM1 3'-untranslated region is associated with methadone efficacy in treating opioid dependence. Pharmacogenomics J 18: 173-179.

11. Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, et al. (2003) A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology 28: 1546-1552.

12. Crews KR, Gaedigk A, Dunnenberger HM, Klein TE, Shen DD, Callaghan JT, Kharasch ED, Skaar TC. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for Codeine Therapy in the Context of Cytochrome P450 2D6(CYP2D6) Genotype. *Clinical Pharmacology Therapy* 2012; 91 (2): 321-26.

13. US Food and Drug Administration. Table of Biomarkers in Drug Labeling. Available at https://www.fda.gov/Drugs/ScienceResearch/ucm572698.htm. Accessed February 28,208.

14. Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF, Altman RB, Klein TE. Pharmacogenomics Knowledge for Personalized Medicine Clinical Pharmacology and Therapeutics 2012; 92(4): 414-7.

15. Murray D, Doran P, MacMathuna P, Moss AC (2007) *In silico* gene expression analysis--an overview. Mol Cancer 6: 50.

16. Vamathevan J, Birney E (2017) A Review of Recent Advances in Translational Bioinformatics: Bridges from Biology to Medicine. Yearb Med Inform 26: 178-187.

17. Wei WQ, Denny JC (2015) Extracting research-quality phenotypes from electronic health records to support precision medicine. Genome Med 7: 41.

18. Hollister BM, Restrepo NA, Farber-Eger E, Crawford DC, Aldrich MC, et al. (2017) Development and Performance of Text-Mining Algorithms to Extract Socioeconomic Status from De-Identified Electronic Health Records. Pac Symp Biocomput 22: 230-241.

19. Bowton E, Field JR, Wang S, Schildcrout JS, Van Driest SL, et al. (2014) Biobanks and electronic medical records: enabling cost-effective research. Sci Transl Med 6: 234cm233.

20. Denny JC (2014) Surveying Recent Themes in Translational Bioinformatics: Big Data in EHRs, Omics for Drugs, and Personal Genomics. Yearb Med Inform 9: 199-205.

21. Wang DG, Fan JB, Siao CJ, Berno A, Young P, et al. (1998) Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280: 1077-1082.

22. Witte JS (2010) Genome-wide association studies and beyond. Annu Rev Public Health 31: 9-20 24 p following 20.

23. Stranger BE, Stahl EA, Raj T (2011) Progress and promise of genome-wide association studies for human complex trait genetics. Genetics 187: 367-383.

24. Zhou K, Pearson ER (2013) Insights from genome-wide association studies of drug response. Annu Rev Pharmacol Toxicol 53: 299-310.

25. Denny JC, Bastarache L, Ritchie MD, Carroll RJ, Zink R, et al. (2013) Systematic comparison of phenome-wide association study of electronic medical record data and genome-wide association study data. Nat Biotechnol 31: 1102-1110.

26. Denny JC, Bastarache L, Roden DM (2016) Phenome-Wide Association Studies as a Tool to Advance Precision Medicine. Annu Rev Genomics Hum Genet 17: 353-373.

27. Nelen M, Veltman JA (2012) Genome and exome sequencing in the clinic: unbiased genomic approaches with a high diagnostic yield. Pharmacogenomics 13: 511-514.

28. Bao R, Huang L, Andrade J, Tan W, Kibbe WA, et al. (2014) Review of current methods, applications, and data management for the bioinformatics analysis of whole exome sequencing. Cancer Inform. 13(Suppl 2): 67-82.

29. Diniz WJ, Canduri F (2017) REVIEW-ARTICLE Bioinformatics: an overview and its applications. Genet Mol Res 16(1).

30. Vicini P, Fields O, Lai E, Litwack ED, Martin AM, et al. (2016) Precision medicine in the age of big data: The present and future role of large-scale unbiased sequencing in drug discovery and development. Clin Pharmacol Ther 99: 198-207.

31. Nielsen DA, Utrankar A, Reyes JA, Simons DD, Kosten TR (2012) Epigenetics of drug abuse: predisposition or response. Pharmacogenomics 13: 1149-1160.

32. Roberts JS, Dolinoy D, Tarini B (2014) Emerging issues in public health genomics. Annu Rev Genomics Hum Genet 15: 461-480.

33. Bonetta L (2008) Epigenomics: Detailed analysis. Nature 454: 795-798.

34. Pusch W, Flocco MT, Leung SM, Thiele H, Kostrzewa M (2003) Mass spectrometry-based clinical proteomics. Pharmacogenomics 4: 463-476.

35. Feng Z, Prentice R, Srivastava S (2004) Research issues and strategies for genomic and proteomic biomarker discovery and validation: a statistical perspective. Pharmacogenomics 5: 709-719.

36. Wishart DS (2007) Proteomics and the human metabolome project. Expert Rev Proteomics 4: 333-335.

37. Wishart DS (2016) Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Discov 15: 473-484.

38. Zhu S, Qing T, Zheng Y, Jin L, Shi L (2017) Advances in single-cell RNA sequencing and its applications in cancer research. Oncotarget 8: 53763-53779.

39. Mishra PJ (2012) The miRNA-drug resistance connection: a new era of personalized medicine using noncoding RNA begins. Pharmacogenomics 13: 1321-1324.

40. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, et al. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411: 494-498.

41. Zhang XD, Yang XC, Chung N, Gates A, Stec E, et al. (2006) Robust statistical methods for hit selection in RNA interference high-throughput screening experiments. Pharmacogenomics 7: 299-309.

42. Luizon MR, Ahituv N (2015) Uncovering drug-responsive regulatory elements. Pharmacogenomics16: 1829-1841.

43. Wilhelm BT, Landry JR (2009) RNA-Seq-quantitative measurement of expression through massively parallel RNA-sequencing. Methods 48: 249-257.

44. Folkersen L, Brynedal B, Diaz-Gallo LM, Ramsköld D, Shchetynsky K, et al. (2016) Integration of known DNA, RNA and protein biomarkers provides prediction of anti-TNF response in rheumatoid arthritis: results from the COMBINE study. Mol Med 22.

45. Shenker NS, Ueland PM, Polidoro S, van Veldhoven K, Ricceri F, et al. (2013) DNA methylation as a long-term biomarker of exposure to tobacco smoke. Epidemiology 24: 712-716.

46. Tsaprouni LG, Yang TP, Bell J, Dick KJ, Kanoni S, et al. (2014) Cigarette smoking reduces DNA methylation levels at multiple genomic loci but the effect is partially reversible upon cessation. Epigenetics 9: 1382-1396.

47. Chatterton Z, Hartley BJ, Seok MH, Mendelev N, Chen S, et al. (2017) In utero exposure to maternal smoking is associated with DNA methylation alterations and reduced neuronal content in the developing fetal brain. Epigenetics Chromatin10: 4.

48. Chhabra D, Sharma S, Kho AT, Gaedigk R, Vyhlidal CA, et al. (2014) Fetal lung and placental methylation is associated with in utero nicotine exposure. Epigenetics 9: 1473-1484.

49. Marutha Ravindran CR, Ticku MK (2005) Role of CpG islands in the up-regulation of NMDA receptor NR2B gene expression following

chronic ethanol treatment of cultured cortical neurons of mice. Neurochem Int 46: 313-327.

50. Qiang M, Denny A, Lieu M, Carreon S, Li J (2011) Histone H3K9 modifications are a local chromatin event involved in ethanol-induced neuroadaptation of the NR2B gene. Epigenetics 6:1095-1104.

51. Ouko LA, Shantikumar K, Knezovich J, Haycock P, Schnugh DJ, et al. (2009) Effect of alcohol consumption on CpG methylation in the differentially methylated regions of H19 and IG-DMR in male gametes: implications for fetal alcohol spectrum disorders. Alcohol Clin Exp Res 33: 1615-1627.

52. Hwang CK, Song KY, Kim CS, Choi HS, Guo XH, et al. (2007) Evidence of endogenous mu opioid receptor regulation by epigenetic control of the promoters. Mol Cell Biol 27: 4720-4736.

53. Chorbov VM, Todorov AA, Lynskey MT, Cicero TJ (2011) Elevated levels of DNA methylation at the OPRM1 promoter in blood and sperm from male opioid addicts. J Opioid Manag 7: 258-264.

54. Anier K, Malinovskaja K, Aonurm-Helm A, Zharkovsky A, Kalda A (2010) DNA methylation regulates cocaine-induced behavioral sensitization in mice. Neuropsychopharmacology 35: 2450-2461.