Clinical pharmacogenomics for anti-epileptic drug response in epilepsy patient management in India

By

DEBLEENA GUIN
Department of Biotechnology
(Registration Number **– 2k17/PhD/BT/01)**

Under the supervision of
Prof. Yasha Hasija
&
Joint-supervision of
Dr. Ritushree Kukreti (CSIR-IGIB)

Submitted
in fulfilment of the requirements of the degree of
DOCTOR OF PHILOSOPHY (Ph.D)
to the

DELHI TECHNOLOGICAL UNIVERSITY
SHAHBAD DAULATPUR, DELHI - 110042, INDIA
JANUARY 2021

Dedicated to the patients enrolled in the study

&

To my parents for their profound untold sacrifices and perpetual support

CERTIFICATE

This is to certify that the thesis entitled titled **"Clinical pharmacogenomics for anti-epileptic drug response in epilepsy patient management in India"** submitted by **Ms. Debleena Guin** to **Delhi Technological University (Formerly DCE)**, for the award of the degree of "Doctor of Philosophy" in Biotechnology is a record of bonȧ fide work carried out by her. Debleena Guin has worked under my guidance and supervision and with joint supervision with Dr. Ritushree Kukreti, Chief Scientist, CSIR-IGIB has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached requisite standards.

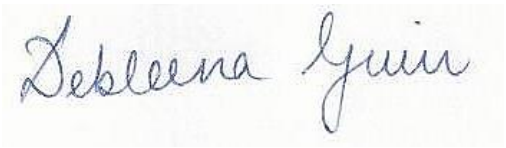
The results contained in this thesis are original and have not been submitted to any other university or institute for the award

Prof. Yasha Hasija
Supervisor/ Guide
Professor,
Dept. of Biotechnology,
Shahbad Daulatpur,
Main Bawana Road,
Place: Delhi, India
Date: 13.10.2023

Dr. Ritushree Kukreti
Joint Supervisor/Co-Guide
Chief Scientist,
Functional Genomics Unit,
CSIR-Institute of Genomics and Integrative Biology
Mall Road,
Place: New Delhi, India
Date: 13.10.2023

DECLARATION BY THE CANDIDATE

I declare that the matter embodied in this thesis entitled "**Clinical pharmacogenomics for anti-epileptic drug response in epilepsy patient management in India**" is the result of the investigations carried out by me at **Delhi Technological University** & **CSIR-Institute of Genomics and Integrative Biology** for the degree of Doctor of Philosophy during the period from 1st August, 2017 to 19th September, 2023 under the supervision of Dr. Yasha Hasija and joint supervision of Dr. Ritushree Kukreti. This work has not been submitted for the award of any other degree, diploma, associateship or membership of any University or other Institution of higher learning. I further declare that the material obtained from other sources has been duly acknowledged and cited.



Debleena Guin

Regn. No.: 2k17/PhD/BT/01
Delhi Technological University (DTU),
Shahbad Daulatpur, Main Bawana Road
&
CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB)
Mall Road,
New Delhi, India

Date: 13/10/2023
Place: New Delhi

Acknowledgements

I shall forever be grateful to my supervisor and mentor, ***Prof. Yasha Hasija, Professor, Dept. of Biotechnology, DTU.*** *She has been the kindest guide, I could have every wished for. My tenure during Ph.D was a rollercoaster ride with many lows and a few highs. She has given me all the space and laid her deepest faith in me to fall and stand back straight again and start running to strive excellence. Her scientific ways to deal with my challenging experiments gave a new direction to my thinking and approach. Her warmth during my tough times gave me immense courage and motivation to fight again. I will always be indebted to you for your kindness and support.*

I have no words to express my gratitude to my 'mentor for life' and my doctorate joint-supervisor, ***Dr. Ritushree Kukreti, Chief Scientist, CSIR-IGIB****. Under her supervision, I have learned ways to systematically approach any scientific objectives, tackling and transforming complexities to simpler explanations. Ma'am, the basic values that you inculcated about kindness, empathy, hard work and discipline has shaped me into a different individual. You are my personal example of 'work is worship'. You have always encouraged me to reach higher in life and never settle. I thank you for having trust in me for carrying out my thesis in my own direction; for giving me space and freedom. It was a matter of supreme luck, pride and honour to work under your guidance.*

I express my kind regards and gratitude to ***Prof. Jai Prakash Saini, Vice chancellor, Delhi Technological University****, and* ***Dr. Souvik Maiti Director, CSIR-IGIB*** *for giving me the excellent environment and infrastructure to carry out my research work. The resources, the environment and the expertise for the best faculties I received, was my sheer privilege.*

I am extremely grateful to the other faculty members of department of biotechnology, DTU. I am also highly grateful to ***Dean (PG), DRC chairman, DRC members, my SRC members (Prof. Pawan Dhar, Dr. Sangeeta Sharma, Dr. Nitin Puri, Prof. Pravir Kumar)*** *and all the faculty members of the Department of Biotechnology, DTU for their comments, valuable suggestions and guidance during the entire tenure of my Ph.D.*

My special deep regard for ***Dr. S. Ramachandran****. Sir, you gave the opportunity and strength to survive in this institute when I was at the tip of a breakdown. Thank you for everything that you provided, knowing or unknowingly. Thank you for making me familiar to programming languages and teaching me from scratch. You bestowed a new vision to my understanding of biological science and opened the doors of big data analytics.*

My acknowledgement and respect to our collaborators at Institute of human behaviour and allied sciences (IHBAS), whose clinical experience and insight gave a new direction to my study design. I would also like

to thank the attendants and the hospital staff for their aids in recruiting patients and collecting data for my work. Finally, I would like to acknowledge the participation of enrolled patients and their family members, without them this study would not be possible. This study is from them and for them always.

*My sincere and heartfelt gratitude to all the non-scientific and administrative officials in both the institutes. I thank the Indian council of medical research (**ICMR**) for my fellowship.I am very thankful to the staff of the Department of Biotechnology specially **Mr. Chhail Bihari (CB Sir), Mr. Jitendar Singh (Jitendar Sir)** for their kind, and helpful reminders for completing all the administrative formalities.*

*My bow down respect to my seniors, **Dr. Sandeep Grover, Dr. Harpreet Kaur, Dr. Ajay Jajodia, Dr. Puneet Talwar.** Even being continents apart, they were never out of reach for the silliest of help required. Also my admiration to **Dr. Ruchi Baghel** and **Dr. Gurpreet Kaur Grewal** for showing us the right path during obstructions and uplift us, professionally and personally.*

*My research work and thesis writing would never have been possible without my beloved friends, **Ankit Srivastava** and **Neha Kanojia.** You guys are my family at IGIB. Thank you for being there to celebrate all the 'highs' and pull me out of all the lowest moments. I can't thank you enough. Cheers to all the moments we shared together! My friend **Chitra,** helped me understand the disease and always improved me scientifically. My friend, **Samiksha Kukal**, has taught me how soft and kind attitude can take me higher in life. My younger friends in the lab **Priyanka Singh** and **Sarita,** you are like my children. Thank you for being my reach out person, always. My other younger friends, **Manish Mishra, Shivangi, Saroj Yadav, Pooja Singh, Priyanka Paul , & Nitin ,** you were a great family to live with throughout this difficult journey. You all made it splendid. Special thanks to my friend, **Jyoti** (at SR lab), thank you for being patient with me while teaching R and **Bharathram** (Faruq lab) for introducing me to the wonders of high performance computing and troubleshooting the silliest to the strongest issues throughout our analysis.*

*My sincere gratitude and appreciation to **Dinesh Bhaiya** and **JP Bhaiya.** Without you, this lab can barely function.*

*I would like to thank all my DTU 2017 batch mates, **Niharika, Lakhan, Avnish** and others for all the kind support and help in all possible ways.*

*I have some precious gems in life, my friends-like- family, my best friend, **Ritu**, you are my childhood sweetheart. Thank you for being there, always! My soul-sister, **Prerna**, even though we are ocean apart, you are just a click away. My sweeter than sweetest, **Mishtu** for making it lively with all the fun we have around the darkest corners.*

*I dedicate my thesis to **parents**. Thank you, **Dad**, for believing in me and giving me more than everything I wanted ever. You are incomparable, truly! Thank you for imbibing in me the value, that work comes first. Thank you for believing me when everyone else raised a finger. Your silence efforts will never go waste, I promise. Thank you **Mamma** for the smallest of sacrifices you made to give us this life today. I will make you proud some day! Thank you for being just a call away, at the oddest of hours. Thank you, **Bonnie**, my little sister, for giving me the purest form of love. Thanks you for taking turns to be the elder one, when I act silly. My other mother, whom I came across during this tenure itself. She has been my role model for empowerment. Thank you for giving me more power every day. Last but not the least, my husband, **Tathagata,** thank you for being just there. Without the slightest of doubt, you have been there like a shadow. In all these years together, I learnt that marriage is all about companionship, being around in all of it- bad, boring, good & happy.*

Last, but not the least, thanks to all the people whose smallest efforts have made our lives easier and comfortable, from housekeeping, to security, to my domestic helps; to all who have not been mentioned by name but have given me all the support and help in their own way. They gave the luxury of choice of what I wanted to pursue.

My heartfelt thanks to each one of you!!

Debleena Guin

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

5-FU	5-Fluorouracil
6-MP	6-Mercaptopurine
ADME	Absorption distribution metabolism and excretion
ADR	Adverse drug reactions
AED	Anti-epileptic drugs
AFR	African
ALL	Acute lymphoblastic leukaemia
AMR	American
ANNOVAR	ANNOtate VARiation
AS	Activity score
AUC	Area under curve
AZA	Azathioprine
BBB	Blood brain barrier
BMI	Body mass index
BRAINEAC	Brain eQTL Almanac
CADD	Combined Annotation Dependent Depletion
CAE	Childhood absence epilepsy
CBZ	Carbamazepine
CI	Confidence interval
CPIC	Clinical pharmacogenetic implementation consortium
CPNDS	Canadian Pharmacogenomics Network for Drug Safety
CTD	Comparative toxicogenomic database
DAT	Dopamine transporters
dbSNP	Database of SNPs
DILI	Drug induced liver injury
DME	Drug metabolising enzyme
DPWG	Dutch Pharmacogenetics Working Group
DRE	Drug resistant epilepsy
DT	Drug transporters
DYPD	Dihydropyrimidine dehydrogenase
EAS	East Asian
EEG	Electroencephalogram
EMA	European medical association
EpiGAD	Epilepsy genetic association database
eQTL	Expression quantitative trait analysis
ESC	Embryonic Stem Cells
EUR	European
FDR	False discovery rate
FN	False negative
FP	False positive
FUMA	Functional mapping and annotation of genetic associations
GABA	Gamma aminobutyric acid
GAS	Genetic association study
GO	Gene ontology

GSA	Global screening array
GTCS	Generalized Tonic-Clonic Seizure
GTEx	Genotype-Tissue Expression
GWAS	Genome-wide
HCSC	Health Care Service Corporation
HER	Herceptin
HET	Heterozygosity
HGMD	Human gene mutation database
HGNC	Human gene nomenclature committee
HLA	Human leukocyte antigen
HSS	Hypersensitivity syndrome
HWE	Hardy Weinberg equilibrium
IBS	Ingenuity by descent
ICD-10	International classification of diseases v10
IFN	Interferon
IHBAS	Institute of human behaviour and allied sciences
ILAE	International league against epilepsy
INR	International normalised ratio
iPSC	Induced pluripotent stem cells
ISM1	Isthmin 1
LD	Linkage disequilibrium
LTG	Lamotrigine
MAF	Minor allele frequency
MAGMA	Multi-marker analysis of genoMic annotation
mEH	Microsomal epoxide
MeSH	Medical subject heading
MS	Multiple sclerosis
MT	Multi-therapy
MTHFR	Methylenetetrahydrofolate reductase
mTOR	Serine/threonine protein kinase
NCBI	National centre for biotechnology information
NIH	National institute of health
OMIM	Online Mendelian Inheritance in Man
OR	Odds Ratio
PB	Phenobarbital
PC	Principal component
PD	Pharmacodynamic
P-gp	p-glycoprotein
PGRN	Pharmacogenomic research network
PGx	Pharmacogenomics
PharmGKB	Pharmacogenomic knowledgebase
PHT	Phenytoin
PK	Pharmacokinetic
PMDA	Pharmaceuticals and Medical Devices Agency
PREPARE	PREemptive Pharmacogenomic Testing for Preventing Adverse Drug Reactions

PTZ	Pentylenetetrazol
PWE	Patients with epilepsy
QC	Quality control
RBC	Red blood cells
RCT	Randomised controlled trials
RNPGx	French National Network (Réseau) of Pharmacogenetics
ROC	Receiver operating characteristic
SAS	South Asian
SCAR	Severe cutaneous adverse reactions
SJS	Steven Johnson syndrome
SNP	Single nucleotide polymorphism
SPTLC3	Serine palmitoyltransferase long chain base subunit 3
STATA	Statistics and data
TEN	Toxic epidermal necrolysis
TN	True negative
TP	True positive
TPMT	Thiopurine methyltrasnferase
TSS	Transcription start site
TssA	Active transcription start site
Tx	Transcription site
Tx/Wk	Weak transcription
U-PGx	European Ubiquitous Pharmacogenomics
US-FDA	The United states- Food and drug administration
VPA	Valproic acid
WHO	World health organisation

Abstract

ABSTRACT

Inter-individual variability in drug response, broadly including drug efficacy and its safety, is an increasing problem globally. Such variability has detrimental effect on patients leading to increased financial burden to reduced quality of life. Genomic factors being one of the reasons for this variability, and is explored in the field of study called 'pharmacogenomics' (PGx). A broader definition of PGx, 'is the study of genomic technologies to enable the discovery and development of novel drugs and the optimization of drug dose and choice in individual patients to maximize efficacy and minimise toxicity. The efficacy of different drugs have been reported to vary from 25% to 80%. It is famously stated that more than 90% of the drugs only work in 30 to 50% of the people. And in terms of drug toxicity, adverse drug reactions (ADRs) affect about 15% of people in hospital. The aim of PGx is to define the underlying genetic mechanisms and ultimately to implement pharmacogenetic testing to improve treatment outcome. Another advantage of understanding the genetic basis of variable drug response can be used as a tool to expand the use of existing drugs for new indications as well as for identification of new drug targets or drug development.

After three decades of research in PGx, there are vital gaps in achieving translational efficiency when advancing towards clinical implementation. With the enormous amount of articles published every year (approximately 6 lakh articles in PubMed so far), less than 1% reaches to clinical trials (5491 randomised controlled trials). And among these only 133 genes are known with PGx outcome and are included for drug labelling warnings approved by the United Stated food and drug administration (FDA) for 363 drugs. This large translational gap needs to reduce. Secondly, most of the PGx markers established (163 markers for 118 drugs) are used to predict drug toxicity because clear and discrete phenotypic end-points are available for assessment like skin rashes, liver toxicity. But only a handful of markers (73 markers for 20 drugs) are known to predict drug response due to heterogeneous end-points like drug clearance rate, drug/metabolite ratio, and metabolizer status. More homogenous patient cohorts studying drug response outcomes are required to identify genome-based markers for poor response. And lastly, so far after several candidate gene studies, genome-wide association (GWAS) are shaping the future of genetic association models and it should attempt to explore the genome of patients across different ethnic groups but unfortunately, most of the GWAS are confined to western population like American (AMR) and European (EUR). Only a few in East Asian (EAS) encompassing Chinese and Japanese ethnicity, and no GWAS in African (AFR)

or South Asian (SAS) group so far. It is inevitable to screen the genome of all global populations to identify conclusive markers predicting PGx outcomes. Thus, our study was aimed to elucidate PGx markers to prognose therapeutic phenotypes in patients with epilepsy (PWE) & develop a platform for evidence-based testing for clinical implications.

In this study, we first developed a semi-automated text mining approach, using R-package, pubmed.mineR, to retrieve published articles with PGx information in the form of disease–drug–gene- genetic polymorphism relationships to obtain PGx related data for epilepsy treatment for easier therapeutic guidelines. Further we evaluated our approach by comparing its performance (precision and accuracy) with the other available benchmark datasets like PharmGKB and compared the results retrieved with the FDA approved PGx markers used for drug labelling to weigh its clinical ability and accuracy. We identified 2304 PGx relationships pertaining to 1753 disease types and 666 drugs. Our approach showed performance precision of 80.6% with benchmark datasets like Pharmacogenomic Knowledgebase (PharmGKB) (90.4%), Online Mendelian Inheritance in Man (OMIM) (60.0%), and comparative toxicogenomics database (CTD) (72.9%). From a total of 2,304 PGx relationships identified, a total of 127 (68%) are coinciding with the 362 US-FDA approved 362 pharmacogenomic markers used in drug labelling, indicating that our approach has a greater precision in data extraction with PGx information for drug response prediction.

Subsequently, we performed genome-wide genotyping on 789 PWE of North Indian origin to identify genetic variants significantly associated with poor response to commonly prescribed anti-epileptic drugs (AEDs) like phenytoin (PHT), carbamazepine (CBZ) or valproic acid (VPA). This GWAS was performed using commercially available Illumina Infinium Global screening array (GSA)-24 v2.0 with psych customization. On performing quality control (QC) based on different parameters to exclude out the poor quality single nucleotide polymorphisms (SNPs) and samples, we conducted logistic regression using age, sex and 2 principal components (PC1, PC2) as covariates in PLINK 1.9 assuming an additive model and evaluated the association of each imputed SNP. Our GWAS of AED response revealed suggestive evidence for association at 29 genomic loci ($p < 5.0 \times 10^{-5}$) but no significant association reflecting its limited power. The suggestive associations highlight candidate genes that are implicated in metabolism of AEDs are known targets to these drugs. The top SNP rs60633642 associated with overall poor response to AED [OR (95% CI) = 1.98(1.50-2.60), $p < 1.185 \times 10^{-6}$] is an intergenic variant (*SPTLC3*; *ISM1*) down-regulating the expression of *SPTLC3* gene in different tissues, most significant at brain caudate basal ganglia

tissue ($p<0.0003$). Functional annotation of these genomic loci based on enrichment analysis revealed functions like regulation of K^+ transmembrane transport, neuron development, Ca^{2+} transport to be enriched with maximum fold enrichment (12 fold, $p< 5.0 \times 10^{-6}$). Likewise, genetic variants associated with poor response to phenytoin, carbamazepine, valproic acid within the suggestive cut-off were four, thirteen and eleven, respectively. Our study is the first of its kind, investigating genetic association of AED response specific to Indian population using a genome-wide approach. The findings from this study upon replication and diagnostic predictability assessment can be used for upcoming pharmacogenetic studies.

Lastly, we estimated the diagnostic accuracy of these identified PGx markers for which we overlapped our GWAS results with already published markers known with PGx response to AEDs as well as our findings from text mining in our study. In conclusion, 88 commercial PGx marker are known related to AED response. Among these 19 SNPs overlapped with our GWAS findings for overall poor response. Assessing the diagnostic predictability of these 19 markers showed moderate accuracy (50-60%). These markers are promising candidates for PGx application after appropriate validation and replication. Eight out of these 19 genetic variants are already in use for drug labelling approved by the FDA. Strengthening the fact that genome-based markers can be exploited for application in precision medicine in epilepsy treatment.

In conclusion, our study provides a robust text mining semi-automated R-package for retrieving promising PGx variants. These variants were screened in our population specific cohort highlighting significant loci associated with poor response to AEDs like phenytoin, carbamazepine and valproic acid. Although, further replication in independent sample cohort can strengthen statistical power, or functional validation of the associated loci could help provide mechanistic insights for biological relevance to pharmaco-response. Eleven out of the 19 SNPs identified from our GWAS data have moderate diagnostic accuracy are promising candidates which upon validation can be used for PGx application in prognosis of poor response in epilepsy specific for our population.

Chapter 1

Introduction, Aim & Objectives

1.1 Introduction

Inter-individual variability in drug response, broadly including drug efficacy and its safety, is an increasing problem globally. Such variability has detrimental effect on patients leading to increased financial burden to reduced quality of life. Genomic factors being one of the reasons for this variability, and is explored in the field of study called 'pharmacogenomics'. PGx elaborates how the role of genes or their genetic variants (involved in the pharmacokinetic/ pharmacodynamic pathways of the drug) can ultimately impact drug response. However, in this new age of high throughput technologies and complex disorders, the research has become increasingly multi-dimensional, involving multiple genes (and/or variants). A better understanding of the molecular aspect of variability in drug action from the genomic perspective can provide important insights into individual genetic determinants of therapeutic response (**Figure 1.1**) and evidence-based tailored treatment or clinical guidelines for epilepsy patient management.

Applications of pharmacogenomics can range from predicting drug dose, improving drug efficacy, to minimize drug toxicity. In the past two decades of abundant academic research and publications in this field, several success stories have paved their way towards clinical testing. The best example being the 'poster-child' of pharmacogenomics, warfarin which is metabolised by CYP2C9 and VKORC1 which is inhibited by warfarin determines the dose requirement of the drug. Patients carrying the loss-of-function variant of CYP2C9, in one copy or two, require lower doses to achieve therapeutic response of anti-coagulation (Bourgeois, 2016). Similar examples for drug efficacy markers are known for drugs like clopidogrel, tamoxifen, metformin, codeine, etc. About one-third of patients have reduced enzyme activity due to the presence of loss-of-function variants of CYP2C19 (Pereira, 2019), an enzyme that metabolises the prodrug, clopidogrel which is given as an anti-platelet agent in patients with ischemic heart diseases. As a result, patients show poor efficacy and increased risk of ischemic events(Shuldiner, 2009a). Advances in HLA pharmacogenomics have established extensive research towards understanding immune mediated adverse drug reactions (ADRs). Some classic examples are skin hypersensitivity reactions in response to carbamazepine linked to HLA-B*15:02 variant. This variant has been widely replicated globally and is presently used in drug labelling information and genetic testing (Ferrell, 2008).

The gap between such enormous build of published articles in pharmacogenomic, to promising markers that can be used in actionable PGx testing is still wide and persistent.

Therefore, it is our foremost goal to screen these drug-gene relationships from the literature for better characterization of their potential for clinical utility. Analysing such related data from the literature, we a robust, fast and accurate platform to retrieve population-specific genetic polymorphisms that correlate with drug response. These variants can be crucial for diagnosis, treatment, and prevention. Such considerations may benefit clinicians, researchers and most importantly the patients. There are already available databases that demands manual curation of biomedical literature to obtain PGx relationships for humans. The gold standard being the PharmGKB database, followed by the table of PGx markers used for drug labelling information approved by the United States Food and drug administration (US- FDA). Thus to enable clinical implementation, all types of evidence should be taken into account and evaluated carefully to optimize patient outcomes. Screening through all the biomedical literature published so far in pharmacogenomics, the largest part includes drugs administered for neoplasms (34%) followed by neurological disorders (14%), mental and behavioural disorders (11%), cardiovascular disorder (10.2%), metabolic (7.5%), blood and other related disorders (5.5%) and others (including infectious diseases, congenital or autoimmune disorders, pain, reproductive, eye, ear, bones- related, hormonal and lymphatic system related disorders) (18.5%). Besides, based on the data of top 200 drugs prescribed in the US (in year 2020), 20% of them are for neurological disorders (ClinCalc). Thus, we decided to take up neurological disorders for our pharmacogenomic work. Additionally, the recent world health organization (WHO) report (2013), reveals that of the one billion people affected with neurological disorder worldwide, 65 million suffer from epilepsy and 24 million from Alzheimer's disease and other dementias (WHO, 2022). Thus we considered epilepsy for our study.

Epilepsy (G40) is an umbrella term, included under 'diseases of nervous system' in international classification of diseases (ICD-10 version 2019) that comprises of a spectrum of complex neurological disorders with a ranging symptom of seizure occurrence in common (Organisation, 2010). It includes localisation-related or generalised seizures, special epileptic syndromes, and status epilepticus. The treatment of epilepsy, most commonly includes prescription of anti-epileptic drugs. They primarily function to control seizures. The treatment outcome vary considerably among patients with same epilepsy symptoms when administered with the same dose of the AED.

Even after 40 years of the discovery of genetic variability playing a role in the treatment of phenytoin for epilepsy, there are several crossroads for PGx to make its way through the clinics. Researches done so far on association of particular genes and its variants with seizure

control and adverse drug reactions have not provided unifying conclusions. This reflects limitation in methodology or study design in the past. The quality of evidence is lessened due to lack of an accurate phenotype definition for pharmacogenomic studies, inadequate sample sizes and also replicability.

Apart from this, implementation of this scientific knowledge for clinical use is another major setback. Patients carrying CYP2C9*2 or *3 allele have been known to reduce the metabolism of phenytoin in European population, however it is still not routinely used to screen patients (Franco V, 2015). May be due to limited availability of genetic testing, patients' willingness to perform the test, financial constraints and other socio-economic factors alike. This gap is beyond the reach of this study to be addressed therefore, focussing on the robustness of the scientific evidences achieved so far the field of PGx in epilepsy has been suggested to be one of the favourable areas to have consistency, large amount of genetic data available, greater degree of *in vitro* and *in vivo* evidences to substantiate the functional importance (Leschziner GD, 2007). One of the prominent advancement in this field has been the US Food and drug administration (FDA) approval of HLA- B*1502 allele for carbamazepine induced skin reactions as ADR of the drug used for drug labelling (FDA, 2018; P Brent Ferrell, 2008).

One further issue is that identifying an 'associated' genetic variant do not necessarily imply causation of the phenotype. Therefore, more direct evidences of the functional relevance of that variant is needed to accept that factor contributing to pharmacoresistance of AEDs. Several attempts in such direction have been made to correlate the multifaceted challenge of AED pharmacoresistance with single nucleotide polymorphisms (SNPs) in genes like *CYP2C9, CYP2C19, CYP3A4, CYP1A1, EPHX1, UGT1A4, UGT2B7, ABCB1, ABCC2* and *SCN1A* have multiple evidences to alter the pharmacokinetic or pharmacodynamic of the commonly prescribed AEDs like CBZ, PHT, lamotrigine (LTG) and so on.

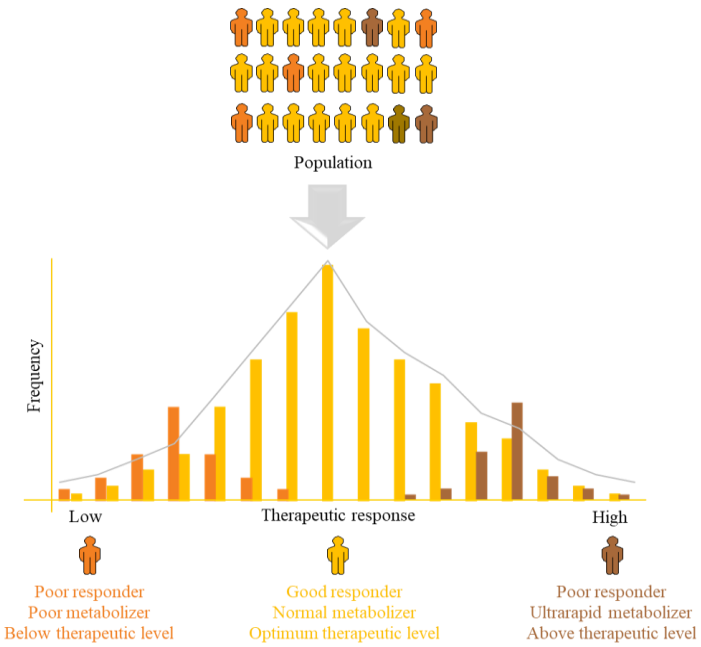


Figure 1.1: Profile of variable drug response as influenced by single gene variant (in bars) or multiple variants (in grey) in a sample cohort. Genetically determined variability in drug response often involve single gene variant common in a population and associated with relatively large effect sizes and distinct pharmacogenomic phenotypes like metabolizer phenotype (poor metabolizer, normal metabolizer and ultrarapid metabolizer), therapeutic level (below therapeutic level, optimum therapeutic level, above therapeutic), response to drug therapy (good responder, poor responder).

1.2 Rationale and Hypothesis

Despite the availability of more than 25 antiepileptic drugs, around 30% of people with epilepsy do not respond to its therapy, thereby being refractory, a medically intractable condition wherein patient does not respond to even multidrug therapy (S., 2010). A critically high percentage that has not changed in decades, in spite of all the genetic or molecular information available. This can be primarily because all the current pharmaceutical agent can merely reduce the incidence of seizures ("anti-ictogenic"), and they do not interfere with the natural course of the disease. Failure to respond to AEDs results in long-term negative effects on health care as well as social domains such as education, employment, marriage etc. (Jennum P, 2016). This non-responsiveness to AEDs encouraged researchers to adopt the approach of pharmacogenetics (Pirmohamed., 2001). From a pharmacogenetic perspective, there are few robust genetic findings with established evidence in epilepsy. For example that Asian patients with a particular HLA allele, HLA-B*1502, are at a higher risk for Stevens-Johnson syndrome when using carbamazepine, are helpful to increase our knowledge how genetic variation affects the treatment of epilepsy, or for CYP2C9 genotyping to identify individuals at risk for serious skin reactions from phenytoin is less compelling. The FDA has a list of 517 gene-drug associations that are currently used for drug labelling (FDA., 2022b) and its table of

pharmacogenomic associations lists 121 drug-gene interactions (FDA., 2022a). Based on the factual so far, there are approximately 136 FDA-approved drugs with PGx information in their labelling. Among these several biomarkers are available for adverse drug reactions of AEDs and none of the biomarker for drug efficacy. Apart from this the available PGx markers are broadly classify into markers predicting ADRs and drug response. From literature that discrete ADR markers are available but there are inconclusive and a very few markers available to predict drug response. Thus, discrete genetic variants identifying drug response outcome for specific drugs are elusive. Lastly, most of the genome wide studies published till date include the western population, American and European. No studies in African and Asian population are published till date, Thus, our study is an attempt to bridge these gaps in order to identifying the population specific markers predicting drug response in epilepsy. This would help the clinicians/ medical practitioners make efficient decision regarding prognosis, diagnosis, and treatment.

PGx markers has potential to prognose therapeutic phenotypes in patients with epilepsy and thus can be used for evidence-based testing for clinical applications in predicting drug-specific outcomes prior treatment. Hence, we aim to identify such markers specific for our population which can be used for epilepsy patient management in India.

1.3 Objectives:

1. **To screen pharmacogenetic (PGx) markers from global literature and build a resource of drug response genes for clinical implementation.**

   In this objective we aim to develop a semi-automated text mining approach to retrieve the complete PGx resource integrating disease–drug–gene-polymorphism relationships to derive a global perspective of epilepsy for ease in therapeutic approaches. Further we evaluated our approach by comparing its performance (precision and accuracy) with the other available benchmark datasets like PharmGKB, etc. We, then extended our effort to compare the results retrieved with the FDA approved PGx markers used for drug labelling to weigh its clinical ability and accuracy. This approach is a scalable and state-of-art approach in curation for PGx clinical utility.

2. **Identification of these PGx markers in north Indian population to assess their correlation with anti-epileptic drug response using high throughput genome-wide screening.**

Using the genome-wide genotyping approach to find the genetic markers we adopted global screening array (GSA), a genome wide approach which is a next-generation genotyping array used for population scale genetics, variant screening, precision medicine and pharmacogenomics. It contains 7,60,502 markers and 44,877 customized markers. GSA contains all clinically relevant variations which are associated with at least one trait and somehow involved in regulating human physiology, we have opted this platform. Hence, it is a good approach to find genome wide susceptibility loci for epilepsy. It is a three day protocol in which firstly, DNA having concentration 50 ng/µl is amplified, fragmented using enzymatic processes, precipitated, re-suspended using specific reagents and then hybridised with the bead chip probes. After hybridisation, single base extension occurred and staining is performed. At the final step of experiment imaging is done using iScan. Then data is generated in ".idat" files. A comprehensive genome-wide association analysis was performed which broadly include (i) data pre-processing and QC check (ii) statistical and association analysis using tools like Genome studio and PLINK/R software. Further, Post-GWAS analysis was also performed for identification annotation of independent associations within our data using functional mapping and annotation of genetic associations (FUMA).

3. Development of PGx panel with efficacy/ toxicity marker for pharmacogenetic testing towards pilot implementation.

This objective is committed to identify potential translating PGx markers into actionable prescribing guidelines, standardize PGx testing and thereby improve patient treatment outcomes. For this we estimated the diagnostic accuracy of the identified markers which would be essential for developing PGx panel.

Chapter 2

Review of Literature

2.1 Pharmacogenomics: a brief introduction

Inter-individual variability in drug response, broadly including drug efficacy and its safety, is an increasing problem globally. Such variability has detrimental effect on patients leading to increased financial burden to reduced quality of life. Genomic factors being one of the reasons for this variability, and is explored in the field of study called 'pharmacogenomics'. A broader definition of pharmacogenomics, 'is the study of genomic technologies to enable the discovery and development of novel drugs and the optimization of drug dose and choice in individual patients to maximize efficacy and minimise toxicity. The efficacy of different drugs have been reported to vary from 25% to 80%. Allen Roses in 2003 famously stated that more than 90% of the drugs only work in 30 to 50% of the people (Connor, 2003). And in terms of drug toxicity, ADRs affect about 15% of people in hospital. The aim of pharmacogenomics is to define the underlying genetic mechanisms and ultimately to implement pharmacogenetic testing to improve treatment outcome. From a clinical perspective, pharmacogenomics targets to shift from the conventional notion of 'one-drug-fits-all' towards a more individualised choice, based on the patients' details like demographics (age, sex, body mass index (BMI), ethnicity, diet, etc), clinical parameters like (comorbidity, disease type, duration, family history, and so on), pharmacological (including the pharmacokinetic parameters), and of course, the genetic construct of the individual.

The substantial progress made in genotyping and sequencing technologies, statistical genomic analysis, better clinical trial designs, as well as collaborative research efforts have driven the discovery of genetic variants associated with drug response. Advancing towards the foremost application of pharmacogenomics, which is in predicting drug dose, improving drug efficacy, predicting the activation of pro-drug and preventing adverse drug reactions by prospectively genotyping individuals for at-risk alleles. Another advantage of understanding the genetic basis of variable drug response can be its use as a tool to expand the application of existing drugs for new indications as well as for identification of new drug targets or drug development (**Figure 2.1**).

Conceptually, there are two pathways by which an individual respond towards an administered drug. The pharmacokinetics (PK), i.e. factors that influence the concentration of the drug that reaches the therapeutic site. Pharmacodynamics (PD) describes the variability in drug action not directly attributed to the concentration of the drug, but may reflect the variability in interaction of the drug or its metabolite with other molecules. Earlier studies

determined the clinical implications of drug response within gene families encoding drug metabolising

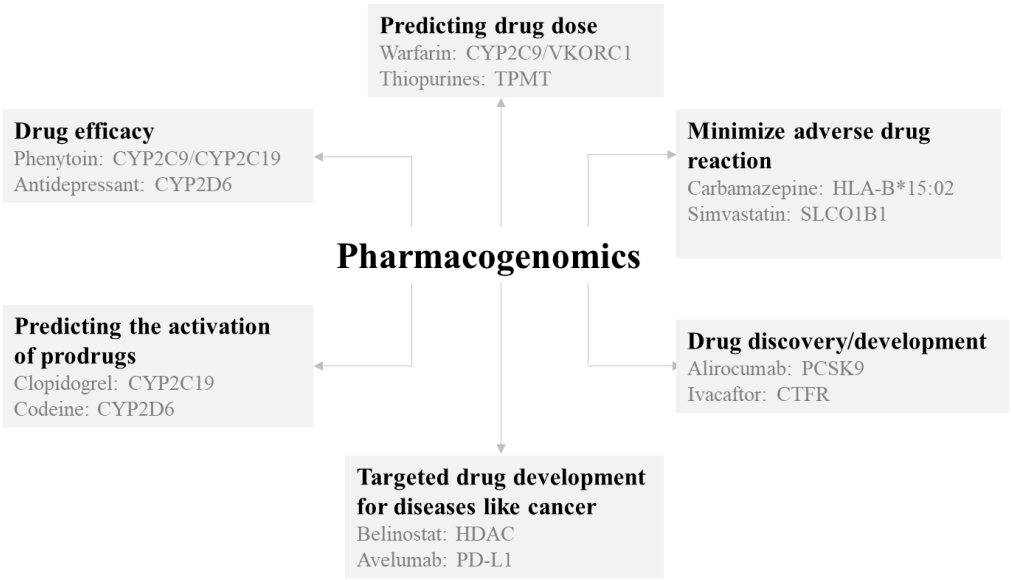


Figure 2.1: Scope of pharmacogenomics. Pharmacogenomics is important for predicting drug response, improving drug efficacy, predicting activation of prodrug molecules, for targeted drug development (for diseases like cancer), evidence-based drug discovery or development and in minimizing adverse effects of drugs. Two examples in each category are represented (Pirmohamed, 2023).

enzymes (DMEs), drug transporters (DTs) and drug targets. This can be because of simpler measurable phenotype, like serum drug concentration, drug-to-metabolite ratio, contributing to variable clinical response and easier identification of PK outliers from the population in whom higher or lower drug concentrations were correlated to drug efficacy or adverse drug response. Genetic variants within or near genes that encode that enzymes are known with phenotypes based on the enzyme activity. For example, the thiopurine methyltransferases (TPMT) enzymes catalyses methylation of thiopurine drugs like azathioprine. The most common variant allele of this enzyme, TPMT*3A with minor allele frequency of 5%, making one out of every 300 European individual, a homozygous carrier of this variant (Szumlanski C, 1996) . This variant includes two non-synonymous alterations in TPMT gene, which when translated forms misfolded TPMT protein and thus rapidly degraded. As a consequence, a homozygous carrier subject when given thiopurine drugs at the conventional dose, leads to ten-fold overdose due to enzyme inactivity causes cytotoxicity like myelosuppression. (Tai HL, 1999) (Wang L, 2003) (Wang L, 2005).

The other scenario where genetic variants in PK gene have large effect is with administration of pro-drugs. Prodrugs are pharmacologically inactive compounds that are bio-

activated by enzymes to show therapeutic effect. The most popular example is clopidogrel, an antiplatelet drug. This is metabolised into active drug by CYP2C19. Patients harbouring the loss-of-function variant CYP2C19*2 or CYP2C19*3 (Pereira, 2019) show high reactivity and increased risk of ischemic effects (Shuldiner, 2009b).There is a large spectrum of these pharmacogenomic effects. Thus, an increased dose showed therapeutic effect in heterozygous carriers of the variant, CYP2C19*2. Contrastingly, a dose increase in homozygous carriers showed no anti-platelet activity due to complete loss of enzyme activity(Mega JL, 2011). Hence, dose determination for drugs can be based on enzyme activity to control the efficacy of the drugs. Carriers of loss-of-function variants, heterozygous or homozygous, requires a lower dose administration to achieve overexposure. A dew gain-of-function variants are also known which are associated with excess drug response. For example, CYP2C19*17 has been associated with excessive bleeding on clopidogrel therapy (Sibbing D, 2010).

Pharmacodynamic variations also influence drug response. The classic example identified are the loss-of-function variants in VKORC1 associated with warfarin resistance, an absence of rise in international normalised ratio (INR) even on large dose of warfarin administration. Of the earliest known evidence of drug response with PD mechanisms are variants reducing G6PD function caused a high incidence of haemolytic anaemia in African-American soldiers during World War II, when given Rasburicase (Relling MV, 2014a). Most other PD variants are studied with respect to drug safety and toxicity.

ADRs are mainly of two types, Type A ADRs are caused due to pharmacological consequences of the drug and are dose dependent, with reduction of dose leading to improvement in ADR (Rawlins, 1991). The type B are mostly idiosyncratic reactions which are not directly related to the pharmacological consequences. Many of these are immune-mediated response and substantial progress has been made in regard to HLA pharmacogenomics. The cytotoxicity in regard to TPMT gene variant that we discussed previously is a type A ADR. Haemolytic anaemia caused due to G6PD deficiency is another such example. The most common type B ADR are those associated with drug hypersensitivity reactions, including Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), hypersensitivity syndrome (HSS) and drug-induced liver injury (DILI). For example, HLA-B*5701 with abacavir-induced drug hypersensitivity, HLA-B*1502 in carbamazepine (CBZ)-induced SJS/TEN, allopurinol associated severe cutaneous adverse reactions (SCAR) with HLA-B*5801 (Chun-Yu Wei, 2012). Novel findings suggest that drugs and their metabolites

interact with specific HLA molecules and T cell receptors leading to clonal T cell proliferation and cytokine secretion resulting in tissue injury (White, 2015) (Jaruthamsophon, 2021)

Over the decades, we have now progressed towards agonistic genome-wide association studies (GWAS), to decode the entire spectrum of drug response enabling the identifation of novel variants, new biological/ molecular mechanisms and explain the genomic component of drug response in a specific population. Rigorous efforts in this field have highlighted the importance of ancestry in drug response phenotypes. However, <10% GWAS published so far investigated drug response (McInnes, 2021) due to shortcomings like poor sample size, lack of clear definition of pharmacogenomic phenotypes, and replicability. Nonetheless, GWAS have been able to successfully identify predisposing genetic loci due to large effect size (Maranville, 2016). A review article by McInnes G et al (2021) has meticulous captures the different aspects covered in the GWAS of PGx studies so far (McInnes G, 2021). Association from GWAS only serves as the beginning of our journey in understanding the role of genomics in drug response (Lavertu, 2018). The discovered associations are often not causal, rather in linkage disequilibrium (LD) with the causal variant. Hence, fine mapping of GWAS findings in pivotal, such that they can be used for diagnostic applications. More importantly, findings must be replicated by subsequent studies in external cohort to confirm such association (Schaid, 2018). Many collaborative efforts, and guidelines have been formed to fulfil the goals of PGx into the clinic.

2.2 Pharmacogenomics: from discovery to clinical applications

Much progress has been made from identifying pharmacogenes with variable drug response, characterising them based on the specific phenotype and determining their clinical utility (like diagnostic accuracy, cost effectiveness, robust association, and risk assessment). Although implementation should be accompanied by continuous monitoring with real situations for constant improvement in optimizing the tests. To facilitate clinical implementation of pharmacogenomics, a few key points are: usage in all healthcare settings focussing on drugs with actionable information, appropriate funding, modifiable testing and recommendations with time, investment in infrastructure for education and training of clinicians, policymakers, patients and researchers, efficient research in other fields like ethical, legal and social issues, outlay clear prescription guidelines to minimize error and maximize cost effectiveness (Society., 2022).

To date, several successful attempts have been made to facilitate clinical implementation of pharmacogenomics. With the collaborative efforts of the US National Institute of Health (NIH) along with the pharmacogenomic research network (PGRN) (Relling, 2017), formed the first pharmacogenomic database, PharmGKB, with manual curation of such evidence from biomedical literature (Gong, 2021). At the foremost drive, the FDA incorporated pharmacogenomic drug labelling for over 250 drugs, mentioning the impact of genotype with severe or life threatening response outcomes (FDA., 2022b) (**Table 2.1**). In early 2020, the FDA updated this information and released a table of pharmacogenetic associations listing associations in three groups: i) support therapeutic management recommendations, ii) indicate a potential impact on safety and response, and iii) potential impact on PK properties.

The Clinical pharmacogenetic implementation consortium (CPIC) (Relling MV, 2014a) was formed to form the clinical recommendation guidelines for interpretation and translation of genotype results into prescribing decision for 450 drug-gene interactions. Eighty three of these (22 genes with 63 drugs) are annotated with highest level of evidence and have prescription guidelines. These 63 drugs for a large proportion of prescribed drugs (Alshabeeb, 2019; Dunnenberger, 2015) thus, around 35-65% of the population is exposed to at least one of these prescribed drugs with PGx indication (Chanfreau-Coffinier, 2019; Krebs, 2019)

Implementation of pharmacogenomics has been a far-fetched dream in the past decade. The main reasons being perceived lack of clinical utility, inability to access genotyping tests, cost effectiveness, interpretation of test results, prescribing actions for patients carrying variant allele, issues of data confidentiality, regulatory guidelines. To address these concerns, numerous PGx initiatives have been undertaken. The Canadian Pharmacogenomics Network for Drug Safety (CPNDS), the Dutch Pharmacogenetics Working Group (DPWG) and the French National Network (Réseau) of Pharmacogenetics (RNPGx). The European Ubiquitous Pharmacogenomics (U-PGx) consortium has undertaken a prospective study in seven European centres with almost 7,000 patients randomly allocated to either standard care or genotype-guided care (van der Wouden, 2017).This study include a panel of 44 variants encompassing 12 genes for 42 drugs (van der Wouden, 2020). A striking outcome of this study, showed that genotype-guided care reduced ADRs by 30%, establishing the first randomised evidence for PGx panel based testing (Swen, 2023).

The clinical use of PGx testing has been primarily adopted by two approaches: point-of-care testing or pre-emptive testing. The point-of-care testing includes an alert signal from

electronic health record (EHR) when patient is administered a drug. Rapid turnaround testing is performed and results and dose recommendations are provided accordingly. A consensus to embrace the pre-emptive genotyping strategy was adopted by all these participating organisations. This practically means, a patient requiring a PGx testing which undergo genotyping on a commercial panel containing a number of genetic variants. This data would be then stored in EHR for future use if the patient require anther drug for which response is influenced by PGx variants. This strategy has been widely opted by including St Jude Children's Research Hospital (Hoffman, 2014), Vanderbilt University Medical Center (Van Driest, 2014) and the Mayo Clinic (Matey, 2022).

Several clinical trials to assess the outcome of pharmacogenomic testing. TAILOR-PCI (NCT01742117) and POPular Genetics (NCT01761786) are comparing the effect of a pharmacogenomically informed strategy to conventional strategies in the use of clopidogrel and other antiplatelet therapies. A large trial, dal-GenE (NCT02525939), is underway to screen approximately 35 000 patients to identify around 6000 with the predicted response allele, and to then randomly assign these patients to dalcetrapib or placebo. The largest multi-centric RCT so far designed by the European U-PGx group, the PREemptive Pharmacogenomic Testing for Preventing Adverse Drug Reactions (PREPARE) is evaluating a pre-emptive pharmacogenomic testing strategy in 12 genes to reduce the incidence of ADRs related to 43 target drugs (C.-T. A. van der Wouden CH, Cecchin E, et al., 2017). IGNITE is currently planning an evaluation of panel-based testing for management of depression, chronic pain, and acute postoperative pain (Orlando, 2019). The whole genome programmes include Genome England, aiming to sequence up to 5 000 000 whole genomes, and the US All of Us Program, recruiting 1 000 000 participants (investigators, 2019).

Execution of pharmacogenomics into hospital settings is likely to be a major driver of introduction of genetic testing into clinical practice. This is just one of the components progressing towards precision medicine. Several multimodal algorithms (incorporating clinical and genetic aspects), integration of multi-omics data, cross-border collaborative research and clinical networks, improved financial and regulatory infrastructure and most importantly, the conglomeration of all these into digital therapeutic applications are insurmountable. Such opportunities can revolutionize the human health metrics.

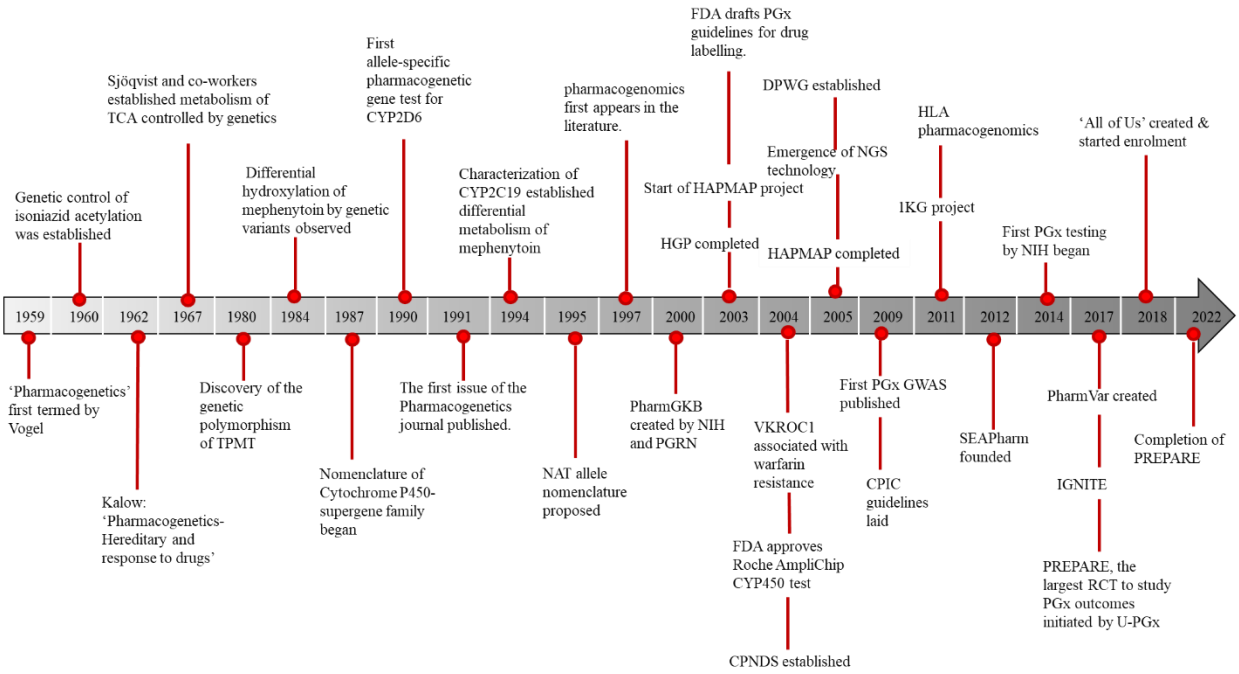


Figure 2.2: Major milestones of pharmacogenomics (UA, 2004).

2.3 Application of pharmacogenomics in different diseases/disorders

With the gradual evolution of genomic medicine and extensive research in broad areas of disease pathogenesis, its diagnosis and treatment with the implementation of pharmacogenomic protocol in personalised therapeutics. Cardiovascular disorders (25%-30%), cancer (20%-25%) and brain disorders (10%-15%) represent over 70% of morbidity and mortality in developed countries. Individuals with such chronic disorders require treatments for long period of time. Thus, determining markers for therapeutic response and minimize risk of ADR and apply this knowledge in a clinical context are the ongoing scenario of pharmacogenomic research. A brief background of the pharmacogenomic story so far from each disease perspective is elaborated below.

2.3.1 Cancer

Oncology is considered to be the field of medicine in which pharmacogenomics and personalised medicine is perhaps most established. A significant number of pre-emptive genetic tests are now routinely undertaken prior to anticancer drug administration. Quite invariably, oncology indications represent 126/364 (35%) of all Food and Drug Administration drug label warnings related to pharmacogenomic markers (FDA., 2022b). Examples where there is clinical utility include genotyping or phenotyping for G6PD to prevent rasburicase-induced red blood cells (RBC) haemolysis, and TPMT to prevent thiopurine-induced bone marrow

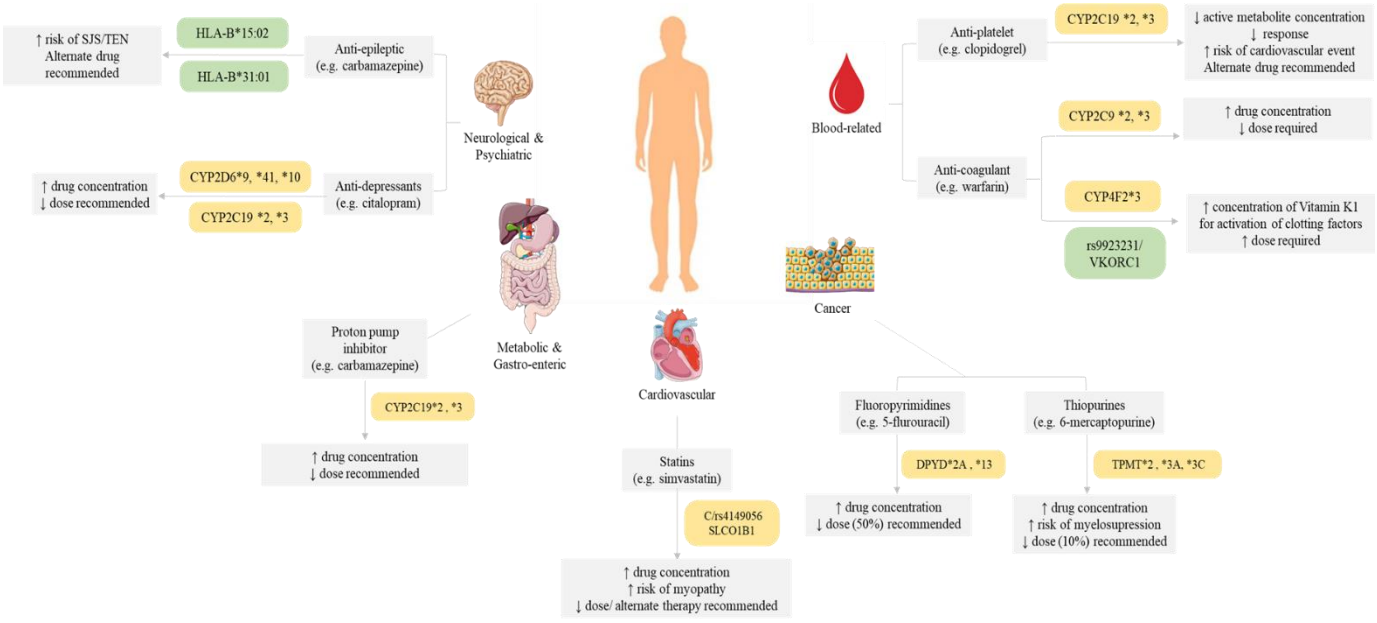


Figure 2.3: Clinical implementation of pharmacogenomics across different disease categories. Commonly prescribed drugs in each disease category and their clinical implementation according to CPIC guidelines used for genotype-guided testing to manage treatment outcomes. Most of these recommendations belong to the category of 'testing required' or 'testing recommended'.

suppression. Other associations such as CYP2D6 status in determining the efficacy of tamoxifen are more controversial because of contradictory evidence leading to variability in clinical implementation (Carr DF, 2021).

6-Mercaptopurine is used in the treatment of acute lymphoblastic leukaemia (ALL), metabolised by TPMT to an inactive methylmercapturine resulting in less parent drug available and toxic metabolites. Variant alleles of TPMT (*2,*3A and *3C) are associated with low enzyme activity thus, individuals carrying 2 loss-of-function alleles are at significantly increased risk of life-threatening myelosuppression as a result of increased metabolite exposure (WE., 2004). More recently, GWAS identified variants in NUDT15 that strongly influence thiopurine intolerance in ALL patients (Yang JJ, 2015). NUDT15 catalyses the conversion of thiopurine metabolite to the less toxic metabolite. Defect in NUDT15 gene lead to risk of myelosuppression. The SNP, rs116855232 (p.R139C), causes a complete loss of enzymatic activity and ultimately severe myelosuppression. Whilst the influence of inherited TPMT dysfunction on thiopurine-induced intolerance is of greater importance in European or African ancestry, NUDT15 risk alleles seem to be more important in those of Asian and Hispanic ethnicity. 5-fluorouracil (5-FU), a prodrug indicated for the treatment of colorectal cancer, breast cancer and other gastrointestinal tract cancers. The rate-limiting enzyme for 5-FU

catabolism is dihydropyrimidine dehydrogenase (DPYD). DPYD*2A and DPYD*13 have the most deleterious impact on DPYD activity heterozygous carriers are designated an activity score (AS) of 1, and homozygotes with AS of 0, when wild type carriers have AS 2. Variants rs75017182 and rs67376798 are thought to moderately reduce DPYD activity and so heterozygotes are given an AS of 1.5 (Lunenburg CATC, 2020). Approximately ~7% of Europeans carry at least 1 reduced function DPYD variant (Amstutz U, 2018b). CPIC guidelines for Fluoropyrimidine recommends a 50% reduction in starting dose in patients with a DPYD AS of 1–1.5 (heterozygous intermediate metabolisers) and avoiding fluoropyrimidine therapy when possible in those with an AS of 0–0.5 (poor metabolisers)(Amstutz U, 2018a). Other such indications for pharmacogenetic testing are well established for variants of G6PD causing enzyme deficiency manifesting haemolytic anaemia when patients are given rasburicase (Relling MV, 2014b). Irinotecan and UGT1A1*28 predisposing patients towards severe ADR like neutropenia (Innocenti F, 2004).Tamoxifen and poor metaboliser genotype of CYP2D6 have been shown to have reduced efficacy and therefore alternate therapy or increased dose is recommended (Goetz MP, 2018) (Zembutsu H, 2017). This observations have contributed towards several initiatives utilizing PGx testing, the European U- PGx consortium, the PREPARE clinical trial (C.-T. A. van der Wouden CH, Cecchin E, et al. , 2017). eMERGE (Gottesman O, 2013), IGNITE (Orlando, 2019), PG4KDS (Hoffman JM, 2014) and ACCOuNT (Program., 2018).

Table 2.1 List of clinically validated PGx biomarkers in oncology and their level of recommendation by different PGx- regulatory bodies for related drugs

Variant	Gene	Drug	CPIC Level	PharmGKB Level of Evidence	FDA	EMA	Swiss Medic	HCSC	PMDA	Ref
rs3918290, rs55886062	*DPYD*	capecitabine	A	1A						(Caudle *et al.*, 2013); (Amstutz *et al.*, 2018)
rs3918290, rs55886062, rs67376798	*DPYD*	fluorouracil	A	1A						(Caudle et al., 2013); (Amstutz et al., 2018)
UGT1A1*28	*UGT1A1*	irinotecan	A	1A						
rs116855232	*NUDT15*	mercaptopurine	A	1A						(Caudle et al., 2013; Relling *et al.*, 2011); (Relling *et al.*, 2019)
TPMT*2, *3A, *3C	*TPMT*	mercaptopurine	A	1A						(Relling et al., 2011); (Caudle et al., 2013);(Relling et al., 2019)
Class II and III variants	*G6PD*	rasburicase	A	1A						(Relling *et al.*, 2014);(Gammal *et al.*, 2023)

CYP2D6 PM	*CYP2D6*	tamoxifen	A	1A						(Goetz *et al.*, 2018)
rs116855232	*NUDT15*	thioguanine	A	3						21270794;23422873;30447069
TPMT*2,*3A,3C	*TPMT*	thioguanine	A	3						21270794;23422873;30447069
UGT1A1*60, UGT1A1*28	*UGT1A1*	belinostat	B							
CYP2C19 PM	*CYP2C9*	erdafitinib	B/C							
rs121434568	*CYP2D6*	gefitinib	B/C	3						
HLA-DRB1*15:02,	*HLA-DRB1*	lapatinib	B/C	3						
UGT1A1*6, UGT1A1*27, UGT1A1*28, UGT1A1*1, UGT1A1*6	*UGT1A1*	nilotinib	B/C	3						
HLA-B*57:01	*HLA-B*	pazopanib	B/C							
UGT1A1*28	*UGT1A1*	pazopanib	B/C	3						
rs113488022	*G6PD*	dabrafenib	C							(Gammal et al., 2023)
rs9272105, HLA-DQA1*01:03, HLA-DQA1*02:01	*HLA-DQA1*	lapatinib	C	3						
DPYD c.1129-5923C>G	*DPYD*	tegafur	C	1A						23988873;29152729
rs113488022	*G6PD*	trametinib	C							36049896
	ITPA	interferon alfa-2b, recombinant	C/D	3						
rs45445694,rs11280056,rs699517	*TYMS*	capecitabine	D	3						
rs4444903	*EGF*	cetuximab	D	3						
rs1872328	*ACYP2*	cisplatin	D	3						
rs3212986,rs3212986.rs11615,rs11615,rs735482,rs11615,	*ERCC1*	cisplatin	D	3						
rs3754446	*GSTM1*	cisplatin	D	3						
rs10517, rs1800566,	*NQO1*	cisplatin	D							
rs2228001,rs2228001,rs2228000,rs2228001	*XPC*	cisplatin	D	3						
rs1695	*GSTP1*	fluorouracil	D	3						
rs1800566	*NQO1*	fluorouracil	D	3						
rs11280056	*TYMS*	fluorouracil	D	3						
rs3772809,rs3772810,rs2291078,	*UMPS*	fluorouracil	D	3						
UGT1A1*28	*C8orf34*	irinotecan	D	3						
UGT1A1*28	*SEMA3C*	irinotecan	D	3						

rs396991	*FCGR3A*	rituximab	D	2B						
EMA: European medical association, HCSC: PMDA: Pharmaceuticals and Medical Devices Agency Red- Testing required; Yellow- Testing recommended; Green- Actionable PGx; Blue-Informative PGx										

2.3.2 Cardiovascular disease

Over the past decade, multiple pharmacogenomic evidence is accumulating from observational studies and randomised controlled trials (RCTs). A few genotype-guided pharmacogenetic testing has also emerged recently. The most common among them being CYP2C19 genotyping for clopidogrel response, CYP2C9 and VKORC1 for warfarin dosing, SLCO1B1 variants for statin prescribing. These variants are FDA approved for drug labelling and CPIC guidelines are available for each of these drug-gene pairs, and a number of healthcare centres are implementing these into clinical practice (Z., 2020).

The CYP2C9 gene involved in the metabolism of warfarin and VKORC1 gene regulating the oxidation state of vitamin K is associated with different sensitivity to warfarin. Pharmacogenomic recommendations for systemic concentration of the drug and dosage requirement. Patients with CYP2C9 genotype leading to decreased drug clearance and VKORC1 genotype resulting in increased drug sensitivity. These patients are recommended to receive lower than typical warfarin dosage (Johnson JA, 2011). Clopidogrel, a prodrug, is metabolised by CYP2C19. Variants of this gene result in enzyme deficiency leading to decreased circulating concentrations of active metabolite, and subsequently suppressed inhibition of platelet activation and aggregation. This report eventually led to FDA labelling and CPIC recommending alternative antiplatelet therapy is recommends for poor metabolizers (Scott SA, 2013).

Apart from the above two drugs, statins are the major lipid lowering drugs with higher efficacy and minimal ADRs. The only common side effect is myopathy which limits its usage. Risk factors for myopathy include higher statin doses, interaction with other drugs, renal or liver dysfunction, and SLCO1B1 genotypes. Early studies of statin pharmacokinetics demonstrated that the transporter, SLCO1B1, regulates liver uptake of the drug, and variants in SLCO1B1, were associated with higher simvastatin concentrations (Pasanen MK, 2006). The 521T>C (p. Val174Ala) polymorphism was found to be related to myopathy caused by statins. Simvastatin has the most data on its association with genetic testing result (Ramsey LB, 2014). Thus, patients with CT or CC genotypes are recommended a lower dose of simvastatin or use of other statins such as pravastatin or rosuvastatin (Roden DM, 2018). β- Blockers are widely used for hypertension, cardiac arrhythmia, and myocardial infarction, have been

associated with variable response due to CYP2D6, ADBR1, and ADBR2 genes. Loss-of-function variants in CYP2D6 are associated with phenotypes of poor metabolizers for β-blockers, which is reflected in FDA label warning.

Table 2.2: List of clinically validated PGx biomarkers in cardiovascular and their level of recommendation by different PGx- regulatory bodies for related drugs

Variant	Gene	Drug	CPIC Level	PharmGKB Level of Evidence	FDA	EMA	Swiss Medic	HCSC	PMDA	Ref
rs4149056	SLCO1B1	atorvastatin	A	1A						(Cooper-DeHoff *et al.*, 2022)
CYP2C19 PM	*CYP2C19*	clopidogrel	A	1A						(Scott *et al.*, 2011); (Scott *et al.*, 2013); (Lee *et al.*, 2022)
rs4149056	*SLCO1B1*	pitavastatin	A	1A						(Cooper-DeHoff et al., 2022)
rs4149056	*SLCO1B1*	pravastatin	A	1A						(Cooper-DeHoff et al., 2022)
rs4149056	*ABCG2*	rosuvastatin	A	1A						(Cooper-DeHoff et al., 2022)
rs4149056	*SLCO1B1*	rosuvastatin	A	1A						(Cooper-DeHoff et al., 2022)
rs4149056	*SLCO1B1*	simvastatin	A	1A						(Wilke *et al.*, 2012); (Ramsey *et al.*, 2014); (Cooper-DeHoff et al., 2022)
CYP2C19 PM	*CYP2C9*	avatrombopag	B/C							
rs1801252 rs1801253	*ADRB1*	carvedilol	B/C	3						
CYP2D6 PM	*CYP2D6*	carvedilol	B/C	3						
rs11198893 rs4752292 rs915120 rs10787959 rs2230345 rs3740563 rs2230345	*GRK5*	carvedilol	B/C							
CYP2D6 PM	*CYP2D6*	lofexidine	B/C							
CYP2D6 PM	*ADRB1*	metoprolol	B/C	3						
CYP2D6 PM	*CYP2D6*	metoprolol	B/C	1A						
CYP2D6*2, CYP2D6*3	*GRK5*	metoprolol	B/C							
CYP2D6 PM	*CYP2D6*	nebivolol	B/C							
NAT2*4	*NAT2*	procainamide	B/C							
CYP2D6 PM	*CYP2D6*	propafenone	B/C	1A						
CYP2D6 PM	*CYP2D6*	propranolol	B/C	4						
CYP2C19 PM	*CYP2C9*	aspirin	C							(Theken *et al.*, 2020)
G6PD A-202A_376G	*G6PD*	aspirin	C	3						(Gammal et al., 2023)
G6PD A-202A_376G,	*HLA-DPB1*	aspirin	C	2B						
CYP3A4*20, CYP3A4*22	*CYP3A4*	atorvastatin	C							

CYP3A5*3,	*CYP3A5*	atorvastatin	C							
CYP3A4*20, CYP3A4*22	*HMGCR*	atorvastatin	C							
G6PD A-202A_376G,	*G6PD*	nicorandil	C							(Gammal et al., 2023)
CYP3A4*20, CYP3A4*22	*CYP3A4*	pravastatin	C							
CYP3A5*3, *6, *7	*CYP3A5*	pravastatin	C							
CYP2D6 PM	*CYP2D6*	quinidine	C							
	CYP3A4	rosuvastatin	C							
	CYP3A5	rosuvastatin	C							
	HMGCR	rosuvastatin	C							
rs1045642	*ABCB1*	simvastatin	C	3						
CYP3A4*20, CYP3A4*22	*CYP3A4*	simvastatin	C							
CYP3A5*3, *6, *7	*CYP3A5*	simvastatin	C							
rs6065	*GP1BA*	aspirin	D	3						
rs730012, rs730012	*LTC4S*	aspirin	D	3						
rs7412	*APOE*	atorvastatin	D	2B						
rs20455	*KIF6*	pravastatin	D	2B						
rs10455872, rs10455872, rs10455872	*LPA*	pravastatin	D							
rs4149056	*COQ2*	rosuvastatin	D	3						
rs4149056	*LPA*	rosuvastatin	D	3						
rs5882	*CETP*	simvastatin	D	3						
rs17244841	*HMGCR*	simvastatin	D	3						
rs10455872, rs10455872, rs10455872	*LPA*	simvastatin	D							
	ADD1	spironolactone	D	3						

Data obtained from CPIC and PharmGKB annotations accessed as on 18 march 2023. Recommendations are: Red- Testing required; Yellow-Testing recommended; Green- Actionable PGx; Blue-Informative PGx

2.3.3 Metabolic disorder and gastrointestinal disorders

Not much have been explore in this field with respect to PGx research**.** Diabetes mellitus being the third most prevalent disorder, is poorly explored through the lens of PGx response. Previous candidate approaches were mainly focused on the DMEs and DTs responsible for the PK of the drugs. Genetic variations in genes like SLC22A1 and SLC47A1 are widely studied with metformin PK, with low transport activity and no consistent effect on glycaemic control. Sulfonylureas are mainly metabolised by CYP2C9. Patient carrying LOF variants of CYP2C9 have higher drug exposure leading to consistent observations of greater glycaemic response (Zhou K, 2016). Some other antidiabetic agents including α-glucosidase

inhibitors, GLP-1 receptor agonists, DPP4 inhibitors, and SGLT2 inhibitors have not shown strong pharmacogenomic evidence and inconsistent findings (Zeng, 2020).

Within gastroenterology and hepatology, there has been little clinical application of pharmacogenomics. Patients with relapsing ulcerative colitis or Crohn's disease are treated with azathioprine (AZA) or 6-mercaptopurine (6-MP), with are primarily metabolised by TPMT and follows the genotype-guided administration (as discussed under section 2.3.1 cancers). There are numerous well known genetic variants used for guided treatment a few approved by the US-FDA for proton pump inhibitor used in acid-related gastrointestinal disease like omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole. CYP2C19 is the main enzyme responsible for elimination of these drugs. The Poor metaboliser genotype of CYP2C19 (*2 and *3) are commonly used for drug labelling for dose adjustment of these drugs (Saito, 2006). Ethnic differences are observed with respect to these alleles *2 and *3 and genotype frequency. Poor metabolisers are seen in 13 – 23% of Asian populations, and 2 – 5% of Caucasian populations. Thus, genotyping may only be relevant for Asian origin people, in whom ≤ 20% may be slow metabolisers (OMIM, 2021).

Table 2.3: List of clinically validated PGx biomarkers in metabolic and gastrointestinal diseases their level of recommendation by different PGx- regulatory bodies for related drugs

Variant	Gene	Drug	CPIC Level	PharmGKB Level of Evidence	FDA	EMA	Swiss Medic	HCSC	PMDA	Ref
CYP2C19 PM	*CYP2C19*	lansoprazole	A	1A						32770672
CYP2C19 PM	*CYP2C19*	omeprazole	A	1A						32770672
CYP2C19 PM	*CYP2C19*	pantoprazole	A	1A						32770672
NA	*NAGS*	carglumic acid	B							
CYP2C19 PM	*CYP2C19*	dexlansoprazole	B	1A						32770672
CYP2C19 PM	*CYP2C9*	lesinurad	B/C							
CYP2D6 PM	*CYP2D6*	metoclopramide	B/C							
CYP2D6 PM	*CYP2D6*	mirabegron	B/C							
NAT2*4	*NAT2*	sulfasalazine	B/C							
CYP2D6 PM	*CYP2D6*	tamsulosin	B/C							
G6PD A- 202A_376G	*G6PD*	chlorpropamide	C							36049896
CYP2D6 PM	*CYP2D6*	darifenacin	C							
CYP2C19 PM	*CYP2C19*	esomeprazole	C	3						32770672
G6PD A- 202A_376G	*G6PD*	gliclazide	C							36049896
G6PD A- 202A_376G	*G6PD*	glimepiride	C							

G6PD A- 202A_376G	*G6PD*	glipizide	C							36049896
G6PD A- 202A_376G	*G6PD*	glyburide	C	3						36049896
CYP2C19 PM	*CYP2C19*	rabeprazole	C	2A						32770672
	CYP2C8	rosiglitazone	C	3						
A- 202A_376G haplotype	*G6PD*	sulfasalazine	C	3						36049896
CYP2D6*2, CYP2D6*10	*CYB5R1*	metoclopramide	D							
CYP2D6*2, CYP2D6*10	*CYB5R2*	metoclopramide	D							
CYP2D6*2, CYP2D6*10	*CYB5R3*	metoclopramide	D							
CYP2D6*2, CYP2D6*10	*CYB5R4*	metoclopramide	D							

Data obtained from CPIC and PharmGKB annotations accessed as on 18 march 2023. Recommendations are: Red- Testing required; Yellow-Testing recommended; Green- Actionable PGx; Blue-Informative PGx

2.3.4 Mental and behavioural disorder

One out of every four individual worldwide experience a mental health condition during their lifetime. Healthcare professionals routinely observe challenges related to such drug prescriptions. The most common one being non-adherence. The high risk of ADR and poor therapeutic response from psychiatric medicines are factors driving poor adherence. Since 2008 when pharmacogenomics was at its forefront, in psychiatric treatment management for clinical purposes (de Leon, 2009). However, due to low clinical utility and contrasting outcomes, the use of pharmacogenetic testing were discontinued (de Leon, 2016). Since then, there has been substantial progress made in pharmacogenomics of antidepressants. The International Society of Psychiatric Genetics (McMahon, 2019) has recognized a stance supported by a recent systematic review of CYP2C19 and CYP2D6 enzyme activity for these classes of medication (Milosavljevic F, 2021). These genes are implicated in the metabolism of 80% of psychiatric medications and are increasingly recognized in regulator- approved drug labels (M€uller, 2013; van Schaik, 2020). This has introduced the FDA approval for drug labelling for the some allelic variants of these genes for drugs like Venlaflaxine, Vortioxetine, Risperidone, Imipramine and others (Pardiñas AF, 2021). A detailed table for pharmacogenetic markers used for clinical purposes is tabulated in **Table 2.4.**

Table 2.4: List of clinically validated PGx biomarkers in psychiatry and their level of recommendation by different PGx- regulatory bodies for related drugs

Variant	Gene	Drug	CPIC Level	PharmGKB Level of Evidence	FDA	EMA	SwissMedic	HCSC	PMDA	Ref

CYP2C19 PM	*CYP2C19*	amitriptyline	A	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	amitriptyline	A	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	atomoxetine	A	1A						30801677
CYP2C19 PM	*CYP2C19*	citalopram	A	1A						25974703
CYP2C19 PM	*CYP2C19*	escitalopram	A	1A						25974703
CYP2D6 PM	*CYP2D6*	nortriptyline	A	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	paroxetine	A	1A						25974703
CYP2D6 PM	*CYP2D6*	pitolisant	A							
CYP2D6 PM	*CYP2D6*	pimozide	A/B							
CYP2D6 PM	*CYP2D6*	venlafaxine	A/B	1A						
CYP2D6 PM	*CYP2D6*	vortioxetine	A/B	3						
CYP2D6 PM	*CYP2D6*	aripiprazole	B	1A						
CYP2C19 PM	*CYP2C19*	clomipramine	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	clomipramine	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	desipramine	B	1A						23486447;27997040
CYP2C19 PM	*CYP2C19*	doxepin	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	doxepin	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	fluvoxamine	B	1A						25974703
CYP2C19 PM	*CYP2C19*	imipramine	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	imipramine	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	risperidone	B	1A						
CYP2C19 PM	*CYP2C19*	trimipramine	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	trimipramine	B	1A						23486447;27997040
CYP2D6 PM	*CYP2D6*	amoxapine	B/C							
CYP2D6 PM	*CYP2D6*	aripiprazole lauroxil	B/C							
CYP2B6*6, *2, *3	*CYP2B6*	bupropion	B/C	2A						
5-HTTLPR	*SLC6A4*	citalopram	B/C	3						
CYP2D6 PM	*CYP2D6*	clozapine	B/C							
HTTLPR	*SLC6A4*	escitalopram	B/C	3						
CYP2D6 PM	*CYP2D6*	iloperidone	B/C	3						
CYP2D6 PM	*CYP2D6*	perphenazine	B/C							

CYP2D6 PM	*CYP2D6*	protriptyline	B/C							
CYP2D6 PM	*CYP2D6*	thioridazine	B/C	3						
CYP2D6 PM	*CYP2D6*	zuclopenthixol	B/C	1A						
rs489693	*MC4R*	aripiprazole	C	3						
rs6311	*HTR2A*	citalopram	C	3						
rs3813929	*HTR2C*	clozapine	C	3						
rs11872992	*MC4R*	clozapine	C	3						
CYP2D6 PM	*CYP2D6*	duloxetine	C							
rs4608	*COMT*	escitalopram	C							
rs1954787, rs12800734	*GRIK4*	escitalopram	C							
CYP2D6 PM	*CYP2D6*	fluoxetine	C	3						
G6PD A-202A_376G	*G6PD*	hydroxychloroquine	C							36049896
CYP2D6 PM	*CYP2D6*	modafinil	C							
rs3813929	*HTR2C*	olanzapine	C	3						
rs11872992	*MC4R*	olanzapine	C	3						
rs17782313, rs489693	*MC4R*	paliperidone	C	3						
rs12800734, rs1954787	*GRIK4*	paroxetine	C							
rs1800497	*DRD2*	risperidone	C	3						
rs3813929	*HTR2C*	risperidone	C	3						
rs489693	*MC4R*	risperidone	C	3						
rs12800734, rs1954787	*GRIK4*	venlafaxine	C							
rs9380524, rs1360780	*FKBP5*	citalopram	D	3						
rs4713916	*FKBP5*	fluoxetine	D	3						
rs1800497	*ANKK1*	olanzapine	D	3						
rs1360780	*FKBP5*	paroxetine	D	3						
rs6295	*HTR1A*	paroxetine	D	3						
rs1800497	*ANKK1*	risperidone	D	3						
CYP2D6 PM	*FKBP5*	venlafaxine	D	3						

Data obtained from CPIC and PharmGKB annotations accessed as on 18 march 2023. Recommendations are: Red- Testing required; Yellow-Testing recommended; Green- Actionable PGx; Blue-Informative PGx

2.3.5 Neurological disorders

In neurology, as in any other clinical specialty, there is a need to develop treatment strategies that allow stratification of therapies to optimize efficacy and minimize toxicity. To date, however, only few genetic variants have been incorporated into clinical algorithms (**Table 2.5**). With respect to drug efficacy and ADRs related to dopaminergic antiparkinsonian medications, significant associations between polymorphisms in dopamine transporters (DAT) or monoamine degradation enzymes (COMT and MAOB) and response to levodopa, Tan and colleagues, reported association between low-activity COMT homozygotes and response to pyridoxine as adjunct therapy to levodopa (Tan EK, 2005). Our previous systematic review identified CA repeats in DRD2 was found to be most significantly associated with dyskinesia, followed by rs1801133 in MTHFR with hyper-homocysteinemia, and rs474559 HOMER1 with hallucination. Accordingly for levodopa efficacy rs28363170, rs3836790 (SLC6A3) and rs4680 (COMT), were important. Individuals with rs3836790 6/6 or rs28363170 10/10 (SLC6A3) genotypes have higher transporter expression leading to lower dopamine levels at the synapse. The COMT haplotype (rs6269-rs4633-rs4818-rs4680) characterises low (ACCG) to high (GCGG) enzyme activity ultimately affecting levodopa metabolism and the synaptic dopamine concentration (Guin, 2017). Another study investigated the DRD2 and DRD3 dopamine receptor polymorphisms and response to the non-ergot dopamine receptor agonist pramipexole, and observed higher response rates in Ser/Ser homozygotes for the DRD3 Ser9Gly polymorphism (Tan EK, 2005). Several genetic association have been explored much it is yet to reach clinical applications due to contrasting findings.

The apolipoprotein-E (APOE) alleles have been the mainstay in Alzheimer's disease genetics. Early work with tacrine suggested reduced drug efficacy in patients carrying APOE ε4 carriers, but later studies observed either no differences or even higher responses in APOE ε4 carriers. Discrepant data also exist for donepezil and differential treatment response in APOE ε4 carriers, while responses to galantamine and rivastigmine appear to be similar between APOE ε4 carriers and non-carriers. Other positive associations have been reported between ACHE polymorphisms (rs2571598) and response to rivastigmine, CHAT (rs733722) and response to donepezil, galantamine and rivastigmine; and CYP2D6 (rs1080985) and response to donepezil. Pharmacogenetic studies investigating ADR suggested genetic variants in ABCB4 may influence tacrine-induced elevation of liver transaminases. Likewise, Studies mu and theta null variants (GSTM1, GSTT1) associated with tacrine-induced liver toxicity have resulted in conflicting results. Despite the inconsistent results observed for most therapies,

APOE genotyping is routinely incorporated into new clinical trials for Alzheimer's disease to evaluate drug efficacy in carriers and non-carriers of the APOE ε4 allele (Chan *et al.*, 2011).

Current literature on PGx of multiple sclerosis (MS), suggests that approximately 30-50% of patients do not respond well to first-line therapies which is hypothesized to be in part attributed to inter-individual genetic variability. MS is clinically heterogeneous and patients suffer from a wide range of symptoms such as muscle weakness, sensory change, pain, ataxia, depression, ataxia, and visual loss. Consequently, responses to several kinds of MS therapies are also heterogeneous. There have been many attempts to identify interferon β therapy-responsive genes(M, 2008). These studies genotyped polymorphisms located in genes that are part of the type I IFN pathway, such as the IFN receptors 1 and 2 (IFNAR1 and IFNAR2), or genes known to be induced by IFN-β. Other studies elucidated the response to IFN-β of HLA class II (DRB1, DQA1 and DQB1) alleles or the HLA-DR2 haplotype was analysed (Cunningham S, 2005; Leyva L, 2005; Weinstock-Guttman B, 2007). Overall, results from these studies revealed either lack of association or weak and poor replicability of candidate associations with the response to IFN-β. So far only one GWAS is published in this regard, which identified genetic loci involved in neuronal repair and growth, and over-representation of genes related to ion channels and signal transduction pathways such as γ-aminobutyric or glutamate receptor genes (Byun E, 2008). In spite of several attempts, none of the markers reached up to clinical applications.

Table 2.5 List of clinically validated PGx biomarkers in neurology and their level of recommendation by different PGx-regulatory bodies for related drugs

Variant	Gene	Drug	CPIC Level	PharmGKB Level of Evidence	FDA	EMA	Swiss Medic	HCSC	PMDA	Ref
HLA-A*31:01	*HLA-A*	carbamazepine	A	1A						23695185;29392710
HLA-B*15:02	*HLA-B*	carbamazepine	A	1A						23695185;29392710
CYP2C9*3	*CYP2C9*	fosphenytoin	A							25099164;32779747
HLA-B*15:02	*HLA-B*	fosphenytoin	A							25099164;32779747
HLA-B*15:02	*HLA-B*	oxcarbazepine	A	1A						29392710
CYP2C9*3	*CYP2C9*	phenytoin	A	1A						25099164;32779747
HLA-B*15:02	*HLA-B*	phenytoin	A	1A						25099164;32779747
CYP2C9*2, CY P2C9*3	*CYP2C9*	siponimod	A	1A						
rs3087374	*POLG*	divalproex sodium	A/B							
CYP2D6 PM	*CYP2D6*	tetrabenazine	A/B							
rs3087374	*POLG*	valproic acid	A/B	3						

CYP2C19 PM	*CYP2C19*	brivaracetam	B	3						
rs3812718	*SCN1A*	carbamazepine	B	2B						
rs3812718	*SCN1A*	phenytoin	B	3						
rs1047891	*CPS1*	valproic acid	B							31151073
	OTC	valproic acid	B							
CYP2D6 PM	*CYP2D6*	amphetamine	B/C							
CYP2D6 PM	*CYP2D6*	brexpiprazole	B/C							
CYP2D6 PM	*CYP2D6*	cevimeline	B/C							
CYP2C19 PM	*CYP2C19*	clobazam	B/C	3						
CYP2D6 PM	*CYP2D6*	deutetrabenazine	B/C							
CYP2D6 PM	*CYP2D6*	dextromethorphan	B/C	3						
CYP2C19 PM	*CYP2C19*	diazepam	B/C	3						
CYP2D6 PM	*CYP2D6*	donepezil	B/C	3						
rs28933389	*BCHE*	mivacurium	B/C							
CYP2D6 PM	*CYP2D6*	valbenazine	B/C							
CYP2D6 PM	*CYP2D6*	galantamine	C	3						
HLA-B*15:02	*HLA-A*	oxcarbazepine	C	3						29392710
rs2234922	*EPHX1*	carbamazepine	D	3						

Data obtained from CPIC and PharmGKB annotations accessed as on 18 march 2023. Recommendations are: Red- Testing required; Yellow-Testing recommended; Green- Actionable PGx; Blue-Informative PGx

2.4 Pharmacogenomics in epilepsy

Apart from the above diseases, the treatment of epilepsy has been one of the model disease for pharmacogenomic studies considering the high prevalence of the disease (Heaney DC, 2002) (JW., 2003) (Banerjee PN, 2009), large inter-individual difference in response to AEDs (S.L. Moshé, 2015), easily measurable outcomes of seizure control and availability of assessment scales to drug response or its related ADRs (Aldenkamp AP, 1997; Baker GA, 1994). There is much heterogeneity in the clinical manifestation of the disease. It includes a number of medical conditions with recurrent seizures being the common characteristic feature. The large number of different syndromes and seizure types as well as highly variable inter-individual response to therapies makes management of this condition often challenging (Striano P, 2020).

Treatment of epilepsy begins with the initial administration of anti-epileptic drugs (AED) based on three level of diagnosis, starting with seizure type, followed by epilepsy type and then epilepsy syndrome classification, considering some other clinical parameters like electroencephalogram (EEG) patterns (Scheffer IE, 2017). In case of failure of initial treatment regime, the physician subsequently move to other drugs or additional poly-therapy. Epilepsy treatment outcome is often characterized by inconsistent drug efficacy, adverse drug response, and dose optimization in patients(Löscher W, 2009). There are several factors contributing to variable treatment response like individual drug metabolism, lifestyle, environmental factors and genetics. Predominantly, variation in response to AED arise from genetic variation in genes involved in drug disposition. These genes affect the pharmacokinetics, or pharmacodynamics of the drug (Madian AG, 2012; Spear, 2001; Wang L, 2011). The current studies associating these genes and their genetic variants with seizure control or adverse events are often limited to specific individualised setting, small study size, inconsistent drug prescription and dosage administration guideline, and difficulties in the validation of findings. Thus, such outcomes have not provided unifying conclusions, specifically for drug efficacy outcomes and hence lacking clinical translation. In contrast, studies in the past identified numerous HLA alleles with a range of ADR mostly affecting skin and liver. HLA screening and PGx-based prescription guidelines are already available for HLA-B*15:02 with carbamazepine induced SJS/TEN establishing clear roadmap for HLA-screening into clinical translation. The identification and validation of genetic factors that reliably predict the efficacy and toxicity of specific drugs for individual patients would significantly improve the current treatment of patients with epilepsy.

Even with the introduction of almost thirty AEDs in the market, the rate of seizure remission remains poor, i.e., approximately 30% patients exhibiting pharmaco-resistance (MJ, 2017; Pohlmann-Eden B, 2013). Though there is marked difference in safety and tolerability of these drugs with time, it is clear that we need an optimised framework to determine the choice of therapy in patients with epilepsy (Marson AG, 2007). The key to improving treatment outcome is certainly to invoke high chances of seizure remission in patients and precision medicine can at best provide clues about prognostication. Like, in case of refractory epilepsy or epilepsy with neuro-developmental deficits, precision medicine may direct them to surgical treatment rather than adhering to pharmacotherapy. Additionally, the identification and validation of genetic factors that reliably predict the efficacy and toxicity of specific drugs for individual patients may allow physicians to predict an accurate treatment or seizure management strategies based on the heterogeneous syndromes of epilepsy. A timeline of

pharmacogenomic improvements for AEDs towards clinical practice is represented in **Figure 2.4.**

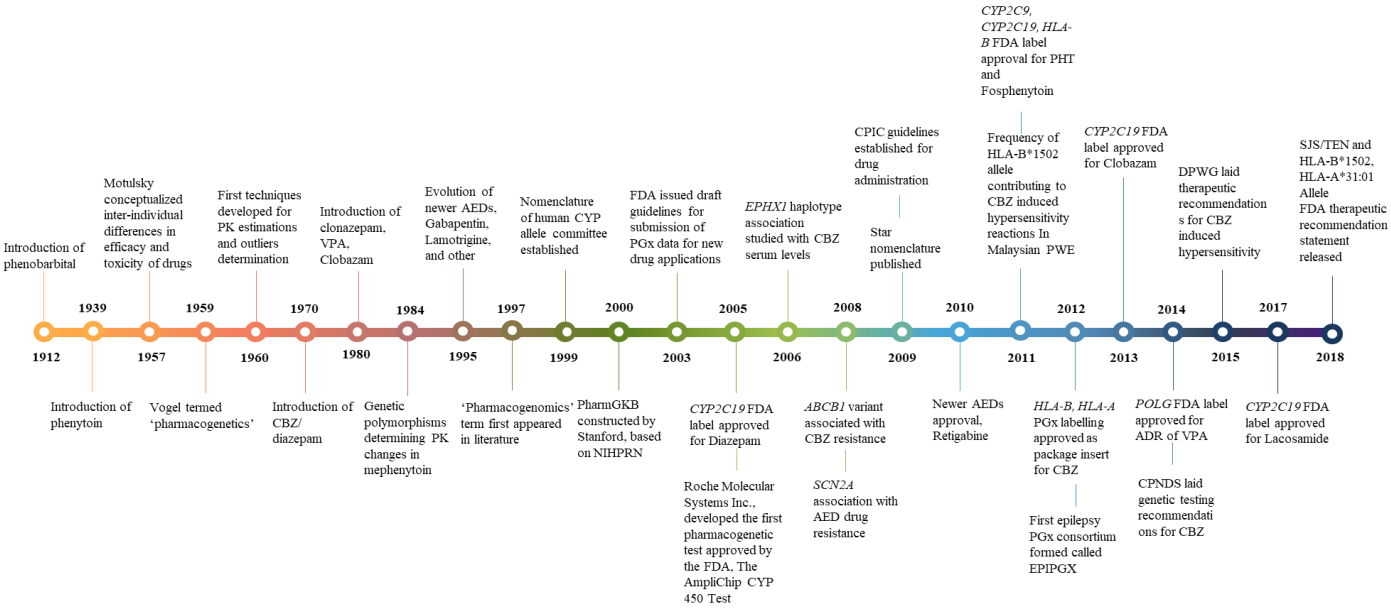


Figure 2.4: Evolution of pharmacogenomics in epilepsy and their way into clinical applications. PHT, phenytoin; PK, pharmacokinetics; CBZ, carbamazepine; VPA, valproic acid; AED,;CYP, cytochrome P450 enzyme; PharmGKB, The pharmacogenomic knowledgebase; NIHPRN, National institute of health Pharmacogenomics Research Network; PGx, pharmacogenomics; FDA, Food and drug administration; CPIC, Clinical Pharmacogenetics Implementation Consortium; PWE, patient with epilepsy; EPIPGX, epilepsy pharmacogenomics consortium; CPNDS, The Canadian Pharmacogenomics Network for Drug Safety ; DPWG, The Dutch Pharmacogenetics Working Group; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis

2.4.1 Anti-epileptic drugs (AEDs) - Evolution, their mode of action, pharmacokinetic and pharmacodynamics of AED

Treatment of epilepsy begins with the initial administration of anti-epileptic drugs (AED) based on three level of diagnosis, starting with seizure type, followed by epilepsy type and then epilepsy syndrome classification, considering some other clinical parameters like EEG patterns (Scheffer IE, 2017). In case of failure of initial treatment regime, the physician subsequently move to other drugs or additional poly-therapy. Patients who do not respond to pharmacotherapy, become drug resistant/ refractory. Such patients are treated with ketogenic diet or surgical interventions (resective surgery of localized seizure-related tissue, vagus nerve stimulation). Epilepsy treatment outcome is often characterized by inconsistent drug efficacy, adverse drug response, and dose optimization in patients (Löscher W, 2009). There are several factors contributing to variable treatment response like individual drug metabolism, lifestyle, environmental factors and genetics. Predominantly, variation in response to AED arise from

genetic variation in genes involved in drug disposition. These genes affect the pharmacokinetics, or pharmacodynamics of the drug (Madian AG, 2012; Spear, 2001; Wang L, 2011).

Currently, a total of 24 AEDs are available in the market **(Figure 2.7)**. The serendipitous discovery of first line conventional AEDs and exploration of their mode of action led to discovery of targeted-based newer AEDs since the 1990s. The first effective AEDs in use were the bromides which were discovered in 1857. Bromides were mainly available as sedatives but were later also used for treating epilepsy, however, were discontinued as they cause impotence in men. The discovery of phenobarbital (PB) was a landmark in AED development which started the era of animal model testing for drug discovery. AEDs can be classified into two groups based on their entry in the market, 1) conventional, and 2) newer AEDs. The conventional AEDs may be considered as those which were introduced into the market before 1980s (Krall *et al.*, 1978; Shorvon, 2009a) while the newer ones were introduced after 1990s and are still adding.

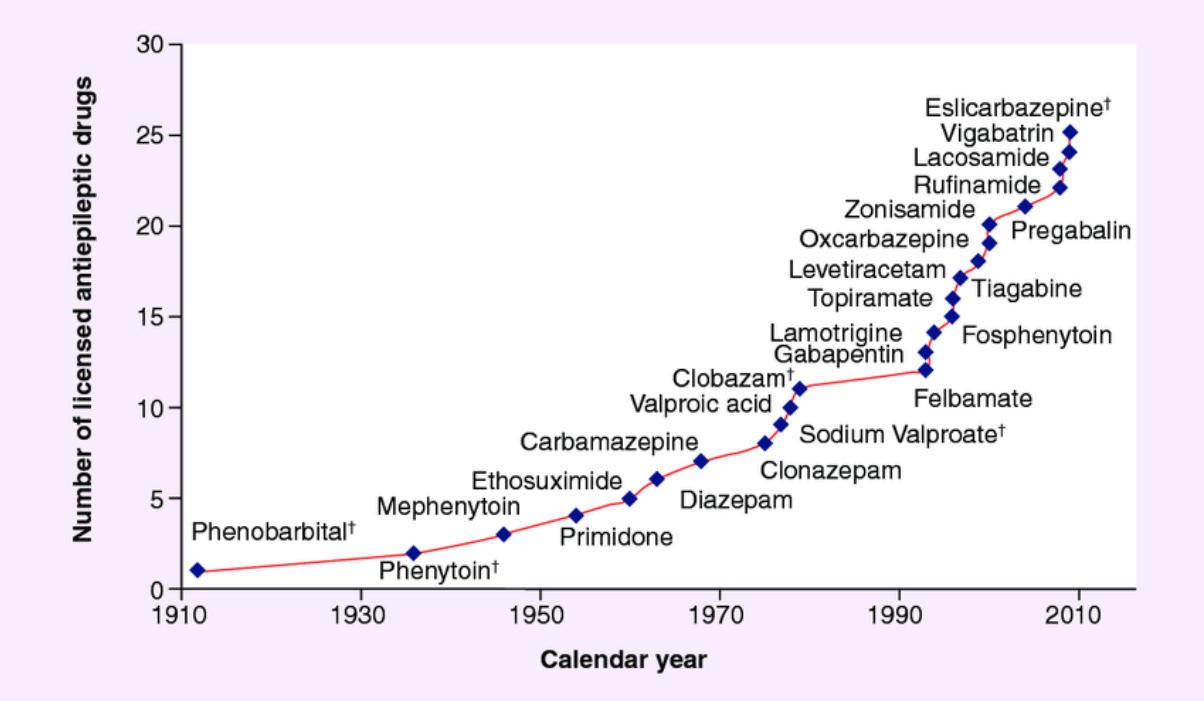


Figure 2.5: Evolution of pharmacogenomics of anti-epileptic drugs according to their year of FDA approval for administration in epilepsy (Cavalleri GL, 2011).

2.4.1.1 Conventional AEDs

AEDs in this group were discovered serendipitously before the 1980s. The drugs were relatively toxic causing wide variety of adverse drug reactions (ADRs) in some patients. The anticonvulsive effect of **PB** came into existence in the year 1912 when Alfred Hauptmann, a physician discovered that the seizures of PWE are susceptible to this drug (Porter *et al.*, 1992). He noted that PB is more effective and less toxic than the bromides. After PB, experimental

evaluation of promising anticonvulsant chemicals started. Researchers started the use of animal models for the drug development by the means of electroshock technique for producing convulsions in animals (Spiegel, 1936). Merritt and Putnam screened a group of compounds and discovered the anticonvulsant properties of diphenylhydantoin, now called **PHT**. Later, Food and Drug Administration (FDA) approved the use of PHT as an AED in 1953. **CBZ**, another AED, was first produced by chemist Walter Schindler at Geigy. It was first tested as a drug for depression, pyschosis and trigeminal neuralgia. Later in the year 1968, FDA approved the use of CBZ as an AED. In 1962, Pierre Eymard serendipitously discovered the anticonvulsant properties of **VA** while using it as a solvent for a number of other compounds that were being screened for anticonvulsive activity. VA was found to prevent the pentylenetetrazol (PTZ)-induced convulsions in rats. FDA approved this drug in the year 1972.

2.4.1.2 Newer AEDs

The AEDs of this generation are an outcome of extraordinary advances in the basic science of pharmacology. This era marked the introduction of many new AEDs as well as second and third generation AEDs for epilepsy treatment after the 1990s. These drugs were based on the concept of selectively targeting one of the possible seizure generation mechanism (Loscher *et al.*, 1994). Despite the encouraging and welcoming advantages in terms of ADRs of new AEDs, the concern of efficacy could not be improved and they were prescribed in combination with the conventional ones (Shorvon, 2009b). This category includes AEDs such as gabapentin, lamotrigine, topiramate, levetiracetam, oxcarbazepine, etc.

Table 2.6: Characteristics of clinically approved AEDs in different generations of their discovery

Sl. No.	Drug (Year of FDA approval)	Active metabo-lite	Mode of action	Mode of administration	FDA indications	Genes			Efficacy (≥50% seizure reduction)	ADRs
						Targets	DMEs	DTs		
1	Potassium bromide^ (1857)	Not metabolised	Activation of GABA receptor(Löscher W, 2013)	Adjuvant	GTCS, myoclonic seizure(Löscher W, 2013)	GABA receptor gene family	-	-	31% (in childhood patients with severe epilepsy and generalized tonic-clonic seizures, at 45 mg/kg) (Korinthenberg R, 2007)	Acts as sedative (Löscher W, 2013)
2	Phenobarbital^ (1912)	p-Hydroxy pheno barbital	Increase amount of time Cl^- channels open, activation of GABA receptor	Mono	Partial seizures (Löscher W, 2013)	*GABRA1, CHRNA4, CHRNA7, GRIA2, GRIK2,* Glutamate receptor ionotropic	*CYP2C19, CYP2C9, CYP2B6, CYP2C8, CYP3A4, CYP1A2, CYP2A6, CYP2E1,*	*ABCB1, ABCC3, ABCB11, ABCC1, SLCO2A1, ABCC2*	73.6% (mean efficacy) (Yasiry Z, 2014)	Somnolence, skin hypersensitivity, depression, behavioural problem, leukopenia, aplastic anemia, Megaloblastic

			(Löscher W, 2013)			(NMDA) gene family, NR1I2	*CYP3A5*, *CYP1A1*, *CYP2C18*, *CYP3A7*, *CYP4B1*, *UGT1A1*, *UGT2B7*			anemia, Osteoporosis, Cognition impaired (Löscher W, 2013)
3	Phenytoin (1938)	Hydroxy-phenytoin (Parke-Davis, 2009a)	Na^+, Ca^{2+} channel inhibitor, potentiate GABA receptor (Löscher W, 2013; Parke-Davis, 2009a)	Mono	generalized tonic-clonic (grand mal) and complex partial (psychomotor, temporal lobe) seizures	*SCN5A*, *SCN1A*, *NR1I2*, *SCN1B*, *SCN3A*, *KCNH2*, Voltage-dependent L-type calcium channel gene family, *SCN2A*, *SCN8A*, GABA receptor gene family	*CYP2C19*, *CYP2C9*, *CYP2C8*, *CYP2B6*, *CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP11B1*, *UGT1A1*, *UGT1A6*, *UGT1A9*, *CYP1A2*, *CYP2A6*, *CYP2D6*, *CYP2E1*, *EPHX1*, *UGT1A4*, *COMT*, *NQO1*, *CYP2C18*	*SLCO1C1*, *ABCB1*, *ABCC2*	50.2% (mean efficacy of patients receiving phenytoin) (Yasiry Z, 2014)	Dizziness, Seizure aggravation, skin hypersensitivity, Encephalopathy, Depression, Behavioral problems, leukopenia, aplastic anemia, Megaloblastic anemia, Osteoporosis, Cognition impaired (Parke- Davis, 2009a)
4	Trimetha-dione* (1946)	Dimetha-dione (Laboratories, 1999)	Ca^{2+} channel inhibitor (Löscher W, 2013)	Mono	petit mal seizures that are refractory (Laboratories, 1999)	*CACNA1G*	*CYP2E1*, *CYP3A4*, *CYP2C8*, *CYP2C9*, *CYP2C19*,	-	-	Teratogenic, skin rashes, Blood Dyscrasias, renal and ocular dysfunction, Lupus-and Myasthenia-like Syndromes (Laboratories, 1999)
5	Primidone (1954)	phenobarbital and phenyl-ethyl-malonamide(Pharmaceuticals, 2009)	bind centrally with voltage-gated Na^+ channels, Activates GABA -A receptor complex with chloride ionophore (Lenkapothula N, 2022; Löscher W, 2013)	Mono, Adjuvant	grand mal, psychomotor, and partial epileptic seizures (Pharmaceuticals, 2009)	*GABRA1*, *GABRA2*, *GABRA3*, *GABRA4*, *GABRA5*, *GABRA6*, *CHRNA4*, *CHRNA7*, *GRIA2*, *GRIK2*, GABA receptor gene family	*CYP2C9*, *CYP2C19*, *CYP2E1*, *CYP3A4*, *CYP1A2*, UGT family	*TRPM3*	80% (at 25 mg/kg/day within 5days) (Sapin JI, 1988)	Sedation, drowsiness, Ataxia, diplopia, and nystagmus, dizziness, vertigo, epigastric pain, megaloblastic anemia, respiratory depression, polyuria, skin rash, facial edema (Lenkapothula N, 2022; Pharmaceuticals, 2009)
6	Peganone/ Ethotoin (1957)	N-deethyl and p-hydroxyl-ethotoin (Inc, 2010)	Na^+, Ca^{2+} channel inhibitor, potentiate GABA receptor(Löscher W, 2013)	Adjuvant	tonic-clonic (grand mal) and complex partial (psychomotor) seizures (Inc, 2010)	*SCN5A*, *NR1I2*	-	-	83% (initial dose of 100mg/day increased by 50mg per week, till maintenance dose)(LIVINGSTONS, 1961)	chest pain, nystagmus, diplopia, fever, dizziness, diarrhea, headache, insomnia, fatigue, numbness, skin rash, and

										Stevens-Johnson syndrome (Löscher W, 2013)
7	Ethosuximide (1958)	Mono-hydroxy-ethosuximides	T-type Ca^{2+} channel inhibitor (Hanrahan B, 2022; Löscher W, 2013)	Mono, Adjuvant (in other forms of epilepsy coexist with absence epilepsy) (MM., 2010)	absence (petit mal) epilepsy (Parke-Davis, 2009b)	*CACNA1G*	*CYP2E1*, *CYP3A4*, CYP3A subfamily	-	-	nausea, vomiting, diarrhea, and anorexia, drowsiness, lethargy, insomnia, and hiccups (Parke-Davis, 2009b)
8	Diazepam (1963)	N-desmethyldiazepam, Temazepam(Hoffmann-La Roche, 2016)	GABA potentiation(Hoffmann-La Roche, 2016; Löscher W, 2013)	Adjuvant	Status epilepticus, partial and generalised seizure (Löscher W, 2013)	GABA receptor gene family	*CYP2C9*, *CYP3A7*, *CYP3A4*, *CYP2C19*, *CYP3A5*, *CYP2B6*, *PTGS1*, *CYP2C18*, *CYP2C8*, *CYP2E1*	*ABCB1*	90% (patients stopped having seizures in acute repetitive seizures given diazepam rectal gel)(Fakhoury T, 2007)	drowsiness, fatigue, muscle weakness, and ataxia (Hoffmann-La Roche, 2016)
9	Carbamazepine (1964)	carbamazepine-10,11-epoxide (Corporation, 2009b)	Na^{+}, Ca^{2+} channel inhibitor, increase GABA transmission (Löscher W, 2013)	Mono, adjuvant (Corporation, 2009b)	Partial seizure, generalised tonic-clonic seizure, mixed seizure(Corporation, 2009b)	Voltage-gated sodium channel gene family, *CHRNA4*, *NR1I2*	*CYP3A4*, *CYP2C8*, *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2B6*, *CYP3A5*, *UGT2B7*, *UGT1A1*, *UGT1A6*, *UGT1A7*	*ABCB1*, *RALBP1*, *ABCC2*	39% (complete freedom from seizure) and 36% (>50% seizure reduction) at 600mg/ day given o patients with generalised seizures (Callaghan N, 1985)	Seizure aggravation, gastrointestinal distress, skin Hypersensitivity, Aplastic anemia, Cognition impaired, weight gain (Corporation, 2009b)
10	Valproic acid (1967)	4-ene-Valproic acid (Inc., 1978)	Na^{+}, Ca^{2+} channel inhibitor, potentiate GABA receptor, Glutamate (*NMDA*) inhibitor (Löscher W, 2013)	Mono, Adjuvant(Inc., 1978)	GTCS (primary and secondary), absence, atonic and myoclonic seizure, complex partial seizure(Inc., 1978)	*ACADSB*, *HDAC9*, *OGDH*, *ALDH5A1*, Sodium channel protein gene family, *HDAC2*, *PPARA*, *PPARD*, *PPARG*	*CYP2A6*, *CYP2B6*, *CYP2C9*, *CYP3A5*, *PTGS1*, *CYP1A2*, *CYP2C19*, *CYP3A4*, *UGT1A4*, *UGT1A8*, *UGT1A10*, *UGT1A6*, *UGT1A3*, *UGT2B7*, *UGT2B15*, *UGT1A9*, *UGT1A1*	*SLC22A6*, *SLC22A8*, *SLC22A5*, *SLC16A1*, *SLC22A7*, *SLCO2B1*	45% (drug vs placebo in monotherapy to complex partial seizure patients) (Inc., 1978)	Gastrointestinal distress, encephalopathy, teratogenicity; weight gain, Thrombocytopenia, Pancreatitis, retinal dysfunction (Inc., 1978)
11	Clonazepam (1968)	7-amino-clonazepam(Inc, 2013)	GABA potentiation(Inc, 2013)	Mono, Adjuvant	Lennox-Gastaut syndrome (petit mal variant),	GABA receptor gene family, *NR1I2*	*CYP3A4*, *CYP2E1*, *NAT2*	-	23% (drug vs placebo, primarily as treatment in partial	Somnolence, dizziness, coordination abnormal, ataxia,

					akinetic and myoclonic seizures (Inc, 2013)				complex seizures) (G.L., 1990)	Dysarthria, psychiatric, respiratory and other gastrointestinal problems (Inc, 2013)
12	Clobazam (1975)	N-desmethylclobazam, 4'-hydroxyclobazam (Lundbeck, 2011)	GABA potentiation(Lundbeck, 2011)	Adjuvant	Lennox–Gastaut Syndrome (Lundbeck, 2011)	GABA receptor gene family	*CYP3A4*, *CYP2C19*, *CYP2B6*, *CYP2C18*, *CYP2D6* (Lundbeck, 2011)	*SLC6A1*, *SLC6A11*, *ABCB1*	52% (drug vs placebo with 1mg/kg/day) (Gauthier AC, 2015)	constipation, somnolence or sedation, pyrexia, lethargy, and drooling (Lundbeck, 2011)
13	Progabide^ (1985)	GABA, gabamide	GABA potentiation(G., 1984)	Adjuvant(Löscher W, 2013)	Partial and generalised seizure, Lennox–Gastaut syndrome, myoclonic seizures (Löscher W, 2013)	*GABBR1*	*CYP3A4*	*ABCB1*	63.9% in partial epilepsy, 62.2% in primary generalised and 57.1% with secondary generalized epilepsy (Drug vs placebo at mean daily dose of 30.5 mg/kg/day) (Musch B, 1987)	Clinical hepatotoxicity(Löscher W, 2013)
14	Vigabatrin (1989)	Not metabolised	irreversible inhibitor of γ- amino butyric acid transaminase (GABA-T) (Lundbeck, 2009)	Mono, Adjuvant	Infantile spasm, Refractory Complex Partial Seizures (Lundbeck, 2009)	*ABAT*	*CYP2C9*	*SLC36A1*	53% (drug vs placebo at 6g/day) (Lundbeck, 2009)	permanent vision loss, fatigue, somnolence, nystagmus, tremor, vision blurred, memory impairment, weight gain, arthralgia, abnormal coordination, and confusional state (Lundbeck, 2009)
15	Lamotrigine (1990)	2-N-glucuronide (inactive) (GlaxoSmith Kline, 2015)	Na^+ channel inhibitor (GlaxoSmith Kline, 2015)	Mono, Adjuvant	Lennox–Gastaut syndrome, partial-onset seizures, primary generalized tonic-clonic seizures (GlaxoSmith Kline, 2015)	*CACNA1E*, Voltage-gated sodium channel gene family, *ADORA1*, *ADORA2A*, *ADRA1A*, *ADRA2A*, *ADRB1*, *DRD1*, *DRD5*, *DRD2*, GABA receptor gene family,	UGT protein group, *DHFR*	*ABCB1*, *SLC22A2*	72% (drug vs placebo, with lamotrigine add –on with one or two other AED, at maintenance phase) (Biton V, 2005)	Dizziness, headache, diplopia, ataxia, nausea, blurred vision, somnolence, rhinitis, pharyngitis, and rash.(GlaxoSmithKline, 2015)

						HRH1, *OPRK1*, *CHRNA1*, *HTR2A*, *HTR3A*, *GRIA1*				
16	Oxcarbazepine (1990)	10,11-dihydro-10-hydroxy-carbazepine(Corporation, 2000)	Voltage sensitive Na^+ channel inhibitor (Corporation, 2000)	Mono, Adjuvant	partial seizures (Corporation, 2000)	Voltage-gated sodium channel gene family	*AKR1C1*, *AKR1C2*, *AKR1C3*, *AKR1C4*, *CBR1*, *CBR3*, *CYP3A4*, *CYP2C19*, *CYP3A5*	*ABCB1*	32% (J.A.P. Van Parys, 1994)	Dizziness, somnolence, diplopia, fatigue, nausea, vomiting, ataxia, abnormal vision, headache, nystagmus, tremor,and abnormal gait (Corporation, 2000).
17	Felbamate (1993)	p-hydroxy, and 2-hydroxy metabolitesmonocarbamate	GABA potentiation, Glutamate receptor (*NMDA*) inhibitor, Na^+, Ca^{2+} channel inhibitor (Inc, 1993)	Mono, Adjuvant	partial-onset seizures, Lennox–Gastaut syndrome (Inc, 1993)	*GRIN2B*, *GRIN2A*	*CYP3A4*, *CYP2C19*, *CYP2E1*, *CYP2C9*	-	23%*(drug vs placebo starting dosage starting dosage of 1400 mg/day increased to 2600mg/day, drug administered as add-on)(Inc, 1993)	anorexia, vomiting, insomnia, nausea, dizziness, somnolence, and headache
18	Gabapentin (1993)	Not metabolised(MJ., 1994)	Ca^{2+} channel inhibitor (Pfizer)	Adjuvant	partial onset seizures (Pfizer)	*CACNA2D1*, *CACNA2D2*, *CACNA1B*, *ADORA1*, *KCNQ3*, *KCNQ5*	*BCAT1*	*SLC7A5*	18.4% (drug vs placebo at 1200 mg/day)	Somnolence, Dizziness, Seizure aggravation, psychotic episodes, weight gain (Pfizer)
19	Topiramate (1995)	2,3-desisopropylidene topiramate	Na^+, Ca^{2+} channel inhibitor, potentiate GABA receptor, Glutamate (*AMPA*) inhibitor(Janssen Pharmaceuticals, 1996)	Mono, Adjuvant(Janssen Pharmaceuticals, 1996)	Partial and generalised seizure, Lennox–Gastaut Syndrome. (Janssen Pharmaceuticals, 1996)	*GABRA1*, Voltage-gated sodium channel gene family, Glutamate receptor ionotropic (NMDA) gene family, *CA4*, *CA1*, *CA3*, Voltage-dependent L-type calcium channel gene family	*CYP2C19*, *CYP3A4*	*ABCB1*, Tau-protein kinase activity gene family	44.7% (drug vs placebo, at 600mg/day) (Janssen Pharmaceuticals, 1996)	paresthesia, anorexia, weight decrease, fatigue, dizziness, somnolence, nervousness, psychomotor slowing, difficulty with memory, difficulty with concentration/attention, cognitive problems, confusion, mood problems, fever, infection, and flushing.(Janssen

										Pharmaceuticals, 1996)
20	Tiagabine (1996)	5-oxo-tiagabine	GABA potentiation(Cephalon, 2009)	Adjuvant	Partial seizure.	*SLC6A1*	*CYP3A4*, *UGT1A1*	-	33-46% (drug vs placebo, TGB 26–36 mg dose study period of 6 months) (Bauer J, 1995)	dizziness somnolence, depression, confusion asthenia
21	Acetazolamide (1997)	Not metabolised	Carbonic anhydrase inhibitor (Anne T., 2007; Ciccone L, 2021)	Adjuvant(Ciccone L, 2021)	partial, myoclonic, absence and primary generalized tonic–clonic seizures (Anne T., 2007), catamenial epilepsy (Rivera C., 2005)	*CA1*, *CA2 CA3*, *CA4*, *CA7*, *CA12*, *CA14*, *AQP1*	*CYP3A4*	*SLC22A6*	44% (when administered in adjunct with CBZ, at 22 mg/kg/day maximum dose)(Millichap, 1989)	Paraesthesias, dysgeusia, fatigue and gastrointestinal symptoms (Schmickl CN, 2020)
22	Zonisamide (2000)	N-acetyl zonisamide(Pharmaceutical, 2011)	Na^+ channel inhibitor, reduces voltage-dependent, transient inward currents (T-type Ca^{2+} currents) (Pharmaceutical, 2011)	Adjuvant(Pharmaceutical, 2011)	partial seizures	*SCN1A*, *SCN2A*, *SCN3A*, *SCN4A*, *SCN5A*, *SCN9A*, *SCN11A*, *SCN1B*, *SCN2B*, *SCN3B*, *SCN4B*, *CACNA1G*, *CACNA1H*, *CACNA1I*, *CA1*, *CA2*, *CA3*, *CA4*, *CA5A*, *CA5B*, *CA6*, *CA7*, *CA8*, *CA9*, *CA10*, *CA11*, *CA12*, *CA13*, *CA14*, *MAOB*, *MAOA*	*CYP3A4*, *AOX1*, *CYP2C19*, *UGT1A1*, *CYP3A5*	*ABCB1*	41.8% (drug vs placebo, 400 mg administered and primary comparison after 8-12 weeks) (Pharmaceutical, 2011)	Somnolence, Dizziness, gastrointestinal distress, skin hypersensitivity, behavioural problem, Nephrolithiasis, weight loss, cognition impaired
23	Levetiracetam (2000)	carboxylic acid metabolite (ucb L057) (inactive) (UCB, 1999)	Synaptic vesicle (*SV2A*) modulator (UCB, 1999)	Adjuvant	Partial Onset Seizures, Myoclonic Seizures, Primary Generalized Tonic-Clonic Seizures	*SV2A*, Voltage-gated sodium channel gene family (B., 2008)	-	*ABCB1*	39.8% (drug vs placebo, over the entire randomized treatment period at 3000 mg/day)(Cereghino JJ, 2000)	somnolence, asthenia, infection and dizziness (in adults)(UCB, 1999)
24	Stiripentol (2002)	13 metabolites	Potentiate GABA receptor (BIOCODEX, 2018)	Adjuvant	Dravet syndrome (BIOCODEX, 2018)	GABA receptor gene family, *LDHA*, *LDHB*	*CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP1A2*, *CYP2C9 (BIOCODEX, 2018)*	-	71% (drug vs placebo, responder rate)(Inoue Y, 2014) (BIOCODEX, 2018)	anorexia, loss of appetite, nausea, vomiting, weight loss, reversible neutropenia, insomnia,

										drowsiness, ataxia, dystonia, hyperkinesia, hypotonia (BIOCODEX, 2018)
26	Pregabaline (2004)	Not metabolised	Ca^{2+} channel inhibitor (Pfizer, 2004)	Adjuvant(Pfizer, 2004)	Partial Onset Seizures	*CACNA2D1*	-	*SLC1A1, SLC7A5*	40.6% (drug vs placebo with 10 mg/kg/day dose) (Pfizer, 2004)	dizziness, somnolence, dry mouth, edema, blurred vision, weight gain, and thinking abnormal(Pfizer , 2004)
27	Rufinamide (2004)	Carboxylamide (inactive)	Na^+ channel inhibitor (Eisai, 2008)	Adjuvant	Lennox–Gastaut Syndrome (Eisai, 2008)	*GRM5, SCN9A*	*CES1, CYP3A4 CYP2E1,*	-	32.7% (drug vs placebo Median percent change in total seizure frequency per 28 days)(Glauser T, 2008)	headache, dizziness, fatigue, somnolence, and nausea(Eisai, 2008)
28	Carisbamate (2008)	O-carisbamate glucoronide metabolite, (R)-carisbamate, two minor mercapturic acid metabolites (minor)	Na^+ channel inhibitor (Whalley BJ, 2009)	Adjuvant	Partial onset seizures (Whalley BJ, 2009)	Voltage-gated sodium channel gene family(Zannikos P, 2009)	*CYP2A6, CYP2C9, CYP2C19, ADH1B, ALDH2, UGT1A1, UGT1A9*	*GAT1, CLC* gene family (Whalley BJ, 2009)	36% (drug vs placebo median percent reduction from baseline to the double-blind phase in seizure frequency) (Halford JJ, 2011)	dizziness, headache, somnolence and nausea (Kulig K, 2007)
29	Lacosamide (2009)	O-desmethyl metabolite (inactive) (UCB, 2008)	Enhanced slow inactivation of Na^+ channel(UCB , 2008)	Mono, Adjuvant	Partial-onset seizures.	*SCN9A, SCN3A, SCN10A*	*CYP3A4, CYP2C19, CYP2C9*	-	38.1–41.2% (drug vs placebo at 600 mg/day) (Bauer S, 2017)	diplopia, headache, dizziness, nausea
30	Eslicarbazepine acetate (2009)	Eslicarbazepine (ESL)	Na^+ channel inhibitor (Sunovion, 2013)	Mono, Adjuvant(Sunovion, 2013)	Partial-onset seizures.	*P2RX4*	*CYP3A4, CYP2C19, UGT1A1*	-	34% and 43% (drug vs placebo at ESL 800 and 1200 mg) (Gil-Nagel A., 2009)	Dizziness, somnolence, nausea, headache, diplopia, vomiting, fatigue, vertigo, ataxia, blurred vision, and tremor(Sunovion, 2013).
31	Everolimus (2010)	24 metabolites	Rapamycin (mTOR) inhibitor (Corporation, 2009a)	Adjuvant	Tuberous sclerosis complex associated partial onset seizure(Corporation, 2009a)	*mTOR, FKBP12* VEGF	*CYP3A4, CYP3A5, CYP2C8, CYP2D6 (Kirchner GI, 2004)*	*SLCO1B1, SLCO1B3, SLCO1A2, ABCB1*	64.8% (Drug vs placebo after 12 week in PEP)(Franz DN, 2021)	Stomatitis, infections, asthenia, fatigue, cough, and diarrhea (Corporation, 2009a)
32	Retigabine/ Ezogabine* (2011)	N-acetyl ezogabine	K^+ channel activator	Adjuvant(GlaxoSmi	Partial-onset seizures	*KCNQ2, KCNQ3, KCNQ4,*	*UGT1A1, UGT1A3,*	-	44.3% (drug vs placebo with 1200	Neuro-psychiatric symptoms, QT

			(GlaxoSmith Kline, 2012)	thKline, 2012)	(GlaxoSmith Kline, 2012)	*KCNQ5 (MY. 2013)*	*UGT1A4, UGT1A9, NAT2*		mg/day based on median % reduction in seizure frequency in 28 days) (Brodie MJ; MY., 2013)	prolongation, suicide ideation(Glaxo SmithKline, 2012)
33	Perampanel (2012)	dihydrodiol metabolite, N-acetyl cysteine (inactive)	Glutamate (*AMPA*) agonist (Co, 2012)	Adjuvant	Partial seizure, GTCS (Co, 2012)	*GRIA1*	*CYP3A4, CYP3A5, CYP1A2, CYP2B6*	-	64.2% (drug vs placebo at 8 mg or highest tolerated dose of perampanel in 28 days)(Frenc h JA, 2015)	dizziness, somnolence, fatigue, irritability, falls, nausea, weight gain, vertigo, ataxia, headache, vomiting, contusion, abdominal pain, and anxiety (Co, 2012)
34	Talampanel^ (2012)	-	allosteric antagonist of the AMPA receptor (Howes *et al.*, 2007)	Mono (Langan YM, 2003), Adjuvant (Chappell *et al.*, 2002; Howes & Bell, 2007)	refractory epilepsy (Langan YM, 2003), partial complex seizures (Chappell et al., 2002; Howes & Bell, 2007)	*GRIA1 GRIA2, GRIA3, GRIA4*	-	-	-	Fatigue, dizziness, ataxia, nausea (Iwamoto FM, 2010)
35	Brivaracetam (2016)	carboxylic acid metabolite	Synaptic vesicle (*SV2A*) modulator (Smyrna, 2018)	Mono, Adjuvant(Smyrna, 2018)	Partial onset seizure	*SV2A,* Voltage-gatec sodium channel gene family	*CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, EPH*	-	25.2% (drug vs placebo, with 100mg/day, based on 28day seizure freq) (SA, 2009)	somnolence, dizziness, fatigue, and nausea (Smyrna, 2018)
36	Sulthiame^	Unknown	Carbonic anhydrase inhibitor	Mono, Adjuvant	benign epilepsy of childhood with central temporal spikes, refractory seizure	*CA2*	-	-	81% (drug vs placebo with 5 mg/kg/day (Rating D, 2000)	hyperpnea, paresthesias, and anorexia (Fejerman N, 2012)
37	Cannabidiol (2018)	11-carboxy-CBD	unknown	Adjuvant(Raucci U, 2020)	Dravet syndrome, Lennox–Gastaut Syndrome (Pharmaceuti cals, 2018)	*CNR1, CNR2, GPR12, GLRA1, GLRA1, GLRB, GLRA3, GPR18, GPR55, HTR1A, HTR2A, CHRNA7, OPRD1, OPRM1, PPARG, TRPV1, CACNA1G,*	*CYP2C19, CYP3A4, UGT1A7, UGT1A9, and UGT2B7* (Pharmaceu icals, 2018)	*ABCB1, ABCG2, ABCB11 (Pharmaceu ticals, 2018)*	39% (patients administere d with 15.0mg/kg) (Pamplona FA, 2018; Raucci U, 2020)	somnolence; decreased appetite; diarrhea; transaminase elevations; fatigue, malaise, and asthenia; rash; insomnia, sleep disorder, and poor quality sleep; and infections (Pharmaceutical s, 2018)

						CACNA1H, CACNA1I, TRPA1, TRPM8, TRPV2, TRPV3, TRPV4, VDAC1, HTR3A, ADORA1, PTGS1, PTGS2, ACAT1, HMGCR, GSR, GPX1, IDO1, CYP1B1, NQO1, CAT, SOD1, AANAT, NAAA				
38	Cenobamate (2019)	Not known	Inhibit voltage-gated sodium channel currents, modulator $GABA_A$ receptors (Co.; Zaccara G, 2021)	Adjuvant (Steinhoff, 2021)	Partial onset seizure(Steinhoff, 2021)	Voltage-gated sodium channel gene family, $GABA_A$ receptors (Co.)	*CYP2C19, CYP3A4, CYP3A5, CYP2B6, CYP2E1, CYP2A6, UGT2B4, UGT2B7, CYP2C8* (A., 2022)	-	55.6% (drug vs placebo (SK Life Science, 2022)	hypersensitivity, suicidal ideation, and QT shortening (A., 2022)
39	Fenfluramine (2020)		serotonergic 5-HT2 receptor agonist and σ1 receptor antagonist (Martin P, 2020; Rodriguez-Munoz M, 2018)	Adjuvant	Dravet Syndrome (T., 2019), Lennox-Gastaut syndrome (Knupp KG, 2022)	*SLC6A4, HTR1D, HTR2C, SIGMAR1, HTR2A, HTR2B, HTR1A*	*CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP2D6 (Zogenix, 2020)*	*SLC6A4*	70% (drug vs placebo at 0.7 mg/kg/day) ("A Trial of Two Fixed Doses of ZX008 (Fenfluramine HCl) as an Adjunctive Therapy in Children and Young Adults With Dravet Syndrome," ; Zogenix International Limited *et al.*, 2016) 23.7% (drug vs placebo at 0.7 mg/kg/day)(Knupp KG, 2022)	diarrhea, decreased appetite, fatigue, somnolence, and vomiting (Zogenix, 2020)
40	Ganaxolone (2022)	16-OH ganaxolone	positive allosteric modulators $GABA_A$ receptors (Marinus	Mono, adjuvant	seizures associated with CDD (Marinus Pharmaceuticals, 2022)	$GABA_A$ Receptor (Monaghan EP, 1997)	*CYP3A4, CYP3A5, CYP2B6, CYP2C19, CYP2D6* (Nohria V, 2007)		27·1% (drug vs placebo) (Knight EMP, 2022)	somnolence, pyrexia, salivary hypersecretion , and seasonal allergy (Marinus

			Pharmaceuticals, 2022)							Pharmaceuticals, 2022)

AEDs represented in the chronological order of the FDA approval as for anti-epileptic/ anti-seizure administration. AED, anti-epileptic drug; DME, drug metabolising enzymes, DT, drug transporters; ADR, Adverse drug reactions; Efficacy data is obtained from FDA drug datasheet and for seizure type/ epilepsy syndrome for which FDA administration is approved. All the DMEs, DTs and Drug target data was obtained from https://go.drugbank.com/ as on August 23, 2022. Efficacy is defined as percentage of patients with ≥ 50% decrease in seizure frequency from baseline data in respective study assessed after a given time (and dose) are marked with #. All the efficacy data for drugs are assessed for the specific epilepsy/seizure type for which they are FDA approved for administration. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GTCS, generalized tonic–clonic seizures; JME, juvenile myoclonic epilepsy; NMDA, N-methyl-d-aspartate; SV2A, synaptic vesicle glycoprotein 2A; URTI, Upper respiratory tract infection; The year of approval indicates the year in which the drug was first approved or marketed in the United States or Europe. ^ not FDA approved; CDD, CDKL5 deficiency disorder; PEP, Post extension phase: Patients benefitted from continued everolimus treatment at the end of extension phase continued treatment 105 * Percentage seizure frequency reduction

2.4.2 Genetic factors and response to AEDs

More than 24 AEDs are available in the market with newer drugs also developing constantly, however conventional AEDs such as CBZ, PHT and VPA still remain as the major prescriptions (Sirven *et al.*, 2012). These AEDs are more often accompanied by ADRs than newer AEDs, however, their cost-effectiveness is the major reason behind their preference over the newer ones. Despite advancements in drug therapy management approaches and discovery of newer AEDs many epilepsy patients struggle to achieve seizure control (Piana *et al.*, 2014). It is widely reported that 30% of the PWE fail to respond to AED polytherapy making the disease refractory (Brodie *et al.*, 2000). Inadequate patient response has majorly been because of the large inter-individual genetic variability of patients. There is variability in efficacy and ADRs in patients who were on similar dosages of same medications. This uncertainty in therapy management comprising drug and dose selection, along with managing range of ADRs in each individual patient has highlighted the urgent need to understand the individual genetic profile and develop predictive test for prescribing drugs and dosages in a personalized manner (Cavalleri *et al.*, 2011). An earliest report of 1968 provided evidence of involvement of genetic factors (congenital enzyme deficiency) in response to AEDs (Kutt *et al.*, 1968). Since then, role of number of genes involved in metabolism, transport and target of AEDs have been elucidated in pharmacogenetic studies. Despite many studies results have been inconclusive and conflicting with no recommendations available to date for prediction of antiepileptic dose, drug level and drug response (efficacy) by means of genetic markers/genotypes of individual patients (Dlugos *et al.*, 2006). This hints towards the need for further exploration of AED mechanisms and the hidden facts behind.

2.4.2.1 Drug metabolizing enzymes (DMEs)

The genes involved in metabolism of AEDs (pharmacokinetics) are mainly from Cytochrome P450 (CYP450) superfamily and Microsomal Epoxides (mEH). Of the CYP450,

CYP2C9 is known to be a major metabolizer of PHT and PB followed by *CYP2C19* with comparatively low but significant contribution in VPA metabolism (Anderson, 1998; Jiang *et al.*, 2009; Mehendale, 1995; Riva *et al.*, 1996; Veronese *et al.*, 1991). Another CYP450 *CYP3A4* contributes to the metabolism of CBZ (Kerr *et al.*, 1994; Tomson *et al.*, 1983). Other than *CYP3A4*, *CYP3A5* has also been reported to play role in CBZ metabolism (Park *et al.*, 2009). As there exist a considerable overlap in substrate specificity of CYP3A subfamily gene, therefore *CYP3A7* the third largely expressed enzyme of the CYP3A gene cluster at chromosome 7 after *CYP3A4* and *CYP3A5*, was also prioritized for the present study. Further, of the Microsomal Epoxides, *EPHX1* known to metabolize CBZ was also prioritized (Kerr et al., 1994; Tomson et al., 1983). While conducting pharmacogenetic studies, majority focus has been on AED pharmacokinetic genes (Dlugos et al., 2006). Several functional variants of DME's are known to have significant influence on drug metabolism and drug dosing which ultimately may influence their drug response. Based on the in vitro studies it has been reported that *CYP2C9*3* allele exhibits decreased enzyme activity leading to reduced PHT metabolism (Takanashi *et al.*, 2000). Further in an in vitro study on S-warfarin it was observed that individuals having minor allele of *CYP2C9**2 (R144C) will have decreased clearance of S-warfarinn than the wild type allele (Rettie *et al.*, 1994). It was further observed that patients with *CYP2C9*2* exhibited significant reduction in maximum tolerated doses (Dlugos et al., 2006). Later Tate *et al* performed the genetic association study of *CYP2C9* variants with phenytoin pharmacokinetic parameters, which revealed significantly reduced enzyme activity of *CYP2C9*3* allele leading to lower phenytoin dose requirement (Tate *et al.*, 2004). (Tate & Goldstein, 2004). Other than the *2 and *3 polymorphisms *CYP2C9**5, *6, *8, *11, *1B have also shown impaired phenytoin metabolism leading to increased plasma levels (Allabi *et al.*, 2003; Chaudhry *et al.*, 2010; McCluggage *et al.*, 2009; Ramasamy *et al.*, 2007). Other than phenytoin *CYP2C9* and *CYP2C19* are known to metabolism of Phenobarbital. The influence of *CYP2C19* polymorphism on pharmacokinetic (PK) of Phenobarbital was observed in Japanese population (Yukawa *et al.*, 2006) in case of Asian patients it has been observed that *CYP2C19**1/*3 heterozygous significantly decreases the body clearance of Phenobarbital as compared to *CYP2C19**1/*1 and *CYP2C19**1/*2 (Goto *et al.*, 2007). Based on the *in vitro* and *in vivo* studies exploring functional role of various *CYP3A4* gene variants *CY3A4*1G* (rs2242480) is known to effect *CYP3A4* activity by influencing the metabolism of its substrates, however no significant influence has been observed for CBZ response as well as maintenance doses and adjusted plasma concentrations of CBZ (Dong *et al.*, 2012; Miura *et al.*, 2011; Yun *et al.*, 2013). Another report on Korean ethnicity patients by Park et al reported the influence of *CYP3A5*3* (rs4986910)

variant on steady state serum concentration of CBZ (Piana et al., 2014). Further two common variants of *EPHX1* rs1051740 (c.337T>C) and rs2234922 (c.416A>G) have been demonstrated to influence the metabolic capacity of *EPHX1* both *in vitro* as well *in vivo.* Other than phase I DME's, phase II DME's play significant role in metabolism of selective first line AEDs. UGT's are considered to be the major metabolizers for valproic acid. For example UGT1A3*5 as reported in Chinese population could act as significant determinant of PK variability of valproic acid as these patients require higher doses of valproic acid in order to achieve therapeutic levels (Chu *et al.*, 2012).

2.4.2.2 Drug Transporters

Other than DME's, drug transporters have also been well explored for their possible role in inter-individual variability in AED efficacy. Several in vitro reports of first-line AEDs have demonstrated PHT, CBZ, VPA and PB as substrates of *ABCB1* (P-gp) (Luna-Tortos *et al.*, 2008; Potschka *et al.*, 2001; Weiss *et al.*, 2003). Further evidences have also indicated that PHT and CBZ may also be substrate to another drug efflux transporter *ABCC2* whereas regarding VPA substrate specificity, reports have been controversial (Potschka *et al.*, 2003). It has been hypothesized that over-expression of the drug efflux transporters at the blood brain barrier (BBB) may be the main reason behind drug resistance phenotype in drug refractory epilepsy patients (Lazarowski *et al.*, 2011). For *ABCB1* the majorly studied variants in terms of AED efficacy are rs1128503 (c1236C>T), rs2032582 (c2677T>A) and rs1045642 (c3435C>T). However, the results have been inconsistent but their possible role in response variability may not be undermined (Kwan *et al.*, 2007; Seo *et al.*, 2006; Siddiqui *et al.*, 2003; Zimprich *et al.*, 2004). A recent meta-analysis of 23 studies evaluating the role of *ABCB1* C1236T, G2677T/A and C3435T polymorphisms confirmed these inconsistence (Haerian *et al.*, 2011). In case of *ABCC2* recent reports have highlighted the role of variants c-24C>T and c3972C>T in drug response of Indian and Chinese ethnicity patients (Grover *et al.*, 2012; Qu *et al.*, 2012). The results from other studies have however been inconsistent in case of *ABCC2* with various reports being unable to replicate the findings (Hilger *et al.*, 2012; Kim *et al.*, 2009; Kwan *et al.*, 2011; Seo *et al.*, 2008). However, most recently a meta-analysis of eight studies exploring commonly studied *ABCC2* variants with AED response in 2823 epilepsy cases suggested borderline association of c-24C>T with drug response in Caucasians (Grover *et al.*, 2013).

2.4.2.3 Targets

A common polymorphism in the α1 subunit *(SCN1A)* IVS5-91 G>A has been reported by various studies for association with drug response. It was observed to be significantly associated with

maximal dose or concentration at maintenance dose in PHT and CBZ patients (Tate *et al.*, 2005; Tate & Goldstein, 2004). Same SNP was further reported to be associated with CBZ resistance (Abe *et al.*, 2008). Various studies also failed to replicate the associations (Kumari *et al.*, 2013; Manna *et al.*, 2011; Zimprich *et al.*, 2008). Other than *SCN1A*, studies were also performed for other isoforms of sodium channels such as *SCN2A*, *SCN1B* etc. A recent report by Ma chun et al studied the role of *SCN1A* and *SCN2A* variants with drug resistance in epilepsy patients however, significant association was only observed for *SCN1A* IVS5-91 G>A variant with no significant association for any *SCN2A* variant (Ma *et al.*, 2014). Other than sodium channels gamma aminobutyric acid (GABA) receptor and GABA transporters have also been well studied with epilepsy drug response. Significant association of *GABRA1* rs2279020 (c.1059+15G>A) with drug resistance was observed for patients on treatment with CBZ, PHT or VPA (Kumari *et al.*, 2011). Additionally, a recent study has reported the combined effect of *GABRA1*, *GABRA2* and *GABRA3* SNPs on epilepsy treatment outcome when no single SNP associations could withstand multiple corrections (Hung *et al.*, 2013). Other than GABA receptors glutamate receptors have also been considered as potential candidates/targets for effective therapy management in epilepsy patients (Bialer *et al.*, 2010; Brodie & French, 2000). Further, as levetiracetam story has provided important implications for future drug discovery as well as in reviewing the mode of actions of already existing first line AEDs (Bialer & White, 2010; Brodie & French, 2000). The mode of action of the common AEDs are represented in **Figure 2.6**

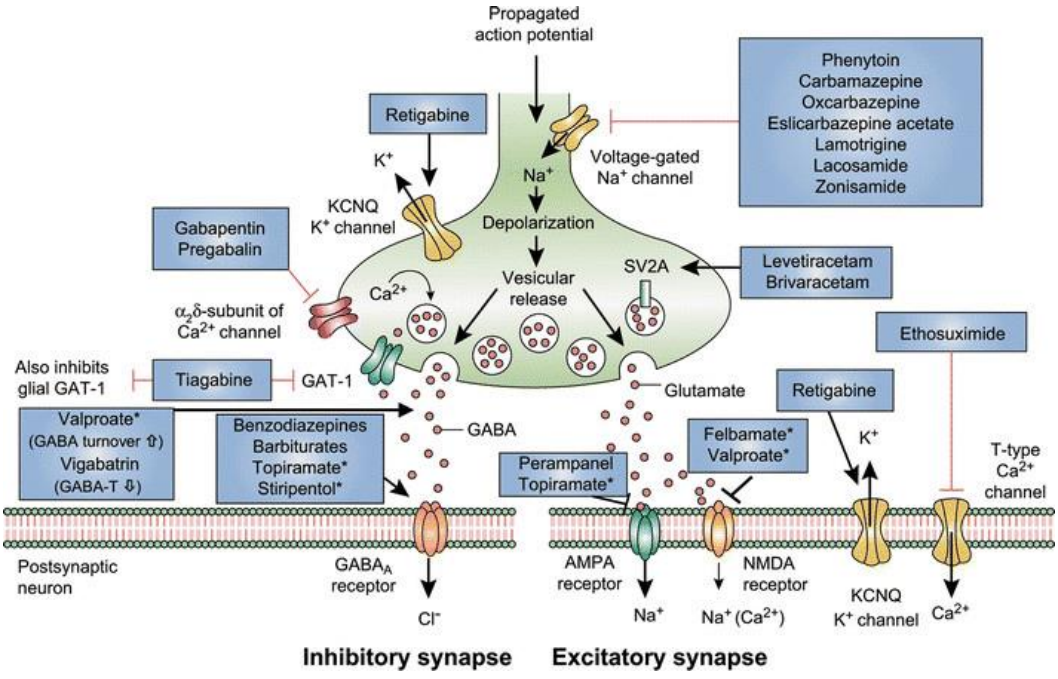


Figure 2.6 Mechanism of action of clinically approved anti-seizure drugs. Drugs marked with asterisks indicate that these compounds act by multiple mechanims (not all mechanisms shown here). GABA-T GABA aminotransferase, GAT GABA transporter, SV2A synaptic vesicle protein 2A, GABA gamma-aminobutyric acid,

NMDA N-methyl-D-aspartate, AMPA α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, KCNQ a family of voltage-gated potassium channels (also known as the Kv7 family).

2.4.3 Gap in present knowledge

In spite of such enormous corpus of pharmacogenomic literature available, data from less than 10% of such articles are used for clinical applications. Establishing an association between a gene or its variants with a drug-related phenotype is merely the first step of biomarker discovery. This lack of progress can be attributed to the common issues of failure to partially or fully replicate research identifying genetic biomarker associations, overestimation of effect of the variants in an underpowered study, inconsistent findings across different study cohorts (M., 2010). Drug response phenotypes are complex to assess, more commonly influenced by a complex interplay between environmental, genetic, and gene–environment interactions. In fact, multiple genes can also have an impact on the predictive value of a genetic biomarker. A further complication is the lengthy and extensive investigation that is required to clinically verify genetic risk factors that are suspected of affecting drug pharmacokinetics and pharmacodynamics (Gervasini G, 2010).With only 3% of published clinical data in this field focusing on phase 2 studies and beyond, there is a lack of evidence-based guidelines for many pharmacogenetic applications(Vijverberg SJ, 2010). In addition, some biomarker tests are in need of phase 3 and 4 research to evaluate whether recommended guidelines have been successful in reducing the burden.

With respect to epilepsy, the challenges in clinical implementation of pharmacogenomics is in multitude. The World health organization (WHO)'s Global Burden of Disease reports epilepsy to have the second most highest burden of all neurological disorders worldwide, in terms of disability of adjusted life years (Murray CJ, 2012). Due to the complex etiology of the disease, seizure occurrence can be sporadic, because of some structural brain lesions, infection,

Despite the availability of many anti-epileptic drugs (AEDs) acting via different modes of action, the overall treatment outcome have not improved. Most patients who attain seizure freedom generally do so with first or second AED administration. Otherwise, the likelihood of achieving seizure freedom reduces substantially with increased disease duration1. More than one-third of patients with epilepsy (PWE) experience seizure recurrent in spite of AED treatment. Such patients are often classified as patients with drug resistant epilepsy (DRE). DRE is a major challenge in epilepsy treatment. Since these patients have minimal chances of seizure freedom based on additional medication trials 2 and they suffer repetitive

seizures leading to neuro-biochemical changes, poor cognitive response and psychological problems which increase the disease burden 3. Therefore, the efficacy of drugs in the early stages of the disease is particularly important because determining efficacy is closely related to the long-term prognosis. Additionally, most of the markers available for clinical translation are related to ADR of AEDs, a few markers for efficacy are known but they lack unifying conclusions. With the advancement in genomic technologies, researchers have moved from the conventional candidate gene approach towards a more holistic genome-wide screening to identify associated genomic loci. So far, ten GWAS are published that are retrieved from the GWAS Catalog as on March 24, 2023 with keyword 'response to anticonvulsant' comprising of 11,872 samples (**Table 2.7**). Limited sample size in PGx studies is a major limitation in exploring the genetic associations. Seven of these includes patients with European ancestry and only three includes East Asian samples. Since genetic architecture is ethnicity- specific, elucidating the genetic landscape with respect to the phenotypic response in very crucial. Like the strong association of HLA variants (HLA-B*15:02, HLA-A*) with carbamazepine induced skin hypersensitivity in South Asian population, GWAS is warranted in different populations to establish population specific markers as well as drug response outcomes. Out of the ten studies, eight of them discusses ADR outcome when treated with any AED, and two discuss drug response which failed to identify SNPs with GWAS significance threshold and none of these loci are currently in clinical use.

Table 2.7: GWAS published till date associated with response to anti-epileptic drugs.

Title	Discovery sample number and ancestry	Variant and risk allele	P-value	OR[95% CI]	Mapped gene	Reported trait	Trait(s)	Study accession
A genome-wide association study of sodium levels and drug metabolism in an epilepsy cohort treated with carbamazepine and oxcarbazepine (Berghuis *et al.*, 2019).	1252 European	rs9833158	1×10^{-6}	1.56[1.32-1.89]	*PLCL2, CDYLP1*	Carbamazepine- or oxcarbazepine-induced hyponatremia in epilepsy	carbamazepine-induced hyponatremia, response to oxcarbazepine, oxcarbazepine-induced hyponatremia, response to carbamazepine	GCST007809
	1252 European	rs4895178	1×10^{-6}	1.55[1.30-1.85]	*RPL23AP44*			GCST007809
	1252 European	rs148646711	1×10^{-6}	1.96[1.48-2.59]	*ANKRD55, FLJ31104*			GCST007809
	804 European	rs1394074	1×10^{-6}	7.75[3.41-17.61]	*OLFML3*	Severe carbamazepine- or oxcarbazepine-induced hyponatremia in epilepsy		GCST007810
	804 European	rs7300380	1×10^{-6}	3.07[1.93-4.87]	*SSPN, ITPR2*			GCST007810
	804 European	rs72894781	7×10^{-6}	3.43[2.01-5.87]	*TEAD3*			GCST007810
Role of Common Genetic Variants for Drug-Resistance to Specific Anti-Seizure Medications (Wolking *et al.*, 2021).	289 European	rs12038219	6×10^{-8}	-	-	Response to phenytoin in focal epilepsy	response to phenytoin	GCST90020117
	289 European	rs28740860	9×10^{-7}	-	-			GCST90020117
	289 European	rs188002	5×10^{-7}	-	-			GCST90020117
	289 European	rs16945236	8×10^{-7}	-	-			GCST90020117
	394 European	rs6552076	6×10^{-6}	-	-	Response to oxcarbazepine in focal epilepsy	response to oxcarbazepine	GCST90020116
	394 European	rs1816237	1×10^{-6}	-	-			GCST90020116
	394 European	rs2944715	3×10^{-6}	-	-			GCST90020116
	394 European	rs34744859	4×10^{-6}	-	-			GCST90020116
	1014 European	rs7811069	2×10^{-6}	-	-	Response to lamotrigine in focal epilepsy	response to lamotrigine	GCST90020114
	1014 European	rs1859577	5×10^{-7}	-	-			GCST90020114
	1014 European	rs2028234	7×10^{-7}	-	-			GCST90020114
	1400 European	rs12468936	3×10^{-6}	-	-	Response to lamotrigine in epilepsy	response to lamotrigine	GCST90020121
	1400 European	rs7811069	8×10^{-7}	-	-			GCST90020121
	1400 European	rs7859863	5×10^{-6}	-	-			GCST90020121
	1400 European	rs28776624	4×10^{-6}	-	-			GCST90020121
	943 European	rs4078065	4×10^{-6}	-	-	Response to carbamazepine in focal epilepsy	response to carbamazepine	GCST90020115
	943 European	rs13150739	9×10^{-7}	-	-			GCST90020115
	943 European	rs4243569	5×10^{-6}	-	-			GCST90020115

Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population (Ozeki T, 2011).	934 East Asian	HLA-A*3101	1×10^{-16}	9.5[5.6-16.3]	-	Adverse response to carbamazepine	response to anticonvulsant	GCST000912
HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans (McCormack *et al.*, 2011).	4052 European	rs1061235	1×10^{-7}	9.12[4.03-20.65]	*HLA-A*	Adverse response to carbamazepine	response to anticonvulsant	GCST001014
Genome-wide mapping for clinically relevant predictors of lamotrigine- and phenytoin-induced hypersensitivity reactions(McCormack *et al.*, 2012).	1372 European	rs10510829	7 x 10-7	-	*FHIT*	Adverse response to lamotrigine and phenytoin	response to anticonvulsant	GCST001431
	1372 European	rs275380	1×10^{-6}	-	*ADAMTS20*			GCST001431
	1372 European	rs6990917	2×10^{-6}	-	*PREX2, NDUFS5P6*			GCST001431
	1372 European	rs285406	5×10^{-6}	-	*ATP6V0D2*			GCST001431
	1372 European	rs183266	5×10^{-6}	-	*IRF2BPL, LINC02288*			GCST001431
	1372 European	rs12230440	3×10^{-6}	-	*ADIPOR2*			GCST001431
	1372 European	rs8083432	6×10^{-6}	-	*ZNF521*			GCST001431
	1372 European	rs7798500	6×10^{-6}	-	*PRKAR2B*			GCST001431
	1372 European	rs17002253	7×10^{-6}	-	*SOWAHB, SHROOM3-AS1*			GCST001431
	1372 European	rs3853240	1×10^{-6}	-	*LINC01947*			GCST001431
	1372 European	rs9919839	2×10^{-6}	-	*DACH1*			GCST001431

	1372 European	rs4569005	5×10^{-6}	-	*NELL1*			GCST001431
Pharmacoresponse in genetic generalized epilepsy: a genome-wide association study (Wolking S, 2020).	886 European	rs6871559	5×10^{-6}	-	*RNU1-76P*	Response to antiepileptic drugs in genetic generalized epilepsy	response to anticonvulsant	GCST010296
	886 European	rs13179734	9×10^{-6}	-	*LINC02064*			GCST010296
	886 European	rs7457112	9×10^{-6}	-	*CNTNAP2*			GCST010296
	886 European	rs1277731	9×10^{-6}	-	*CACNB2*			GCST010296
	565 European	rs78269837	5×10^{-6}	-	*WDR41*	Response to valproic acid in genetic generalized epilepsy		GCST010295
	565 European	rs4292046	5×10^{-6}	-	*EXOC6B, COL6A3*			GCST010295
	565 European	rs6046489	7×10^{-6}	-	*RIN2*			GCST010295
	387 European	rs17650998	9×10^{-7}	-	*KCNMB2-AS1, KCNMB2*	Response to lamotrigine in genetic generalized epilepsy		GCST010294
	387 European	rs10206521	3×10^{-6}	-	*TDRD15*			GCST010294
	387 European	rs1291861	6×10^{-6}	-	*CELF2*			GCST010294
	387 European	rs11794033	8×10^{-6}	-	*RN7SKP120*			GCST010294
	209 European	rs17676256	1×10^{-7}	-	*ANK2*			GCST010293
	209 European	rs12320526	2×10^{-6}	-	*NAV3*			GCST010293
	209 European	rs12734159	3×10^{-6}	-	*LEPR, RN7SL854P*			GCST010293
	209 European	rs7956831	3×10^{-6}	-	*CLECL1, CD69*			GCST010293
	209 European	rs1014085	4×10^{-6}	-				GCST010293
	209 European	rs3756744	4×10^{-6}	-	*SLC27A6, ISOC1*			GCST010293
	209 European	rs7515154	4×10^{-6}	-	*SYDE2, C1orf52*			GCST010293
	209 European	rs72765466	6×10^{-6}	-	*NID1*			GCST010293
	209 European	rs17124115	7×10^{-6}	-	*LINC02396, LINC02395*			GCST010293

	104 European	rs1922809	8×10^{-7}	-	*LRRTM4*	Response to lamotrigine and valproic acid in genetic generalized epilepsy		GCST010292
	104 European	rs4751538	8×10^{-7}	-	*BUB1P1, FOXI2*			GCST010292
	104 European	rs78723182	2×10^{-6}	-	*MAGI2*			GCST010292
	104 European	rs4416719	2×10^{-6}	-	*F13A1*			GCST010292
	104 European	rs1479876	4×10^{-6}	-	*CLSTN2*			GCST010292
	104 European	rs7705566	4×10^{-6}	-	*CDH6*			GCST010292
	104 European	rs8003775	6×10^{-6}	-	*LINC00639*			GCST010292
Role of Common Genetic Variants for Drug-Resistance to Specific Anti-Seizure Medications (Wolking S, 2021).	936 European	rs10191428	2×10^{-6}	-	*TMEM17, RN7SL18P*	Response to levetiracetam in focal epilepsy		GCST90020112
	936 European	rs6455984	3×10^{-6}	-				GCST90020112
	936 European	rs10786411	4×10^{-6}	-				GCST90020112
	2196 European	rs2600151	3×10^{-6}	-		Response to sodium channel-Active anticonvulsant in focal epilepsy		GCST90020113
	2196 European	rs60350499	7×10^{-8}	-				GCST90020113
	624 European	rs11125398	3×10^{-6}	-		Response to calcium-channel-Active anticonvulsants in focal epilepsy		GCST90020118
	624 European	rs73104283	4×10^{-6}	-				GCST90020118
	624 European	rs7092992	4×10^{-6}	-				GCST90020118
	730 European	rs2700204	5×10^{-6}	-		Response to valproic acid in focal epilepsy		GCST90020119
	730 European	rs1952670	9×10^{-7}	-				GCST90020119
	730 European	rs7092992	4×10^{-6}	-				GCST90020119
	1238 European	rs10191428	2×10^{-6}	-	*TMEM17, RN7SL18P*	Response to levetiracetam in epilepsy		GCST90020120
	1238 European	rs9390556	5×10^{-6}	-		Response to levetiracetam in epilepsy		GCST90020120
	1538 European	rs73104283	1×10^{-6}	-		Response to calcium-channel-Active anticonvulsants in epilepsy		GCST90020122
	1302 European	rs3936663	4×10^{-6}	-		Response to valproic acid in epilepsy		GCST90020123

Genetic variation in CFH predicts phenytoin-induced maculopapular exanthema in European-descent patients (McCormack *et al.*, 2018).	524 European 80 East Asian	rs78239784	5×10^{-11}	7[3.2-16]	*CFHR4*	Phenytoin-induced maculopapular exanthema	maculopapular eruption, response to phenytoin	GCST005200
A genome-wide association study of sodium levels and drug metabolism in an epilepsy cohort treated with carbamazepine and oxcarbazepine (Berghuis et al., 2019).	297 European	rs1718641	6×10^{-6}	2.5[1.69-3.70]	*LINC01808*	Oxcarbazepine-induced hyponatremia in epilepsy	response to oxcarbazepine, oxcarbazepine-induced hyponatremia	GCST007812
	1031 European	rs9833158	1×10^{-6}	1.63[1.35-2.04]	*PLCL2, CDYLP1*			GCST007811
	1031 European	rs57143981	2×10^{-6}	1.98[1.50-2.62]	*BTBD11*			GCST007811
	1031 European	rs4817405	2×10^{-6}	1.61[1.33-1.96]	*TIAM1, FBXW11P1*			GCST007811
	1031 European	rs11817796	4×10^{-6}	1.74[1.37-2.19]				GCST007811
	1031 European	rs35648408	4×10^{-6}	1.78[1.39-2.27]	*RUNDC3B*			GCST007811
	1031 European	rs12743242	6×10^{-6}	1.68[1.34-2.11]	*LINC01141*			GCST007811
	1031 European	rs6033092	7×10^{-6}	1.55[1.28-1.87]	*RPS11P1*			GCST007811
Genetic variation in CFH predicts phenytoin-induced maculopapular exanthema in European-descent patients (McCormack et al., 2018).	282 East Asian 964 European	HLA-A*31:01	1×10^{-10}	5.5[3.0-10]	-	Carbamazepine-induced maculopapular exanthema	maculopapular eruption, response to carbamazepine	GCST005198
Shared genetic risk factors across carbamazepine-induced hypersensitivity reactions (Nicoletti *et al.*, 2019).	10726 European	HLA-A*31:01	2×10^{-9}	12.9[5.58-29.78]	-	Carbamazepine-induced reaction with eosinophilia and systemic symptoms	drug hypersensitivity syndrome, response to carbamazepine	GCST008384
	10726 European	HLA-B*51:01	6×10^{-6}	5.72[2.69-12.16]	-			GCST008384
	10726 European	rs187926838	1×10^{-6}	18.2[5.2-63.72]	*ALK*			GCST008384
	10744 European	rs116071718	2×10^{-6}	3.99[2.25-7.05]	*MUC22*	Carbamazepine-induced serious cutaneous adverse reaction	severe cutaneous adverse reaction, response to carbamazepine	GCST008386
	10744 European	rs192543598	2×10^{-12}	18.11[8.03-40.88]	*MICD, HLA-W*			GCST008386
	10744 European	rs187926838	5×10^{-8}	12.1[4.94-29.8]	*ALK*			GCST008386
GWAS identifies two susceptibility loci for lamotrigine-induced skin	1114 East Asian	rs141860749	4×10^{-7}	8.9[NR]	*SYT2*		response to lamotrigine,	GCST002949
	1114 East Asian	rs62270313	1×10^{-7}	4.6[NR]	*EPHB1*			GCST002949

rash in patients with epilepsy (Jang *et al.*, 2015).	1114 East Asian	rs631844	7×10^{-8}	4.9[NR]	*LINC02109*	Epilepsy and lamotrigine-induced maculopapular eruptions	maculopapular eruption, epilepsy	GCST002949
	1114 East Asian	rs71568191	3×10^{-9}	8.9[NR]	*HCG27*			GCST002949
	1114 East Asian	rs1178326	2×10^{-7}	8.1[NR]	*HDAC9*			GCST002949
	1114 East Asian	rs12668095	5×10^{-7}	3.4[NR]	*MRPL42P4*			GCST002949
	1114 East Asian	rs7461897	5×10^{-7}	4.1[NR]	*MIR548H4*			GCST002949
	1114 East Asian	rs13287547	7×10^{-7}	4.8[NR]	*C9orf92*			GCST002949
	1114 East Asian	rs74912790	1×10^{-9}	10.7[NR]	*IFNA13, IFNA6*			GCST002949
	1114 East Asian	rs75078187	2×10^{-7}	8.1[NR]				GCST002949
	1114 East Asian	rs74308953	2×10^{-10}	4.6[NR]	*LINC02578*			GCST002949
	1114 East Asian	rs146173241	1×10^{-7}	7.3[NR]	*GFRA1, CCDC172*			GCST002949
	1114 East Asian	rs139427007	4×10^{-7}	11[NR]	*LINC02409*			GCST002949
	1114 East Asian	rs7328626	8×10^{-9}	9.7[NR]	-			GCST002949
	1114 East Asian	rs9596837	4×10^{-9}	10.1[NR]	-			GCST002949
	1114 East Asian	rs9596863	8×10^{-11}	15.2[NR]	*LINC00558*			GCST002949
	1114 East Asian	rs118166657	1×10^{-7}	7.2[NR]	*PPP2R5E, GCATP1*			GCST002949
	1114 East Asian	rs7495694	1×10^{-9}	7.6[NR]	*ADPGK-AS1, NPM1P42*			GCST002949
	1114 East Asian	rs79007183	3×10^{-10}	14[NR]	*CRAMP1*			GCST002949
	1114 East Asian	rs1429264	9×10^{-7}	8.4[NR]	*COTL1*			GCST002949
	1114 East Asian	rs11663316	6×10^{-7}	4.2[NR]	*NDUFV2, RPS4XP19*			GCST002949
	1114 East Asian	rs17084405	1×10^{-7}	12.1[NR]	*LINC01541*			GCST002949
	1114 East Asian	rs970510	7×10^{-7}	6.5[NR]	*LINC01899*			GCST002949
	1114 East Asian	rs150435906	6×10^{-7}	8.6[NR]	*DNAJC5B*			GCST002949
	1114 East Asian	rs2930491	5×10^{-7}	4[NR]	*ABRA*			GCST002949
	1114 East Asian	rs143543475	4×10^{-7}	11[NR]	*RAB18, PTCHD3*			GCST002949
	1114 East Asian	rs55949311	8×10^{-9}	9.7[NR]				GCST002949
Potential role of regulatory DNA variants in modifying the risk of	162 East Asian	rs4471527	1×10^{-8}	5.8[3.0-11.0]	*LINC02414, LINC02370*	Carbamazepine-induced Stevens-Johnson syndrome or	Stevens-Johnson	GCST90103803

severe cutaneous reactions induced by aromatic anti-seizure medications (Mullan *et al.*, 2022).						toxic epidermal necrolysis (SJS/TEN)	syndrome, toxic epidermal necrolysis, response to carbamazepine	
	197 East Asian	rs4471527	2×10^{-9}	5.7[3.1-10.5]	*LINC02414, LINC02370*	Aromatic antiseizure medication-induced Stevens-Johnson syndrome or toxic epidermal necrolysis		GCST90103802
	197 East Asian	rs199755581-CA	6×10^{-9}	4[2.5-6.5]	*NIPAL2*			GCST90103802
	197 East Asian	rs1297852527	9×10^{-9}	5.4[2.9-9.9]	*SLC9B1P3*			GCST90103802
	197 East Asian	rs77491650	1×10^{-8}	0.3[0.2-0.4]	*DDX12P*			GCST90103802
	197 East Asian	chr4:820728	1×10^{-8}	0.3[0.2-0.5]				GCST90103802
	197 East Asian	rs77542827	3×10^{-8}	6.5[3.1-13.6]	*FRG1JP*			GCST90103802
	197 East Asian	rs778096762	3×10^{-8}	3.2[2.1-4.9]	*LINC02153, GFRA2*			GCST90103802
	197 East Asian	rs374138762	4×10^{-8}	5.2[2.8-9.9]	*LINC02005, Metazoa_SRP*			GCST90103802
	197 East Asian	rs879656274	4×10^{-8}	4.9[2.7-8.9]	*SLC9B1P3*			GCST90103802
	88 East Asian	rs1562468327	6×10^{-10}	9.7[4.4-21.1]	*TAB2, ZC3H12D*	Aromatic antiseizure medication-induced Stevens-Johnson syndrome or toxic epidermal necrolysis in HLA-B*15:02 non-carriers		GCST90103804
	88 East Asian	rs199755581-CA	7×10^{-10}	9.7[4.4-21.3]	*NIPAL2*			GCST90103804
	88 East Asian	chr21:9790175	4×10^{-9}	7.4[3.7-15.0]				GCST90103804
	88 East Asian	rs77542827	1×10^{-8}	17.9[5.1-62.5]	*FRG1JP*			GCST90103804
	88 East Asian	rs1286845082	2×10^{-8}	23.8[5.4-105.5]	*PDLIM1P3, MTCO3P13*			GCST90103804
	88 East Asian	rs1597607761	3×10^{-8}	42[5.5-321.8]	*PDLIM1P3, MTCO3P13*			GCST90103804
	88 East Asian	rs1391213386	3×10^{-8}	11.9[4.3-33.1]	*YEATS2*			GCST90103804
	88 East Asian	rs1211926109-AAT	4×10^{-8}	0.1[0.03-0.2]	*SMIM14-DT, UBE2K*			GCST90103804

Source GWAS catalog as accessed on March 24, 2023. The associated loci are mentioned till suggestive p value (p< 1x 10-6). The study title, ethnicity and sample size, associated variants with risk alleles (if known), their p value and OR (95% CI), the mapped genes, the associated phenotype and their GWAS accession number are mentioned in the table.

Chapter 3

Development of an algorithm to screen pharmacogenetic variations from global literature and build a resource for anti-epileptic drug response genes

3.1 Introduction

With development of in high-throughput genomic technologies, the focus of PGx research has moved from candidate gene approach to large-scale genome-wide screening focussing on unbiased clinical PGx applications. Identifying genes involved in the absorption, distribution, metabolism and excretion (ADME) of drugs is critical in PGx. Such genes also known as pharmacogenes— are important for its pharmacology—that are involved in pharmacokinetic or pharmacodynamics actions (Walker, 2004). Abundant articles are published in various perspectives in this regard. To critically evaluate such data from published articles in labour intensive and time consuming. This delay impedes our ability to identify, evaluate, and use genetics to optimize drug selection and dosing with minimal toxicity (Ventola, 2013).

Generally, we explore the answers to such questions in publications describing the disease–drug–gene relationships of interest in a particular population. Such relationships of clinical importance for drug dosing and administration must be interpreted as a priority. Analyzing such related data from the literature, we need to rapidly identify and develop high-throughput, accurate, and population-specific genetic polymorphisms that correlate with drug response. Such genetic considerations can be expected to be important in diagnosis, treatment, and prevention. Both clinical and research communities have placed emphasis on identifying PGx relationships. Several databases employ manual curation of biomedical literature to provide comprehensive coverage of such disease or drug-related genetic association relationships in humans. Some of them OMIM (Amberger et al., 2009), Human Gene Mutation Database (HGMD) (Stenson et al., 2009), CTD (Davis et al., 2016), Genetics Home Reference (GHR) (http://ghr.nlm. nih.gov/) (National Library of Medicine (US), Genetics Home Reference 2013), and the gold standard database in PGx, the Pharmacogenomics Knowledgebase (PharmGKB) (Whirl-Carrillo et al., 2012).

A fully automated PGx relationship curation system to retrieve clinically relevant information is still far-fetched (Singhal et al., 2016). Several advanced computational approaches with statistical evaluation are known which can reduce manual efforts to curate important PGx relationships from available literature. Experimental noise from different sources reported in different articles, result in a number of important genes or polymorphisms causing heterogeneity in data. Therefore, the recent efforts in (semi-)automated approaches facilitate automated extraction with manual curation of relationships for high quality are critical (Garten et al., 2010). Thus in this objective, we propose to develop an end-to-end semi-

automated pipeline for the extraction of PGx relationships in the form of disease– drug–gene–polymorphism from literature published in different populations. If an article mentions a drug and a genetic association, these articles are screened for their relevance in PGx in context to any drug response. The biological entities like disease, drug, gene, genetic variant obtained by automated extraction were normalized with available up-to-date well curated datasets to exclude the ambiguities. Additionally, we compared our developed pipeline with databases like OMIM (Amberger et al., 2009), CTD (Davis et al., 2016), and PharmGKB (Whirl-Carrillo et al., 2012) to assess the sensitivity and specificity of the thus obtained PGx data. We also calculated the accuracy of each relationship obtained and compared their occurrence within the three datasets. We also performed a validation study by comparing our result with commercially used FDA-approved drug labeling biomarkers (https://www.fda.gov/drugs/science-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling) (FDA, 2018). The final PGx relations extracted were also prioritized for significance in clinical application. The key feature of the study is the use of text mining to tabulate the most important PGx information related to disease or given drugs by studying its variability and impact on individuals, which can be used for future clinical administration.

3.2 Materials & Methods

The schematic representation of the study framework is shown in **Figure 3.1.** It is divided into five broad steps: (1) build a corpus of PGx and related abstracts fetched from PubMed using the Medical Subject Headings (MeSH) query; (2) identify all biological entities in PubMed abstracts (diseases, genes, drugs, polymorphisms, and populations); (3) normalization of the obtained entities from standard databases; (4) validation with available dataset(s) in global context; and (5), evaluation of extracted data and ranking of PGx relationships. This process results in a list of all PMIDs aligned PGx relationships of the form < disease–drug–gene–polymorphism>. We used an in-house built R package, called pubmed. mineR (Rani J, 2015), which was used for pilot phase application for extracting PGx information from all the published articles in PubMed. This package was aimed to text mine data from published literature without dependency on other packages.

3.2.1. Information retrieval

Our search query was formed using MeSH terms: “inter-individual variability,” “pharmacogenomics,” “pharmacogenetics,” and “drug response” specifically for human. All the articles with available abstract were downloaded using the “e-utilities” interface in a .csv format. This corpus now contains articles with PGx information published till date.

3.2.2. Entity recognition

For each PMID, the annotation results of all the biomedical entities mentioned in the abstract, i.e., disease, drugs, gene, and mutation, were obtained using PubTator (K. H. Wei CH, Lu Z, 2013). In PubTator, the four biological entities— disease, chemical, gene, and related mutation annotations—were extracted by DNorm (Leaman R, 2013), DrugBank data, GNormPlus (Wei CH, 2015), and tmVar (H. B. Wei CH, Kao HY, Lu Z., 2013), respectively. Using a dictionary-based content search by the pubmed.mineR package, all the relevant population data was obtained.

3.2.3. Normalization

The annotated articles were then filtered using different criteria, with each entity were normalized to reduce false positives and ambiguity. Gene mentioning normalization was initially assessed with GNormPlus. The annotated genes retrieved were further matched to Human gene nomenclature committee (HGNC) gene names (Yates B, 2017). Unmatched entities were ruled out, based on abbreviated names, unconventional names, unspecified names, and other disambiguation. The baseline system implemented for disease normalization used dictionary lookup method using parent disease terms from International Classification of Diseases (ICD-10): version 2016 (Organisation., 2004). All the arbitrary terms referring to a symptom or any consequence of a disease/ syndrome were excluded, as they resulted in a high error percentage. Drugs were matched with DrugBank IDs for unique drugs (Wishart DS, 2008). In case any other names of the drug like chemical name, brand name or the drug metabolite, were consequently excluded. Finally, the genetic polymorphisms related to the selected genes were retrieved in the final sub-corpus and were matched according to their annotation in dbSNP IDs, for human (ftp site:ftp://ftp.ncbi.nlm.nih.gov/snp/organisms/human_9606/ genotype_by_gene/).

3.2.4. Validation

The validation of the retrieved PGx data from the pipeline was assessed at the entity level as well as based on the PGx relationships obtained.It was carried out independently in two steps. Firstly, all the biological entities obtained were validated for their presence in any of the three benchmark datasets. Secondly, the PGx relationships obtained were cross-validated with the gold standard, PharmGKB. To evaluate this, we compared the performance of the system to that obtained from OMIM, CTD, and PharmGKB and measured the concordance of

the data obtained. A circumstantial error analysis was carried out in terms of specificity, sensitivity, accuracy, precision, recall, and F measure. Test data was data extracted from the developed pipeline and gold standard was that of PharmGKB. A data was considered true positive (TP) data if it is present in gold standard and test data, both and true negative (TN) if data is absent in both gold standard and test dataset. False positive (FP) is absent in gold standard and present in test data, and false negative (FN) is the number of correct, incorrect, and missed associations extracted by the system in comparison with the gold standard, respectively.

3.2.5. Ranking of the PGx relationships

We estimated specificity, sensitivity, accuracy, precision, recall, and F measure for all our observations. Let TP, FP, and FN be the number of correct, incorrect, and missed associations extracted by the system in comparison with the gold standard, respectively. All relationships with a frequency greater than 10 ($f > 10$) that occurred only in our framework were appended directly to the end of the consolidated list. By doing so, the genes extracted from these datasets were assumed to be more relevant than those extracted from our pipeline. This is based on our observation that these datasets are manually curated and annotated with validated results with low noise, hence minimal FP genes. The gene names that overlapped between the developed pipeline and that of these datasets were of prime importance, and their ranks need to be aggregated. We simply raised their rank order for such genes based on the number of occurrence in these three datasets. Ultimately, 2,304 PGx relationships were obtained from our pipeline. These relationships were compared with commercially used FDA-approved drug labelling biomarkers. Of the 2304 markers obtained and validated, 127 were common with the already available 363 FDA-approved pharmacogenomic markers in current use. This marked the reliability of the outcome of our pipeline. In addition, the remaining PGx relationships suggest that although they are not included in PharmGKB, they are of prime clinically importance.

3.3. Results

After PGx related data extraction through pubmed.mineR and critically assessing intrinsic performances of our approach and rigorous error analysis at each step of entity detection, entities absent in text, failure to detect entities, entity normalization error we created a resource for PGx evaluation.

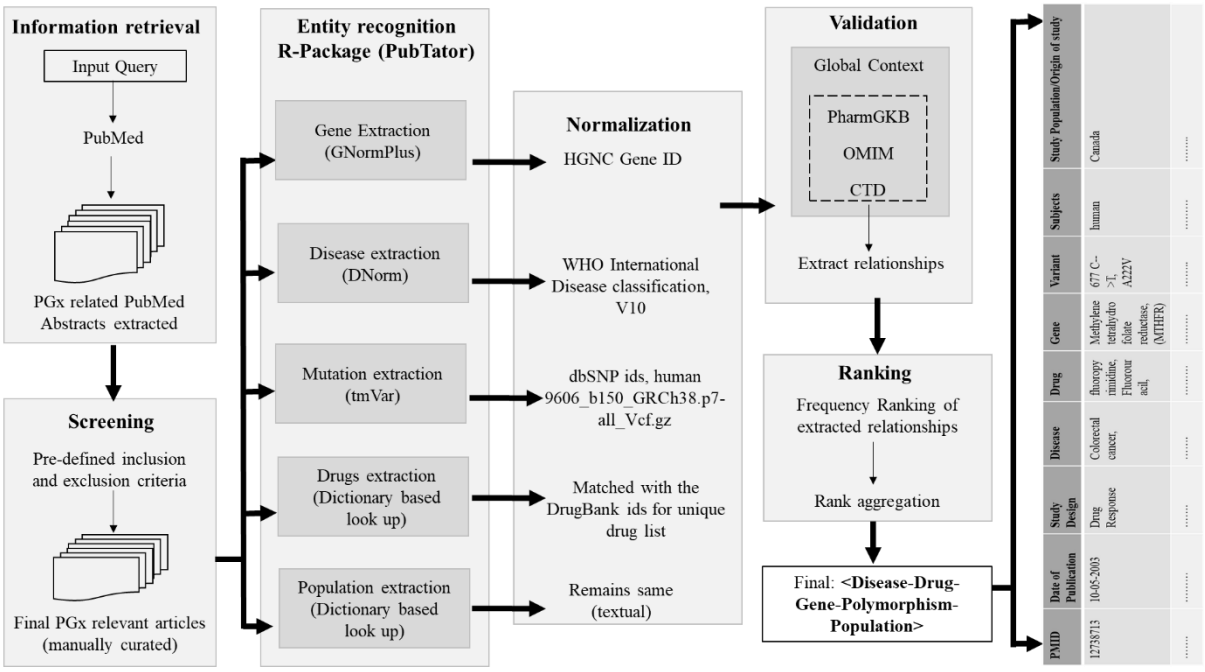


Figure 3.1: Overview of the semi-automated approach developed for PGx test mining. The process of retrieving evidence based sentences from PubMed abstracts using pubmed.mineR which includes information retrieval, entity recognition, normalization, validation, and data integration and ranking.

A total of 180,088 pharmacogenetically relevant articles were identified by our scanning method after stringent exclusion based on our pre-defined inclusion and exclusion criteria. The result were obtained as 2,304 PGx relationships as disease–drug–gene with their frequency of co-occurrences detected in those articles. An example of a few PGx markers with their diagnostic value and predictability has been tabulated in Table 1. This can be queried by disease or drug or gene, and it summarizes gene (or its variant)–drug relationships, categories of evidence, and supporting literature. Ultimately, **Figure 3.2** shows these additional PGx relationships pertaining to global PGx literature that are of promising candidates with clinical importance. The nodes in the left represent disease, the middle nodes the drugs, and the right nodes are genes pertaining the PGx relationship. Each colour of the edges represents a relationship (disease–drug and/or drug–gene) for distinct visualization. The width of the edges represents the number of evidence present. Thus, a thorough categorization with relevance in PGx applications are obtained from the source corpus. This growing resource needs to be tapped for clinical benefits, for drugs of pharmacogenetics significance, and is a core component of pharmacogenetic screening.

3.4. Discussion

New advancements were coming up from creating algorithms to developing computational packages to dig of the deeper details of DNA like associating variations of the genes with clinically relevant phenotypes-disease risk, therapeutic response, adverse effects of drugs (Hansen, Brunak et al. 2009). This study attempts to measure the capability of an R-package based semi-automated text mining system extracting database level annotations from PubMed abstracts for its precision in retrieval of clinically valuable information. Our evaluation of the proposed system pipeline against gold standard annotations extracted from curated database provides insight of the clinical applicability to lay efficient treatment guidelines.

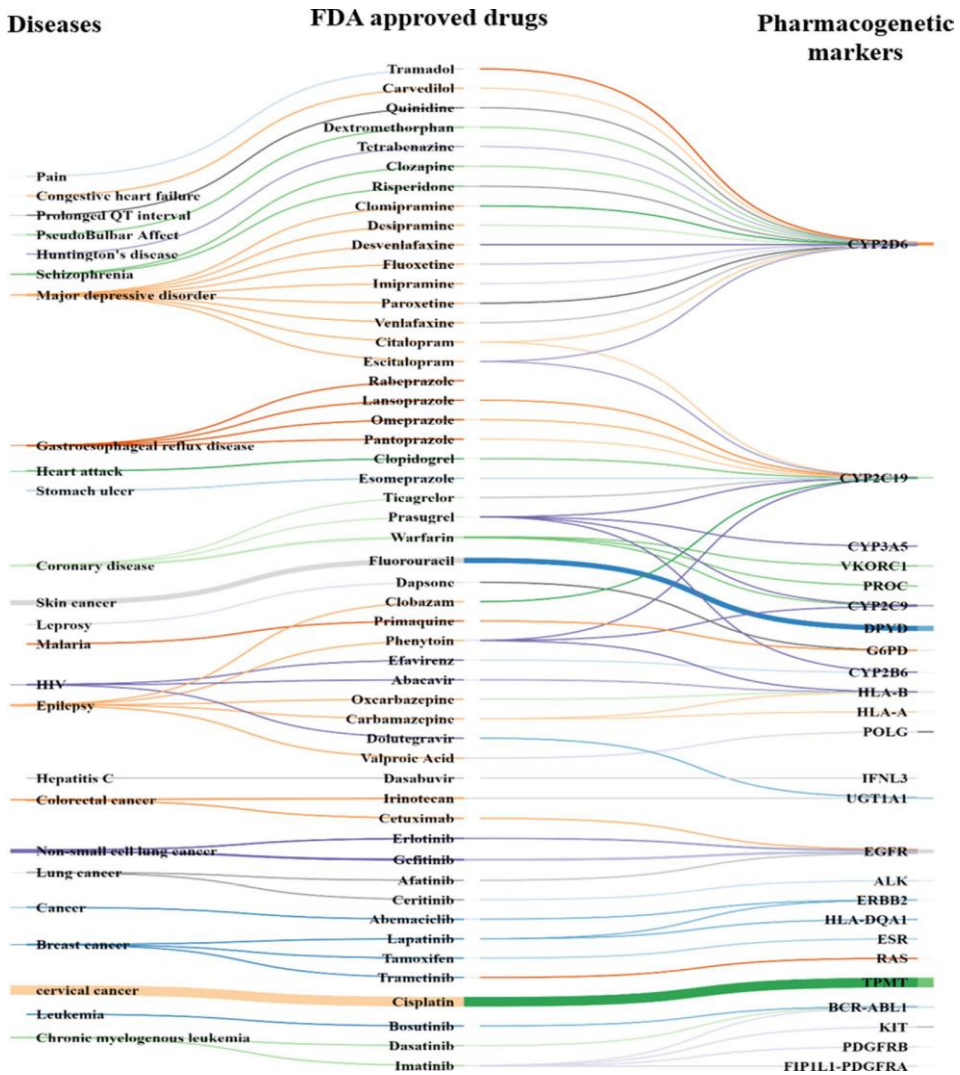


Figure 3.2: A glimpse of few PGx-specific enriched markers obtained from 2304 PGx relations. Disease ontology (left), FDA-approved drug (middle), and pharmacogenes (right) known (i.e., statistical association in clinical-genetics studies) to alter drug response or efficacy or lead to adverse drug response.

Table 3.1: Estimating performance precision of the obtained PGx relationships from our pipeline and comparing them with other benchmark datasets (OMIM, CTD and PharmGKB)

Context type	TP	TN	FP	FN	Sensitivity	Specificity	Efficacy	Precision	Recall	F-measure	Accuracy
Our pipeline with 'PharmGKB'	1509	208	254	78	82.6	86.9	88.2	0.904	0.930	0.923	89.1
Our pipeline with 'OMIM'	2225	-	79	-	78.0	77.5	81.8	0.600	0.681	0.764	59.3
Our pipeline with 'CTD'	1776	153	375	-	70.7	65.5	72.2	0.729	0.803	0.801	79.7
Our pipeline with ('PharmGKB' AND 'OMIM' AND 'CTD')	1875	102	275	75	82.3	84.4	93.3	0.896	0.852	0.828	94.7

PGx corpus from PharmGKB, OMIM and CTD compared to that of our pipeline and the articles extracted in these datasets.TP: True positive, TN: True negative, FP: False positive, FN: False negative

From text mining perspective, this is the first attempt to effectively combine information from multiple sentences to extract quaternary relations between disease, drugs, genes and polymorphism data in the global pharmacogenomic context. Our approach to link association across sentences using entity identity words resulted in substantial performance improvement. Achieving a performance of 89·1 % of overall accuracy when compared with PharmGKB, which when further revised to 94·7 % after detailed comparing it with all the three curated databases. This demonstrates that our approach to some extent addressed the linguistic inference challenge faced by the use of text mining for database curation (Ravikumar Wagholikar et al. 2015).

First of all, even though our algorithm has improved precision when compared with PharmGKB and also ranking of PGx relationships of drug–gene pairs , overall precision is still lower than the gold standard (Rubin, Thorn et al. 2005). Starting from a PGx-specific seed, the algorithm implicitly classifies sentences into PGx-related or non-related. However, if n drugs and m genes co-occur in any PGx article, the algorithm will automatically extracts all n and m possible drug–gene pairs. Extraction algorithm used was probabilistic and do not consider the syntactic relationships between drug entities and gene entities, independently in sentences. Our extraction pipeline cannot extract readymade PGx relationships from the literature, but it can find the sentences with PGx relevant information excluding the several non-specific associations. (Xu 839 and Wang 2013). Therefore, the emphasis on semi-automated approach has been established to construct the exact and complete knowledge resource of PGx specific disease-drug–gene associations from published literature.

Secondly, the results presented in the previous section show the effectiveness of the proposed approach by performance comparison when extracted using our semi-automated method and OMIM. CTD and PharmGKB for extracting correct data string forming disease-drug-gene-mutations. Therefore, we demonstrate an independent comprehensive resource to curate important disease-related PGx relationships. We also analyse our drug-gene pair predictions for known errors and false negatives. Known errors are PGx relationships obtained from our approach but are unrelated or non-specific to the disease, however they are presently associated in any of the 3 datasets. Such cases could be avoided by increasing the weight of target drugs, administered in a particular disease type/category, frequency in comparison to other disease mentions. In a few cases, the documents were not directly related to the target disease. This can be improved by more comprehensive extraction of documents related to the target disease. In other situations, DNorm (Leaman, Islamaj Dogan et al. 2013) tool identifies a non-disease mention as a disease, and the feature set is disturbed due to close proximity of the mutation with the false disease identification. These errors occur due to ambiguous abbreviations or non-standard notations mentioned by authors that resemble biological entity names.

Third, the entire PGx relationship extraction algorithm started with 1753 disease types, 666 drugs, associated with 4132 genes and 33942 polymorphisms collated from 180088 publications filtered out from 60 lakh abstracts. Ultimately, we can rank the 2304 human PGx relationships according to their PGx specificity, we can further improve the precision of the relationship extraction algorithm. We validated comparing the results of our approach with commercially used FDA approved drug labelling biomarkers. Of the 228 FDA approved pharmacogenomic markers, 127 were common with the 2304 markers obtained from our proposed approach. This observation is taken forward for clinical development with therapeutic applications aiming for targeted therapy or drug repurposing. With the keen interest of researchers and clinicians on patient care, like developing new diagnostics techniques, prognostics, prevention strategies/guidelines, and therapies based on pharmacogenomic discoveries for precision medicine. The emergence of translational bioinformatics spans into development of algorithms and computational tools to derive the actual basis of molecular and cellular data with an explicit goal of affecting clinical care. This promise of translational clinical medicine, is progressing with the vision of genome guided medicine (Hauser, Chavali et al.2018).

Chapter 4

Identification of the PGx markers in north Indian population to assess their correlation with anti-epileptic drug response using high throughput genome-wide screening

4.1 Introduction

The predominant mode of treatment in case of all epilepsies are anti-epileptic drug (AED) administration, among individuals with different seizure types, epilepsy syndromes or any other clinical manifestations. Approximately 60% of patients with epilepsy achieve seizure remission, after administration of the first AED at an optimum dose, while 20–30% have a chronic disorder without experiencing significant periods of remission [Annegers, J.F., Hauser, W.A. and Elverback, L.R. (1979) Remission of seizures and relapse in patients with epilepsy. Epilepsia, 20, 729–737.]. Thus, seizure remission is the key concern in epilepsy treatment, minimizing the risk of death and improving quality of life in PWE. While, recent studies have highlighted the role of common SNP variants [1] as well as the enrichment of rare deleterious missense variants in known epilepsy genes in treatment outcomes in epilepsy, very little is known about genetic influences on the prognosis of epilepsy, and to date, genetic effects on epilepsy prognosis are unexplored at the genome-wide level.

Previous genome-wide investigations of AED response associated with ADR, for AED response status, pharmacogenetic findings remain scarce and controversial. Pharmacogenetic findings in childhood absence epilepsy (CAE) showed an association of common variants in the ABCB1 drug transporter as well as in CACNA1H and CACNA1I, subunits of T-type calcium channels, with responder status for the drugs ethosuximide and lamotrigine (LTG) [22]. Genes involved in drug absorption, distribution, metabolism and excretion (ADME) have been in the focus of pharmacogenetic research of AEDs for some time [23–25]. Influence of variants in genes-encoding drug transporters have been shown to influence pharmacokinetic parameters of LTG or VPA [26–28]. Therefore, ADME genes represent prospective locations of genome-wide association.

This study aimed to test whether common genetic variants predict drug response to carbamazepine, phenytoin and valproic acid or overall drug response in a cohort of 789 people with epilepsy that were deeply phenotype regarding clinical presentation and pharmacoresponse. Larger cohorts are vital for increasing sensitivity to detect new genetic associations of small effect.

4.2 Materials and Methods

4.2.1 Ethics Statement:

All the study participants provided written informed consent for inclusion in this research work. The local institutional review boards reviewed and approved the study protocols at each contributing site.

4.2.2. Study population and phenotype definition:

Patients with epilepsy (PWE) were recruited from Outpatient department of Neurology, Institute of Human Behaviour and Allied Sciences (IHBAS), New Delhi, India between 2005 and 2015 after obtaining a complete informed consent (**Figure 4.1**). The sample has previously been described in detail (Rawat *et al.*, 2018) and genetic profile of a subset of this cohort has been previously described in our other papers (Grover *et al.*, 2010; Talwar P, 2017). All the patients were diagnosed and prescribed treatment by an experienced neurologist, based on the latest guidelines of the International league against Epilepsy (ILAE) (Scheffer IE, 2017). Our cohort consisted exclusively of individuals of North Indian origin. All the patients were administered with widely prescribed conventional AEDs [PB, PHT, CBZ, VPA and LEV] and were prescribed for oral administration at a dose within the therapeutic range, that is, 150–1200 mg/day for PHT, 800–1600 mg/day for CBZ, 250–3000 mg/day for VPA, and 30–200 mg/day for PB, 500-1000mg/day. The study was approved by the institutional biomedical research ethics committee. The demographic and seizure-type information was collected by administering a standardized questionnaire including drug-dose information, and other investigations such as the EEG profiling and neuroimaging. Subsequent follow up at 2nd, 4th, 8th, and 12th month within a course of 1 year were evaluated for the drugs being administered and their dosage, the serum drug levels, the frequency of seizure control, the ADRs, and their compliance to AEDs. Patients who did not comply with the treatment regimen or who switched to a second line AED or multi-therapy (MT) in between the course of the study were included in the study but their genetic profile were not analysed. After a year of enrolment in the study, patients were assessed based on their response to the treatment regimen. Patients were categorised into ‘good responder’ including patients who attained complete freedom from seizures during the past 1 year and ‘poor responder’ included those patients who experienced ≥ 1 seizure/s during the same period.

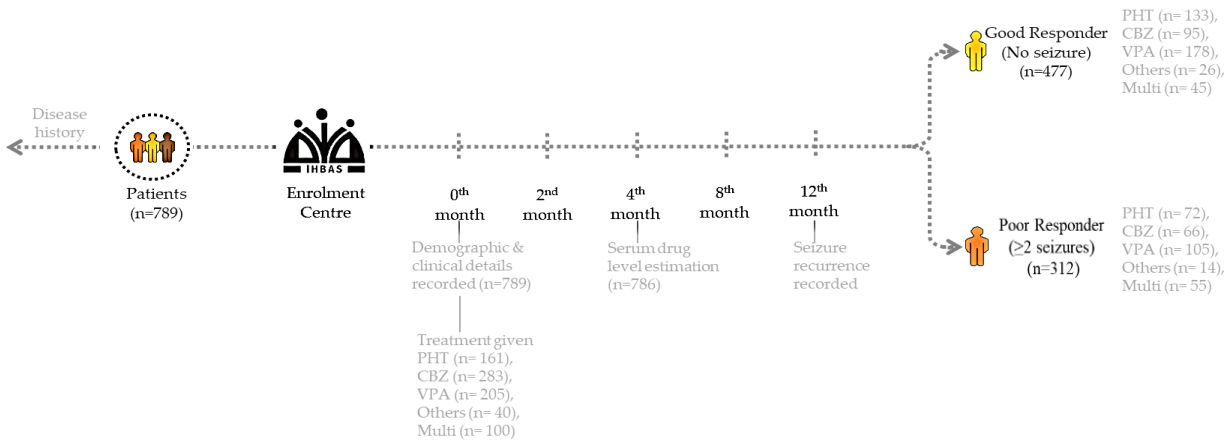


Figure 4.1: Schematic representation of patient enrolment and follow-up at the Outpatient Department of Neurology, Institute of Human Behaviour and Allied Sciences (IHBAS).

Such classification were made based on the number of seizure over the study duration, excluding the initial two months period required for achieving steady-state levels of the AEDs being administered. Based on serum AED levels assessed (discussed in our previous report), patients were classified into therapeutic, below and above therapeutic. The distribution of total enrolled PWE (n=789) during 2005-2015 and their demographic and clinical characteristics are represented in **table 4.1**.

Table 4.1: Demographic and clinical characteristics of total enrolled patients with epilepsy (PWE)

Demographic & clinical characteristics	PHT	CBZ	VPA	PB	LEV	MT	Total	p value
Total PWE (n %)	161 (20.4)	283 (35.9)	205 (26.0)	37 (4.7)	3 (0.4)	100 (12.7)	789 (100)	-
Sex								
Male, n (%)	132 (16.7)	116 (14.7)	138 (17.5)	21 (2.7)	0	68 (8.6)	475 (60.2)	**<0.0001** (b)
Female, n (%)	29 (4.9)	167 (21.2)	67 (8.5)	16 (2.0)	3 (0.4)	32 (4.1)	314 (39.8)	
Age (in year)								
Mean ± SD	22.4±9.5	21.2±8.7	21.3±8.4	25.9±12.1	22.7±8.1	25.1±9.6	22.2±9.2	**<0.001**(a)
Range	5-55	7-56	5-59	12-62	10-46	5-62	18-32	
Median	21	18	20	21	22	20	18	
Age at onset (in year)								
Mean ± SD	16±10.0	15.3±8.3	15.4±8.2	16.5±11.5	8.3±2.5	15.4±9.2	15.5±8.9	0.40 (a)
Range	0.08-54	0.25-48	1-56	4-52	6-11	0-45	0-56	
Median	15	14.25	14	13	9	14	14	
Seizure type, 789 (100%)								
Focal/ partial, n (%)	57 (7.2)	144 (18.3)	48 (6.1)	7 (0.9)	0	46 (5.8)	302 (38.3)	**0.03** (b)
Generalised, n (%)	98 (12.4)	131 (16.6)	153 (19.4)	30 (3.8)	3 (0.4)	48 (6.1)	463 (58.7)	
Mixed and others, n (%)	6 (0.7)	8 (1.0)	5 (0.6)	0	0	5 (0.6)	24 (30.0)	
Epilepsy type, 503 (63.8%)								
Idiopathic	18 (2.3)	24 (3.0)	69 (8.8)	4 (0.5)	3 (0.9)	20 (2.5)	138 (17.5)	0.50(b)
Symptomatic	37 (4.7)	101 (12.8)	46 (5.8)	8 (1.0)	0	41 (5.2)	233 (29.5)	
Crytogenic	33 (4.2)	53 (6.7)	24 (3.0)	5 (0.3)	0	17 (2.2)	132 (16.7)	
Drug response, 789 (100%)								

No seizure (GR)	94(12)	177(22.4)	134(16.9)	24(3.0)	2 (0.3)	45 (5.8)	476 (60.5)	0.61
Recurrent seizure (PR)	67(18.3)	106 (13.4)	72(9.1)	13(1.6)	1 (0.1)	54 (6.8)	313 (39.5)	
Male, 475 (60.2%)								
No seizure (GR)	74(56.1)	73 (62.9)	94(67.6)	15(62.5)	-	30(44.8)	286(60.2)	0.53
Recurrent seizure (PR)	58(43.9)	43(37.1)	45(32.4)	6(25)	-	37(55.2)	189(39.8)	
Female, 314 (39.8%)								
No seizure (GR)	20(69.0)	104(62.3)	40(59.7)	9 (56.3)	2 (66.7)	15(46.9)	190 (60.5)	0.77
Recurrent seizure (PR)	9 (31.0)	63(37.7)	27(40.3)	7(43.7)	1(33.3)	17(53.1)	124(39.5)	
ADR, n (%)	13(1.6)	15(1.9)	23(2.9)	1(0.1)	-	7(0.9)	59(7.5)	-

(a) One-way Anova test; (b) Chi-square test. n: Number of individuals; SD: Standard Deviation. PHT: Phenytoin; CBZ: Carbamazepine; VPA: Valproic Acid; PB: Phenobarbital; LEV: Levetiracetam; MT: Multitherapy. $p<0.05$ [values in bold font]; PWE = People with epilepsy, GR= Good responder; PR = Poor Responder.

4.2.3. Genome-wide genotyping:

We tested whether common genetic variants were significantly associated with drug response to one of the prescribed AEDs. Genomic DNA was isolated from blood samples using a modified protocol of the salting out technique. DNA concentrations were measure using Infinite 200 Pro NanoQuant plate reader™ (Tecan). Genome-wide genotyping was performed for 805379 variants using Illumina Infinium Global Screening Array-24+ v2.0 Kit (multi disease array with psych array- 24 v1-1 customisation) and the chip-wise fluorescent intensity was measured using Illumina iScan™ system. Genotypes were called from the array intensity data, using a custom genotype-calling pipeline using the Illumina proprietary software Genome Studio v2.0.

4.2.4. Genome-wide quality control procedures:

Illumina Genome Studio v2.0 was used to call genotypes, normalize signal intensity data and establish the norm R and norm theta values to cluster A and B allele frequency at every SNP. Quality control filtering of genome-wide genotype data was performed using PLINK v1.9 (Purcell S, 2007) and R v3.4.2. We initially removed all SNPs with a call rate <90%, accounting for the genotyping platform used. We initially performed SNP level QC in Genome Studio2.0 by manually adjusting SNPs that deviated from haploid genome, have GenTrain score <0.6, cluster separation <0.4, and all the SNPs with call frequency 'zero' were excluded. Other QC measures based on AA, AB and BB frequency calls were manually adjusted and finally the samples were again filtered based on call rate >0.95 and .ped/.map files were created with the

remaining sample and SNPs. In PLINK 1.9, the DNA strands calls were reversed as required. Array-specific maps retrieved at the website of Will Rayner at the Wellcome Trust (http://www.well.ox.ac.uk/~wrayner/strand/) were used to update all SNPs positions and chromosome numbers to the Genome Reference consortium human build 37 (GRCh37), and remove all A/T and C/G SNPs to avoid strand issues. We then performed sample-level and SNP-level QC based on the following criteria: (1) we checked for gender mismatch, (2) we removed samples based on race mismatch calculated using a principal component analysis (PCA) plot (**figure 4.2**), (3) a subset of markers independent of each other with respect to LD was created using a window size of 100 markers shifting by 25 markers at a time and removing one half of every SNP pair with genotypic $r^2 > 0.1$ (4) Using this subset of markers, heterozygosity (HET) and (5) identity by state (IBS) was calculated in order to remove all samples with outlying HET values (±4 standard deviations from the median of the whole sample) and one half of all sample pairs with lower call rate (4) an HWE cut-off threshold of $p < 1 \times 10^{-5}$ per marker was applied. Post QC PLINK binary files (.bed, .bim, .fam) containing the sample and marker details were generated for further analysis. We removed the samples and SNPs using MAF filter (>0.01), genotype frequency in SNPs (--geno) and genotype frequency in samples (--mind).

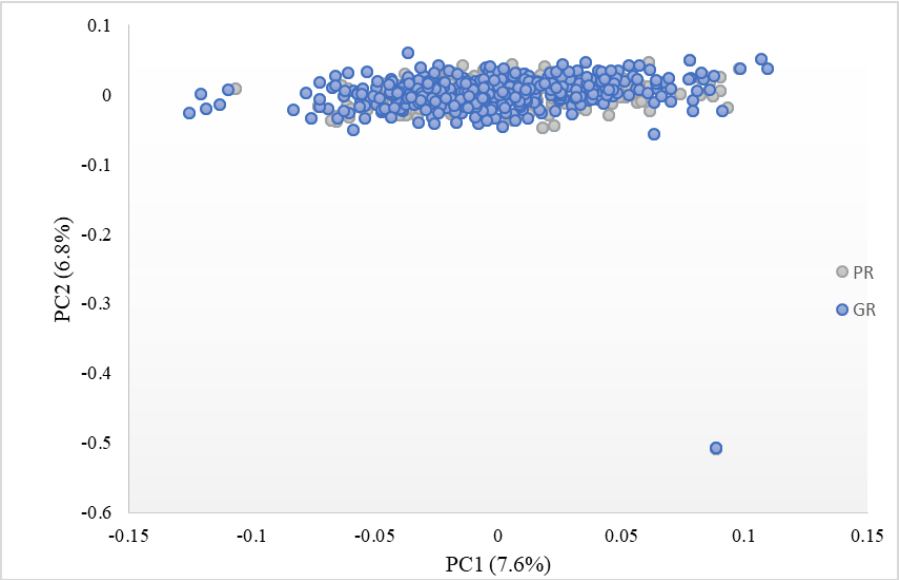


Figure 4.2: The principal component analysis plot of the 746 GWAS samples. The Y and X axes are the first and second dimensions from principal component analysis based on the genome-wide IBS pairwise distances among the 746 GWAS subjects. Grey points represent good response and blue points poor response to AED. The two axes correspond to a reduced representation of 3000 ancestry informative markers (AIMs) SNPs into two dimensions. No clustering pattern was found, indicating that neither substantial population stratification nor cryptic relationship among the 746 subjects was found.

4.2.5. Trans-ancestry genetic correlation analysis:

We estimated the trans-ethnic genetic correlation between North Indian and 1000Genome SAS (**figure 4.3**). We downloaded the vcf file for 1000Genome phase3 data from http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/. All the autosomes and bi-allelic data was retained. This data was converted into PLINK binary files and this data (2504samples) was merged with North Indian (1821samples) using the PLINK command --merge list command. The rare variants were removed using –maf 0.05 and genotype frequency of 0.1 was applied. This analysis was performed on non-imputed North Indian data. On obtaining the .eigenvec and .eigenval data from PLINK, the PCA was plotted in Excel 2013 using PC1 and PC2.

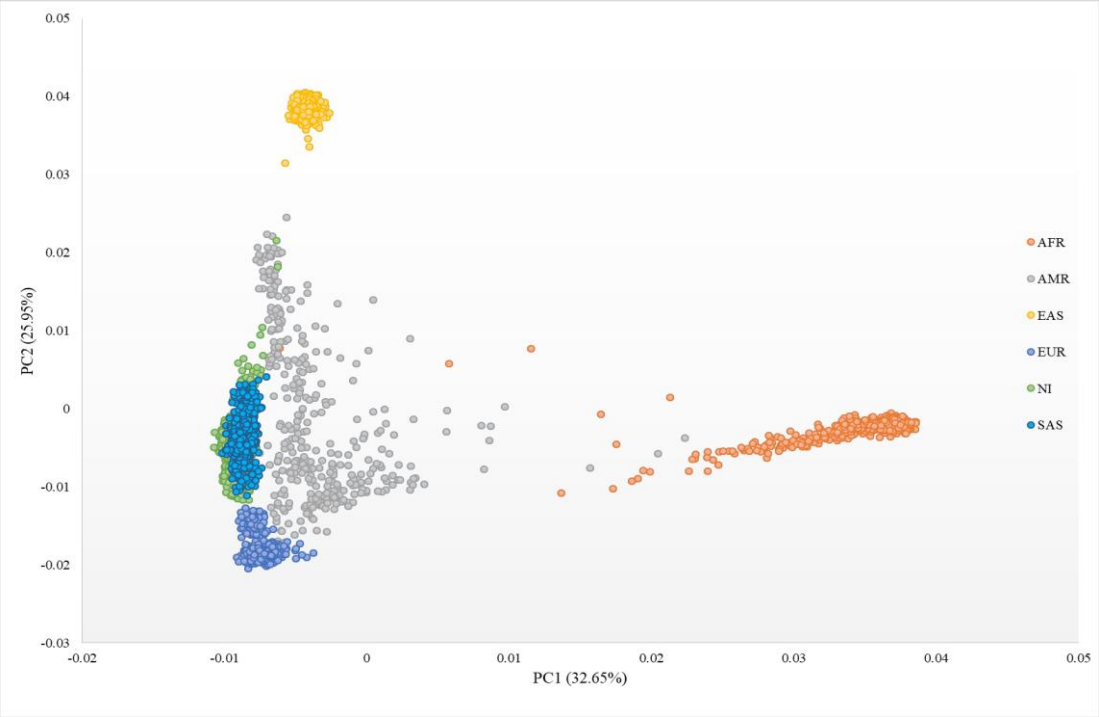


Figure 4.3: PCA comparison to all 1000 Genomes Project super-populations (n case=2504) with our cohort (n case= 1281), unrelated genotyped individuals merged across cohorts and with 1000 Genomes Project[69] population reference samples (AFR: African; AMR: Admixed American; EAS: East Asian; EUR: European, SAS: South Asian). Our samples (NI: North Indian) show complete overlap with 1000G SAS population. Results confirm that the NI and SAS cohorts are consistent with the expected population ancestries.

4.2.6. Genome-wide imputation:

The post QC binary files were split up according to chromosomes for all autosomes and haploid chromosomes (X, Y and mitochondrial). We applied pre-imputation checks according to the scripts available on conform-gt Beagle platform. We used the Michigan imputation server

to perform genome-wide imputation, where we used Eagle 2.4 to pre-phase genotype to produce best guess haplotypes and then perform imputation using minimac4 algorithm and the 1000Genomes project phase 3 integrated variant set 5 (GrCh37/hg19) SAS population as reference panel. All the chromosome-wise .dose files were merged into .pgen files in PLINK 2.0. Post-imputation quality control filters were applied to remove imputed variants with imputation score <0.8, a minimum MAF ≤ 0.01 and without significant ($p > 1 \times 10^{-5}$) deviation from HWE were used in the final analysis, which included 2767974 autosome variants. The detailed inclusion of samples per analysis is described in **table 4.2**.

Table 4.2 Number of samples and SNPs included in each step of the GWAS analysis.

Step	Number of participants	Number of SNPs
Total sample recruited	789	805379
Removing samples based on DNA quality	746	805379
Removing independent samples based on call rate (Call rate<0.90)	716	805379
Removing non autosomal variants	716	799003
Remove SNPs based on SNPs calling quality (GenTrain score <0.7)	716	794052
Cluster separation (<0.50)	716	690260
Removing independent samples based on call rate (Call rate<0.90) and variants based on (Call frequency <0.80)	713	717204
Removing SNPs with strand ambiguous A/T, C/G, gender mismatch, race mismatch	713	717204
LD pruning (50-5-0.5)	713	399189
Exclude related samples & duplicates (relatedness (pi-hat>0.25))	697	399189
HWE ($P>1x10^{-5}$)	697	398177
Check heterozygosity/homozygosity (for sample mix-up/contamination/inbreeding) (Hetrozygosity Mean ± 4SD)	691	398177
Remove SNPs with Missingness > 20%	691	398031
Imputation with imputation score ($R^2>0.3$)	691	14938481
Remove SNPs with $R^2<0.8$	691	2767979
Post imputation QC : Genotype frequency >0.2, mind >0.2, MAF >0.01, HWE outliers ($P>1x10^{-5}$)	691	2197401

4.2.7. Study power

We estimated the study power using Genetic Association study (GAS) power calculator (https://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/), an online tool presented by the University of Michigan. Considering an additive model of genetic association, with a

prevalence of 0.10 for our phenotype, our study had 81.3% power to detect a marker with allele frequency >20% and an alpha level of $5x10^{-7}$ with relative risks (approximated to odds ratio) ≥2 for overall poor response to AED.

4.2.8. Association analysis/ Statistical analysis:

The detailed pipeline opted for the genome-wide analysis is as represented in **figure 4.4.** We conducted additive logistic regression using age, sex and 2 principal components (PC1, PC2) as covariates in PLINK 1.9 assuming an additive model and evaluated the association of each imputed SNP. The p value 5×10^{-8} or 10^{-5} were considered significant or suggestive, respectively. Likewise, we performed logistic regression using the same covariates to evaluate the association of each SNP for each drug type, Phenytoin, Carbamazepine, and Valproic acid. Given the exploratory approach of this GWAS analysis, we did not perform multiple testing for AED response traits- accepting a slightly higher false positive rate in order to present a comprehensive list of candidate loci for each AED response traits. Manhattan and quantile-quantile plots were created using R package qqman. Genomic inflation factors were calculated using. Regional plots were created using LocusZoom webtool (http://locuszoom.org/) based on the GRCh37/ 1000Genomes SAS reference data.

4.2.9. Gene mapping and biological prioritization:

To test whether genes involved in pharmacokinetic or pharmacodynamic of AEDs, were associated as a group with pharmaco-response, we created a set of 256 genes (as mentioned in our previous paper (Guin D, 2019b). We applied MAGMA v1.04 using entire summary statistics for each group and GWAS p values to run the gene-set and gene-level analysis.

4.2.10. Functional annotation of SNPs:

Genomic loci were defined from the SNP-based association results. Functional annotation was carried out in FUMA. Annotations were done for SNP-based and gene-based analysis, with annotations including the ANNOVAR categories, CADD scores, RegulomeDB scores, and 15-core chromatin states and tissue specific eQTL evidence from GTEx v8.0 database. Functionally annotated SNPs were mapped to genes based on the physical position in the genome (FUMA positional mapping), resulting in 25 mapped genes at 12 of the 16 associated loci.

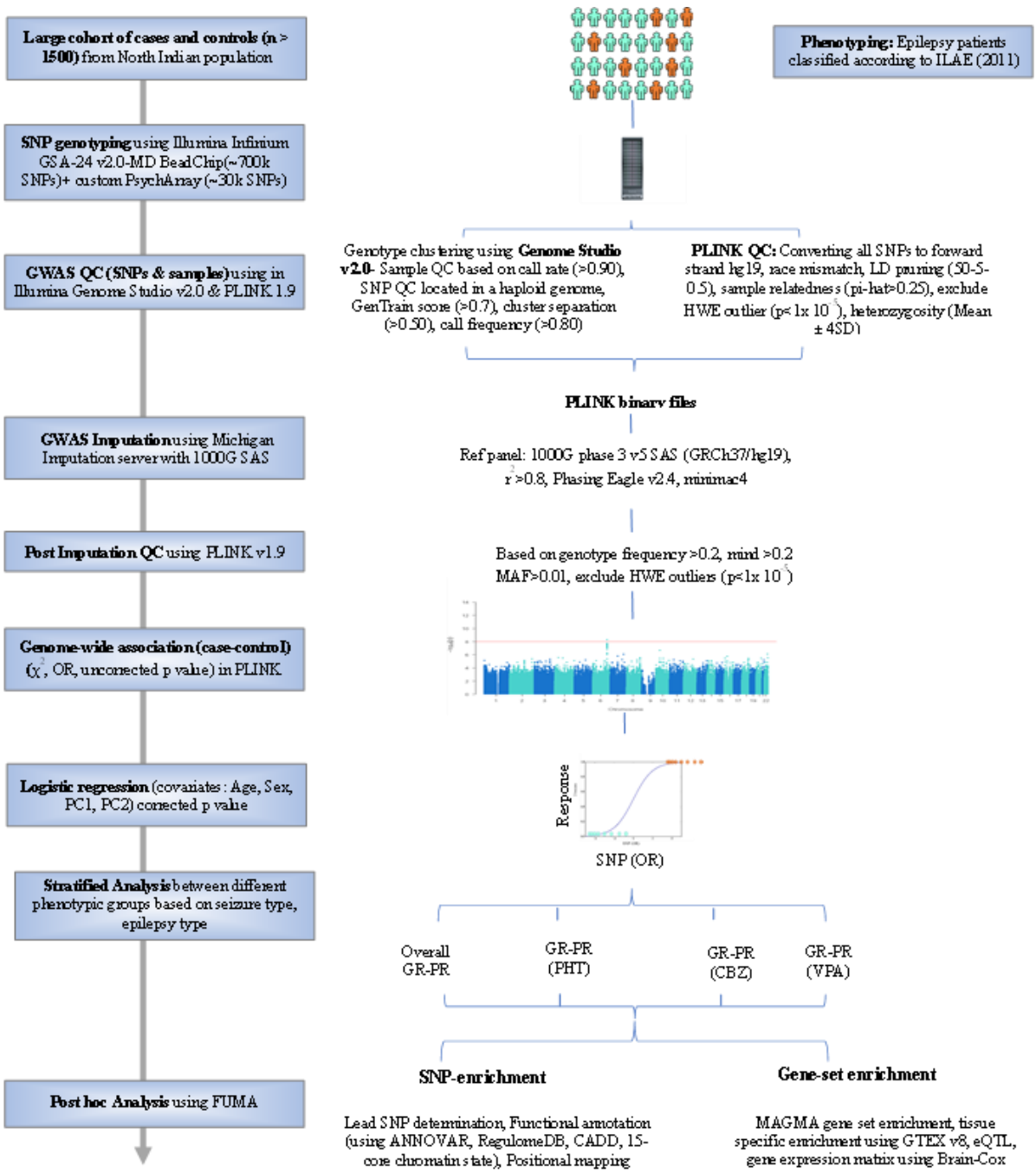


Figure 4.4: The detailed workflow for the GWAS analysis pipeline opted in our study

4.3 Results

4.3.1. Cohort description:

The full dataset included 477 (286 males, 191 females) participants given AED treatment with no seizure recurrence in past l0 months (cases) and 312 (189 males, 123 females) participants with one or more seizures in 10 months after drug administration (controls).

Participants were aged 5 years or over (mean ages of good and poor responders were 22.2 years (S.D. 9.1) and 22.2 years (S.D. 9.4), respectively). After individual level QC, 746 patients were included in the GWAS analysis. The GWAS analysis was majorly divided into four groups, (1) comparing overall poor responders with good responders, (2) poor vs good response in PWE on phenytoin therapy alone, (3) poor vs good response in PWE on carbamazepine therapy alone, (4) poor vs good response in PWE on valproic acid therapy alone. The breakdown at each level of QC for overall group is shown in **figure 4.4**. Comparing the ratio of good to poor responder for different groups, we saw more good responders than poor. Regarding the seizure frequency before treatment with respective AED, we saw PHT and CBZ, a higher frequency of seizure other than generalised tonic clonic seizures (GTCS) in poor responders as compared to good responders. We did not observe this effect for VPA.

4.3.2 Genome-wide association study analysis

The sample cohort and demographics for each phenotype definition are detailed in **table 4.1**. After quality control, **table 4.2** tabulates the details of exclusion of samples and SNPs in each step), 2174963 variants remained for analysis. The detailed pipeline for the GWAS analysis is given in **figure 4.4**. We only considered autosomal SNPs in our analyses. The variance between the PCs were calculated using the .eigenval file. The PCA plot of PC1 versus PC2 is shown in **figure 4.3** with study subjects (in green) overlaid on 1000G SAS ancestral populations, thus we chose 1000G SAS to be the reference population for imputation analysis. To test for hypothesis for poor response to AEDs, logistic regression analyses were performed for four groups, overall poor vs good responder and between these for PWE on PHT, CBZ and VPA. Although we did not observe any GWAS significant ($p < 5.0 \times 10^{-8}$) loci for any of the groups, several crossed the suggestive loci. The strongest association was found in VPA response group for rs4659128, an intronic SNP in TBX15 gene ($p= 2.03 \times 10^{-7}$). Most of the lead loci were either intergenic or intronic which the nearest gene mapped to 1Mb region according to the NCBI RefSeq, represented in Manhattan plots. We obtained 4 SNPs in the overall group, 1, 5 and 1 with PHT, CBZ and VPA poor response, respectively.

The gene-set analysis was performed for each group, using MAGMA on the significant association ($p < 1x\ 10^{-5}$) for 54 tissue specific expression and gene function was identified using gene ontology (GO) analysis for biological processes, molecular functions and cellular components. These genes were prioritised based on a p-value cut-off of 5.0×10^{-3} after false discovery rate (FDR) correction. Other biological evidence for genotype specific tissue-wise

expression was obtained from GTEx v8 and 15-core chromatin state for regulatory potential of non-coding variants. All the results are presented with unadjusted p value cut-off of 1×10^{-3}. The detailed results for each group are discussed in the following sections.

Among the lead loci several represented genes involved in target or transporter for different AEDs administered. Other loci have known functions in neuronal development or associated with neurodevelopmental disorders: CBLN2 and FBXO33 for the overall response, C12orf42 or SLC9A9 for PHT response, TBX15, and SLC25A24 for CBZ response, and PACRG and SYN3 to VPA response. The regional genomic plots of the top results for all GWASs are depicted in locus zoom plots in **figure 4.6**.

4.3.2.1. Overall Good Responder vs Poor Responder:

In the GWAS for overall response (420 Good responders, GR vs 272 Poor responders, PR), no variant passed genome-wide significance. We observed no evidence for a substantial GWAS p value inflation (λ_{median} =1.00745) (**Figure 4.5**). There were 5 SNPs that crossed the suggestive p value threshold. Four of them are intergenic and one intronic. The top genomic locus (lead SNP rs6033642, $p = 1.185 \times 10^{-6}$, OR (95%CI) = 1.98(1.51-2.61) is an intergenic variant lying near *SPTLC3* and *ISM1* gene (**Table 4.3**). This SNP is an eQTL variant –down-regulating the expression of *SPTLC3* gene in different tissues, most significant at Brain Caudate basal ganglia tissue ($p<0.0003$). This SNP is also known to be at weak transcription site (Tx/Wk) in liver, blood and breast. Another SNP, rs71330293, in complete LD ($r^2>0.9$) with the lead SNP lies in the active transcription start site (TSS) in tissues like Blood (all tissues), Brain (Angular Gyrate, Inferior Temporal Lobe, Substantia nigra), Oesophagus, Duodenum, Stomach. The most significant pathway in gene-set analysis of this group is the biological process regulation of potassium ion transmembrane transport (fold enrichment=15, $p<10 \times 10^{-3}$), followed by neuron development (fold enrichment = 7, $p<0.0001$), calcium ion transport (fold enrichment=5, $p<0.00001$). The gene within the suggestive GWAS threshold, shows exclusive differential expression in brain. There are other 14 loci that cross the suggestive p value threshold. Three of them are intronic and rest intergenic and are mapped to protein coding genes like *FBXO33, CBLN2, SORBS1, ERGIC2, ERO1B, ZMIZ1-AS1, FGF12, PLPP3, CDH2, FERMT1,* and *CASC20*. These genes were differentially expressed in most brain tissues.

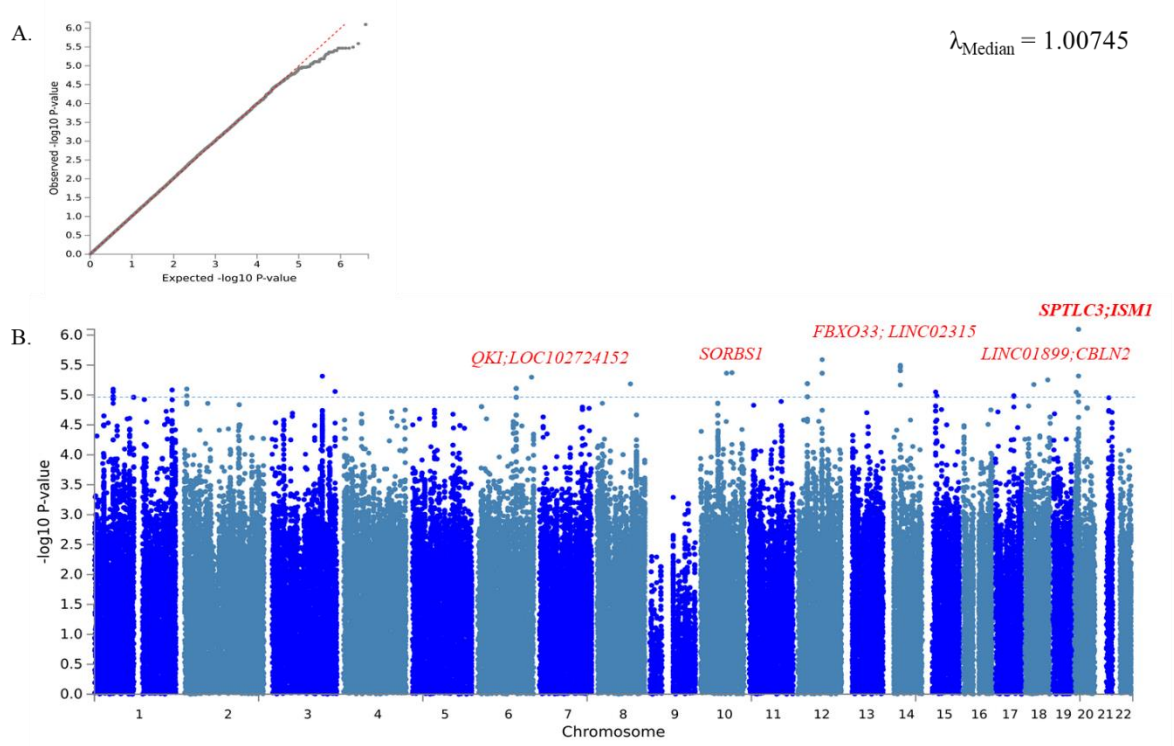


Figure 4.5: Manhattan and QQ plots of genome-wide association analyses for overall AED response. Genomic inflation factors (λ) for the four genome-wide association study analyses. Negative $\log_{10}$ transformed p-values (Y axis) are plotted against chromosomal positions (x-axis).The line represent the suggestive genome-wide significance threshold ($p< 1x\ 10^{-5}$).

4.3.2.2. Good Responder vs Poor Responder on patient with Phenytoin:

In this group, 72 PR and 133 GR were compared were no variants passed GWAS threshold, with no genomic inflation (λ_{median} =1.06036) (**Figure 4.6)**. Only one SNP crossed the suggestive threshold, rs10134329, [$p = 1.004 \times 10^{-5}$, OR (95% CI) = 9.34 (3.48-25.1)] which is an intronic variant in *TTYH3* gene. The Tweety homologs (*TTYH*) gene family are known to form Ca^{2+} and regulating anion channels with potential role in cell adhesion, migration, and developmental signalling (Li B, 2021). According to BRAINEAC database, this SNP show differential expression in brain tissues (cerebellar cortex, occipital cortex, putamen, substantia nigra and intralocular white matter), which is highly correlated with the expression of CARD11 gene (p=0.003) in substantia nigra. This variant is predicted to be present in the strong transcription site (Tx) in most of the tissues. The tissue expression analysis performed by MAGMA for 53 tissue types, supports highest differential expression in brain cerebellar hemisphere and cerebellum. The gene-set enrichment analysis revealed neuron recognition (fold enrichment =45, p< 0.001), followed by cyclic adenosine monophosphate (cAMP) catabolic process (fold enrichment = 42, p<0.001) and its activity to be most significant in this

group. There are other 11 loci that cross the suggestive p value threshold. Three of them are intronic and rest intergenic and are mapped to protein coding genes like *FOXG1-AS1, C12orf42, MIR6078, ANKRD22, STAMBPL1, ARHGEF37, PPARGC1B, SLC9A9, LUZP2A.*

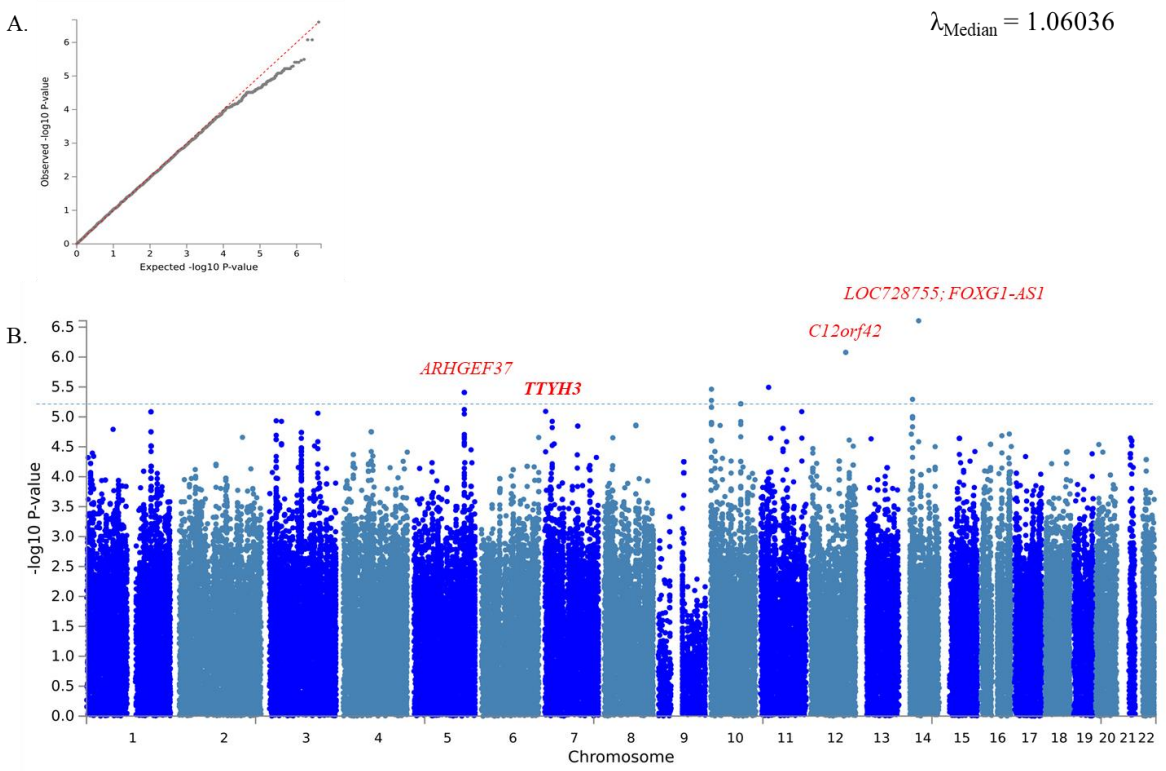


Figure 4.6: Manhattan and QQ plots of genome-wide association analyses for poor response to PHT. Genomic inflation factors (λ) for the four genome-wide association study analyses. Negative $\log_{10}$ transformed p-values (Y axis) are plotted against chromosomal positions (x-axis).The line represent the suggestive genome-wide significance threshold ($p< 1x\ 10^{-5}$).

Table 4.3: Top genome-wide associated loci crossing suggestive threshold ($p < 5 \times 10^{-5}$) for overall AED poor responder

rsID	chr	pos	Non effect allele	Effect allele	MAF	GWAS p	OR (95%CI)	Function	CADD	RDB	Adjusted p	Nearby Genes
rs6033642	20	13199755	G	C	0.2076	8×10^{-7}	1.98(1.50-2.60)	intergenic	11.04	5	1.19×10^{-6}	*SPTLC3;ISM1*
rs970635	14	40123072	T	C	0.3088	3.19×10^{-6}	1.73(1.37-2.19)	intergenic	0.346	6	3.98×10^{-6}	*FBXO33;LINC02315*
rs4891466	18	70017821	T	C	0.2628	5.60×10^{-6}	1.79(1.39-2.30)	intergenic	1.115	NA	4.07×10^{-6}	*LINC01899;CBLN2*
rs76487654	10	97262755	A	AACAC	0.2955	4.24×10^{-6}	1.74(1.37-2.2)	intronic	0.86	NA	4.83×10^{-6}	*SORBS1*
rs1037515723	6	164059731	C	CTT	0.4724	5.05×10^{-6}	1.66(1.34-2.07)	intergenic	0.033	NA	4.94×10^{-6}	*QKI; LOC102724152*
rs1836861	12	29536060	T	C	0.2587	6.47×10^{-6}	0.57(0.45-0.73)	intergenic	5.415	NA	1.10×10^{-5}	*ERGIC2; OVCH1-AS1*
rs6809135	3	153123142	T	C	0.4622	4.85×10^{-6}	1.66(1.33-2.06)	ncRNA intronic	1.12	6	1.28×10^{-5}	*LINC02006*
rs1284129705	12	74562414	G	GA	0.2965	2.57×10^{-6}	0.53(0.41-0.70)	ncRNA intronic	1.35	NA	1.47×10^{-5}	*LOC100507377*
rs61774288	1	57102639	T	C	0.2955	7.99×10^{-6}	1.72(1.35-2.18)	intergenic	2.184	6	1.57×10^{-5}	*LOC101929935*
rs6700066	1	236535458	A	G	0.2853	8.25×10^{-6}	1.80(1.39-2.32)	intronic	1.413	7	1.68×10^{-5}	*ERO1B;EDARADD*
rs1448274664	10	80763023	C	CA	0.2883	4.32×10^{-6}	0.50(0.37-0.68)	ncRNA intronic	1.797	NA	1.76×10^{-5}	*ZMIZ1-AS1*
rs10937534	3	191873224	C	T	0.319	8.75×10^{-6}	0.58(0.45-0.74)	intronic	1.091	5	1.88×10^{-5}	*FGF12*
rs79324826	1	57061340	T	C	0.1329	8.86×10^{-6}	0.40(0.26-0.61)	intergenic	5.195	7	1.89×10^{-5}	*PLPP3;LOC101929935*
rs963788068	18	27610868	A	AT	0.1564	6.69×10^{-6}	1.96(1.46-2.64)	intergenic	0.312	NA	2.14×10^{-5}	*CDH2;MIR302F*
rs6038406	20	6265860	C	T	0.1513	8.99×10^{-6}	2.08(1.49-2.89)	intergenic	2.973	6	2.32×10^{-5}	*FERMT1;CASC20*
rs13001411	2	7821182	T	C	0.226	7.94×10^{-6}	1.77(1.38-2.28)	intergenic	0.195	7	5.48×10^{-5}	*LOC100506274; LOC101929551*
rs1655645	15	29692680	C	T	0.3691	8.93×10^{-6}	1.67(1.33-2.10)	intronic	6.852	NA	7.11×10^{-5}	*FAM189A1*

Chr: chromosome, Pos: position, MAF: minor allele frequency, GWAS p: unadjusted p value calculated using chi-square analysis considering additive genetic model, OR: Odds ratio calculated with respect to effect allele, CI: confidence interval, CADD: Combined Annotation Dependent Depletion, RDB: RegulomeDB, adjusted p: calculated using logistic regression with age, sex and PC1, PC2 as covariates, Nearby Genes annotated by ANNOVAR.

Table 4.4: Top genome-wide associated loci crossing suggestive threshold ($p < 5 \times 10^{-5}$) for poor responder to PHT

rsID	chr	pos	Non effect allele	Effect allele	MAF	gwasP	OR (95%CI)	Function	CADD	RDB	Adjusted p	Nearby Genes
rs6461508	7	2696940	C	T	0.3078	8.09×10^{-6}	0.23(0.1-0.45)	intronic	1.104	4	1.00×10^{-5}	*TTYH3*
rs7971098	14	28154882	C	T	0.456	5.09×10^{-6}	0.32(0.20-0.53)	ncRNA intronic	0.196	6	3.77×10^{-5}	*LOC728755; FOXG1-AS1*
rs1590739488	14	46300328	A	C	0.2137	2.48×10^{-7}	9.34(3.48-25.1)	intergenic	3.642	7	4.98×10^{-5}	*LINC02303; LINC00871*
rs10904170	12	103845157	C	CCATG CCCAG	0.4356	8.36×10^{-7}	3.73(2.18-6.40)	intronic	1.314	NA	5.04×10^{-5}	*C12orf42*
rs7704756	11	122007806	G	A	0.3374	8.17×10^{-6}	3.06(1.86-5.04)	intergenic	12.96	4	5.92×10^{-5}	*MIR100HG*
rs2877849	1	190595160	T	C	0.06851	8.23×10^{-6}	8.59(2.87-25.76)	ncRNA intronic	1.071	6	6.13×10^{-5}	*LINC01720*
rs7897571	10	4061453	G	T	0.273	3.45×10^{-6}	3.86(2.14-6.96)	intergenic	0.097	6	8.66×10^{-5}	*MIR6078; LOC101927964*
rs6461508	7	2696940	C	T	0.3078	8.09×10^{-6}	0.23(0.1-0.45)	intronic	1.104	4	1.00×10^{-5}	*TTYH3*
rs7971098	14	28154882	C	T	0.456	5.09×10^{-6}	0.32(0.20-0.53)	ncRNA intronic	0.196	6	3.77×10^{-4}	*LOC728755; FOXG1-AS1*
rs1590739488	14	46300328	A	C	0.2137	2.48×10^{-7}	9.34(3.48-25.1)	intergenic	3.642	7	4.98×10^{-4}	*LINC02303; LINC00871*
rs10904170	12	103845157	C	CCATG CCCAG	0.4356	8.36×10^{-7}	3.73(2.18-6.40)	intronic	1.314	NA	5.04×10^{-4}	*C12orf42*
rs7704756	11	122007806	G	A	0.3374	8.17×10^{-6}	3.06(1.86-5.04)	intergenic	12.96	4	5.92×10^{-4}	*MIR100HG*

Chr: chromosome, Pos: position, MAF: minor allele frequency, GWAS p: unadjusted p value calculated using chi-square analysis considering additive genetic model, OR: Odds ratio calculated with respect to effect allele, CI: confidence interval, CADD: Combined Annotation Dependent Depletion, RDB: RegulomeDB, adjusted p: calculated using logistic regression with age, sex and PC1, PC2 as covariates, Nearby Genes annotated by ANNOVAR.

.4.3.2.3. Good Responder vs Poor Responder on patient with Carbamazepine:

This analysis was performed between 92 poor and 156 good responder of patients with CBZ with no significant genomic inflation (λ_{median} =1.00823) (**Figure 4.7**). The highest GWAS signal for rs4659128 [p= 2.033 x 10^{-7}, OR (95% CI) = 4.82(2.64-8.79)] which is an intronic variant in *TBX15* gene. These are a class of transcription factors that are known to regulate a variety of developmental processes (Yan, 2023). Substantiating the functional relevance of this SNP, with a RegulomeDB score of 1f, this variant show significant eQTL evidence in almost all tissue. This variant mostly upregulate *WARS2* gene in all tissues. This variant is putatively present at the actively transcribed state (Tx or Tx/Wk) in several tissues like skeletal, muscle, adipose, bone marrow and Enhancer like properties in ESC and iPSC. Another SNP [p= 6.848 x 10^{-6}, OR (95%CI) = 0.361(0.23-0.55)] an intergenic variant near *SLC25A28,* this gene is predicted to be involved in iron import into the mitochondrion. This variant has known to downregulate *ABCC2* in Nucleus accumbens basal ganglia (p=0.023), an important membrane bound transporter of broadly all anti-epileptic drugs and is most in the weak transcription site in blood and epithelial, digestive and foetal brain tissue. The most significant curated biological process in this group is hydrogen peroxide catabolic process (fold enrichment =70, p<0.00001), intracellular signal transduction (fold enrichment= 25, p<0.0001). The other loci associated with poor response to CBZ are annotated in genes like *MBL2*, *PCDH15*, *SLC25A24*, *NYNRIN*.

Table 4.5: Top genome-wide associated loci crossing suggestive threshold ($p < 5 \times 10^{-5}$) for poor responder to CBZ												
rsID	**chr**	**pos**	**Non effect allele**	**Effect allele**	**MAF**	**gwasP**	**OR (95%CI)**	**Function**	**CADD**	**RDB**	**Adjusted p**	**Nearby Genes**
rs4659128	1	119464710	C	T	0.1442	3.90×10^{-8}	4.82(2.64-8.79)	intronic	1.666	1f	2.03×10^{-7}	*TBX15*
rs201566762	2	85175660	TA	T	0.4131	1.64×10^{-6}	0.41(0.28-0.59)	intergenic	1.709	NA	3.25×10^{-6}	
rs73085150	7	22532638	G	C	0.1851	1.56×10^{-6}	3.50(2.05-5.96)	intronic	11.13	6	4.48×10^{-6}	*STEAP1B*
	11	74068812	AG	A	0.4284	6.25×10^{-6}	0.41(0.28-0.61)	intronic	1.235	NA	5.11×10^{-6}	
rs6584298	10	101364049	C	T	0.3395	2.47×10^{-6}	0.36(0.23-0.56)	intergenic	4.144	7	6.88×10^{-6}	*NKX2-3;SLC25A28*
rs6601485	8	10408070	G	C	0.3231	8.60×10^{-6}	0.37(0.24-0.58)	intronic	0.747	4	1.24×10^{-5}	*PRSS55*
rs61889377	11	32543005	A	C	0.3364	9.70×10^{-6}	2.47(1.65-3.72)	intergenic	0.025	5	1.59×10^{-5}	*WT1-AS;EIF3M*
rs374777871	2	228866000	TATAA	T	0.318	4.41×10^{-6}	0.36(0.23-0.56)	intronic	0.268	NA	1.72×10^{-5}	
rs6601485	10	54988100	A	T	0.2188	2.70×10^{-6}	2.68(1.76-4.07)	intergenic	11.63	6	1.85×10^{-5}	*MBL2;PCDH15*
rs12498262	4	150177249	T	C	0.1554	7.63×10^{-6}	3.02(1.83-4.98)	ncRNA splicing	21.6	5	2.80×10^{-5}	*LINC02355*
rs962752548	4	154257268	TTA	T	0.2812	8.24×10^{-6}	2.32(1.60-3.36)	UTR3	9.484	NA	2.86×10^{-5}	
rs543063	1	108732235	G	A	0.3108	6.65×10^{-6}	2.47(1.66-3.68)	intronic	0.63	6	3.21×10^{-5}	*SLC25A24*
rs10169628	2	121135375	G	A	0.1176	7.94×10^{-6}	3.62(2.00-6.55)	intergenic	2.234	5	4.56×10^{-5}	*INHBB;LINC01101*

Chr: chromosome, Pos: position, MAF: minor allele frequency, GWAS pP: unadjusted p value calculated using chi-square analysis considering additive genetic model, OR: Odds ratio calculated with respect to effect allele, CI: confidence interval, CADD: Combined Annotation Dependent Depletion, RDB: RegulomeDB, adjusted p: calculated using logistic regression with age, sex and PC1, PC2 as covariates, Nearby Genes annotated by ANNOVAR.

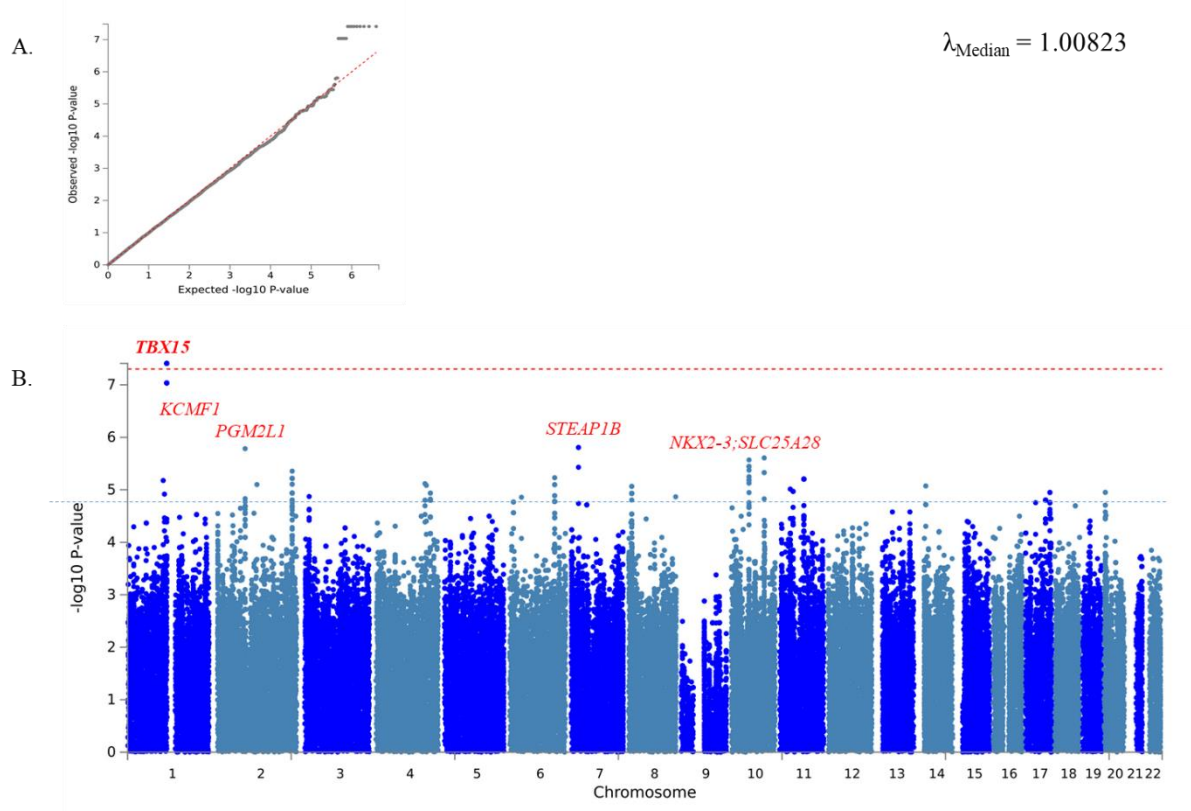


Figure 4.7: Manhanttan and QQ plots of genome-wide association analyses for poor response to CBZ. Genomic inflation factors (λ) for the four genome-wide association study analyses. Negative $\log_{10}$ transformed p-values (Y axis) are plotted against chromosomal positions (x-axis).The red line represent the suggestive genome-wide significance threshold ($p< 1x\ 10^{-5}$).

4.3.2.4. Good Responder vs Poor Responder on patient with Valproic acid

This analysis was performed between 61 poor and 116 good responder of patients with VPA therapy with no significant genomic inflation (λ_{median} =1.03372) (**Figure 4.8**). The top hit in this group is an intronic SNP (rs11569839/ *TNFRSF8*) [p= 9.498 x 10^{-6}, OR (95%CI) = 3.72(2.04-6.79)]. This gene belongs to the TNF receptor superfamily, predicted to enable transmembrane signalling receptor activity by positive regulation of NF-kappa β transcription factor activity, differentially regulating gene expression in brain tissues (most significant in downregulation of *PLOD1* in cerebellar hemisphere (p=0.0018) (**table 4.6**). Interestingly, this variant is correlated with regulation of one of the most important candidate epilepsy gene, *MTHFR* (p=0.0079) and *MTOR (*an AED target gene) (p= 0.043) in temporal cortex and *MTHFR* in cerebellum (p=0.039). This variant is putatively lying in Tx/Wk or Enhancer region in heart and muscle tissues. The other loci is an intergenic variant near synapsin 3 (SYN3) gene. Deficiency of this protein is known to cause epileptic seizures (Schwark R, 2022). Also this is a neuron-specific synaptic vesicle–associated phosphoprotein that has been implicated in synaptogenesis and in the modulation of neurotransmitter release (Morales-Corraliza J,

2010). This SNP is present in the active (TssA) in several tissues. The most important function molecular functions carried out by the associated genes of this group is CCR7 chemokine receptor binding (fold enrichment= 100, p= 0.01).

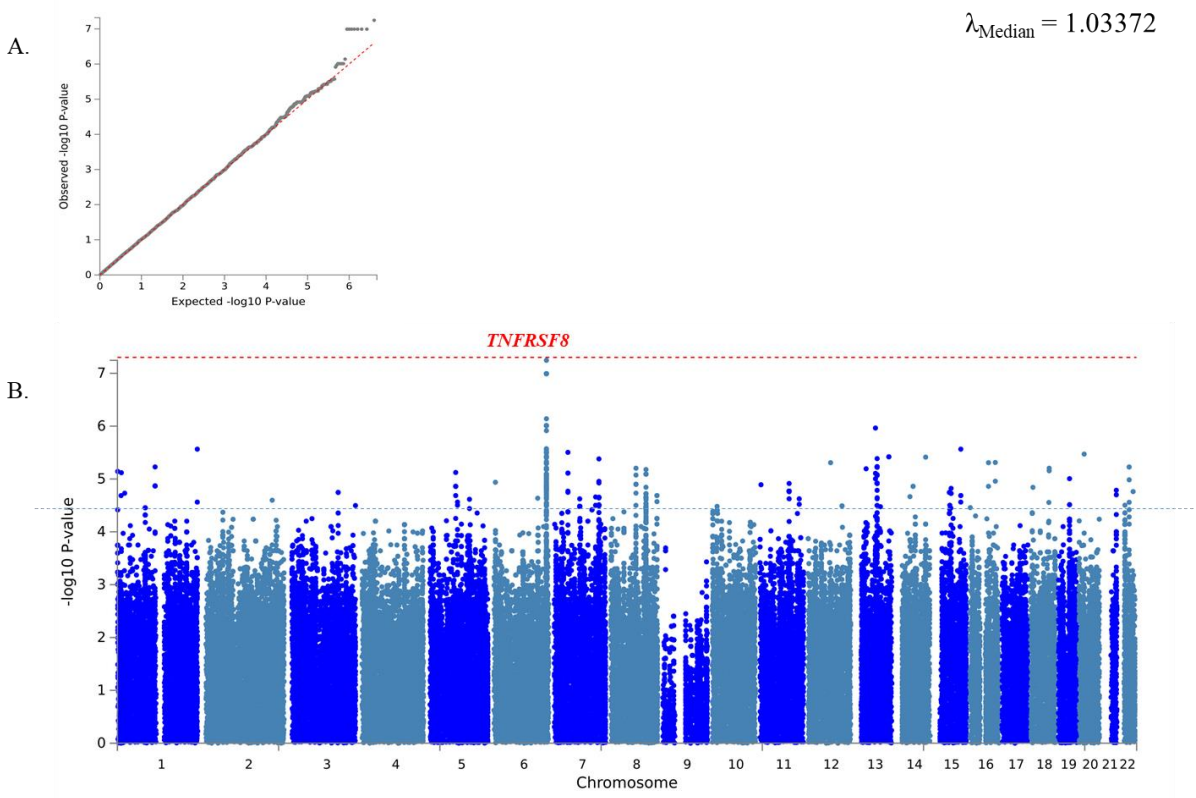


Figure 4.8: Manhanttan and QQ plots of genome-wide association analyses for overall AED response. Genomic inflation factors (λ) for the four genome-wide association study analyses. Negative $\log_{10}$ transformed p-values (Y axis) are plotted against chromosomal positions (x-axis).The line represent the suggestive genome-wide significance threshold ($p< 1x\ 10^{-5}$).

4.3.3 Replication analysis with other GWAS published with AED response

There are only two GWAS published so far establishing AED response (apart from AED related ADRs) (Wolking et al., 2021; Wolking *et al.*, 2020).We aimed to test whether the SNPs described in previous published GWAS on AED response that reportedly associated with overall AED response and drug-wise response for PHT, CBZ and VPA is present in our cohort. Among the genome-wide significant variants identified by (Wolking et al., 2020), a total of 181481 variants were found common with our overall cohort, where rs970635, an intergenic variant present near *FBXO33* and *LINC02315* gene was at highest significance [$p=3.98x\ 10^{-6}$, OR (95%CI) = 1.73(1.37-2.18)]. Likewise, in the VPA group, 296926 variants were common between the two. An intronic variant, rs242076 (between *SYN3* and *TIMP3* gene was the top hit [$p= 1.031 \times 10^{-5}$, OR (95%CI) = 0.35(0.22-0.55)]. The significant loci and the LD SNPs

Table 4.6: Top genome-wide associated loci crossing suggestive threshold ($p < 5 \times 10^{-5}$) for poor responder to VPA

rsID	chr	pos	Non effect allele	Effect allele	MAF	GWAS p	OR (95%CI)	Function	CADD	RDB	Adjusted p	Nearby Genes
rs11569839	1	12157881	G	A	0.1534	7.61×10^{-6}	3.72(2.04-6.79)	intronic	3.239	5	9.50×10^{-6}	*TNFRSF8*
rs242076	22	33229830	A	G	0.4601	5.93×10^{-6}	0.35(0.22-0.55)	intronic	5.047	NA	1.03×10^{-5}	*SYN3;TIMP3*
rs933406127	6	163140575	GA	G	0.3098	5.68×10^{-8}	4.06(2.40-6.87)	intronic	1.111	NA	1.19×10^{-5}	*PACRG*
rs1413065	13	63679702	T	C	0.2812	1.09×10^{-6}	3.33(2.03-5.48)	intergenic	5.436	NA	1.22×10^{-5}	*LINC00448;LINC00376*
rs7192067	13	68829521	T	C	0.407	4.12×10^{-6}	0.33(0.21-0.54)	intergenic	0.148	7	1.33×10^{-5}	*LINC00364;LINC00550*
rs921412	7	138495985	T	A	0.4898	4.17×10^{-6}	0.33(0.21-0.54)	intronic	1.025	6	2.43×10^{-5}	*TMEM213;KIAA1549*
rs1270275	19	35107163	G	T	0.2526	9.86×10^{-6}	3.01(1.83-4.95)	NA	0.82	NA	2.83×10^{-5}	*SCGB1B2P*
rs7192067	16	78864773	T	C	0.1564	4.87×10^{-6}	4.05(2.17-7.61)	intronic	3.516	7	3.06×10^{-5}	*WWOX*
rs7240119	18	57568601	T	C	0.3875	6.25×10^{-6}	2.80(1.78-4.40)	intronic	5.114	4	3.16×10^{-5}	*PMAIP1*
rs12701950	7	42232068	G	A	0.0818	3.14×10^{-6}	5.43(2.51-11.8)	intronic	3.559	6	3.35×10^{-5}	*GLI3*
rs1399610	8	78321942	T	A	0.3875	6.25×10^{-6}	2.80(1.78-4.40)	ncRNA intronic	0.09	6	3.87×10^{-5}	*PEX2;LOC102724874*
rs11850195	14	93326861	C	T	0.2853	3.86×10^{-6}	3.13(1.91-5.13)	intergenic	1.596	6	5.18×10^{-5}	*GOLGA5;LINC02287*
rs112703963	1	859913	G	A	0.09611	7.19×10^{-6}	5.82(2.49-13.59)	upstream	12.3	3a	8.36×10^{-5}	*LINC02593;SAMD11*
rs11850195	1	116879341	C	T	0.1503	5.91×10^{-6}	4.21(2.18-8.12)	intergenic	0.136	5	8.78×10^{-5}	*MAB21L3;ATP1A1*
rs67838640	13	34647926	T	G	0.1503	6.41×10^{-6}	4.30(2.2-8.44)	intergenic	1.849	7	9.06×10^{-5}	*RFC3;LINC02343*

Chr: chromosome, Pos: position, MAF: minor allele frequency, GWAS p: unadjusted p value calculated using chi-square analysis considering additive genetic model, OR: Odds ratio calculated with respect to effect allele, CI: confidence interval, CADD: Combined Annotation Dependent Depletion, RDB: RegulomeDB, adjusted p: calculated using logistic regression with age, sex and PC1, PC2 as covariates, Nearby Genes annotated by ANNOVAR.

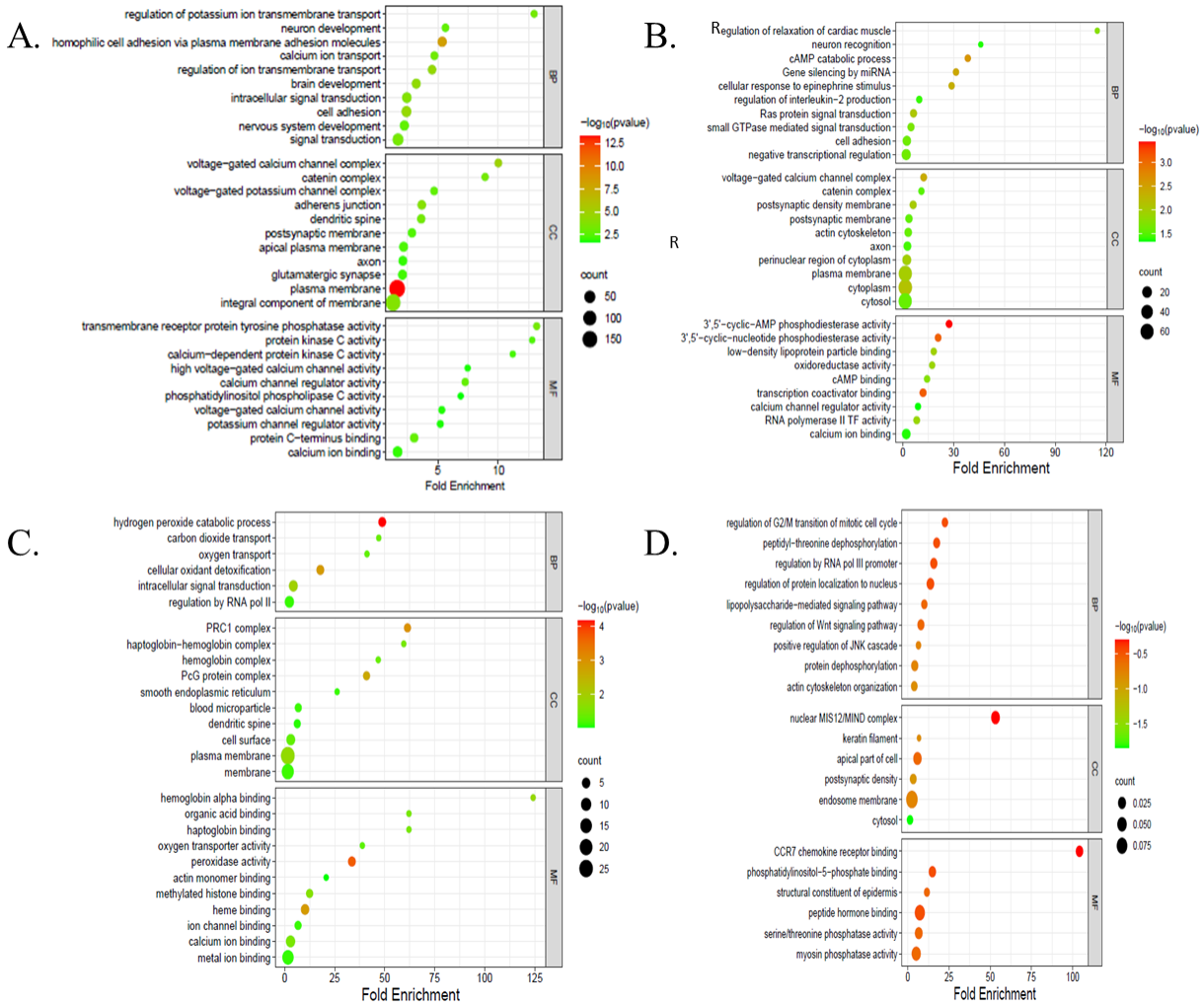


Figure 4.9: The gene set enrichment analysis using input genes from summary statistics with GWAS p threshold $p < 5 \times 10^{-5}$ for GO analysis. Here A. overall AED poor response, B. poor response to PHT, C. poor response to CBZ, D. poor response to VPA.

(r^2>0.8) mentioned in the other GWAS (Wolking S et al. (2021), were tested in our cohort, where rs7092992 (near *MIR4675* and *NEBL*) showed nominal significance in our VPA cohort but the directionality was opposite [p= 0.036, OR (95%CI) = 0.44(0.21-0.97)].Apart from this, we additionally compared our summary statistics with the list of candidate genes prioritised from literature associated with different anti-seizure drugs (Guin D, 2023).

4.4 Discussion

In all the published GWAS so far, no pharmacogenetic marker has been identified for drug response to specific AEDs that crossed the genome-wide significance threshold. In this study we attempted to identify common variants associated with drug response to anti-epileptic drugs, in general, as well as, specific to three commonly prescribed AEDs- PHT, CBZ and

VPA. They are the most frequently used AEDs in epilepsy, particularly in India, and are considered as the first line of treatment (Haroon A, 2012). In our recruited cohort of 789 patients, 39.55% participants were poor responders and 60.45% were good responders. The ratio of good responders to poor responders was higher in the VPA group (35.12%) as compared to CBZ (37.32%) and PHT (40.99%). This observation reflects the superiority of VPA in the treatment of epilepsy. Besides, poor responders had a higher seizure frequency before treatment initiation shows the common clinical observation that individuals with severe epilepsies are less likely to achieve seizure freedom – the cornerstone of the intrinsic severity hypothesis of pharmacoresistance (Rogawski MA, 2013).

Our GWAS results did not reveal strong genetic association with large effect size contributing to genetic variance of overall poor response to AED treatment. Nonetheless, our results show top genomic loci, rs4659128/TBX15 [OR (95% CI) = 4.82(2.64-8.79), $p<2.033 \times 10^{-7}$] and rs11569839/TNFRSF8 [OR (95% CI) = 3.72(2.04-6.79), $p< 9.498 \times 10^{-6}$] to be associated with poor responders to CBZ and VPA treatment respectively. However, these associations did not cross the GWAS significance threshold like previously reported (Wolking S, 2021; Wolking S, 2020). This lack of significant association to drug response outcome underlines the fact that poor response or pharmacoresistance, at large, are complex traits which may or may not be largely controlled by genetics (Balestrini S, 2018). Though, there is some evidence that enrichment of ultra-rare variants in genes associated with pharmacodynamics and pharmacokinetics can modify AED response, but further replication of these results is needed. Other non-genetic risk factors like early onset, abnormal EEG (both slow wave and epileptiform discharges), status epilepticus, symptomatic etiology, febrile seizures, and multiple seizure types, have been proposed for development of non-response (Xue-Ping W, 2019).

Assuming response to AEDs a complex genetic trait, multiple loci with small effect size may be associated with it. Due to limited sample size, our study was underpowered to detect such variants with small effect sizes, though we identified several suggestive loci. Among them, in the overall poor responder group, we identified intergenic SNPs near genes like SPTLC3 and ISM1.SPTLC3 codes for palmitoyl-transferase enzyme which is involved in sphingolipid biosynthesis. Sphingolipids are crucial for proper brain development and functions (Olsen ASB, 2017). Structural variants in this gene are known to be associated with childhood absence epilepsy (Addis L, 2016). The other gene, ISM1, are secretory are pro-apoptotic protein that functions through cell surface high affinity G protein receptors, known

to be associated with intellectual disability and neuronal development (Osório L, 2014). Other SNPs, rs970635 near FBXO33 gene have been identified as a novel susceptibility gene for the Attention-Deficit Hyperactivity Disorder (Sánchez-Mora C, 2015). A study by Flood WD et al (2004) identified this gene to be one of the novel genes responsive to seizure (Flood *et al.*, 2004). Another SNP, rs4891466 near CBLN2 have been previously reported in other GWAS is known to have tissue specific expression in brain, six times higher as compared to other tissues (Jang et al., 2015).

Likewise, for poor response specific to PHT, the top loci associated was an intronic SNP (rs6461508) in TTYH3 gene. This gene encodes for protein that functions as a Ca^{2+} activated large conductance Cl^- channel (Li *et al.*, 2021). They are highly expressed in nervous system, and this gene is upregulated following epileptic events in central neurons and glial cells (Stefaniuk *et al.*, 2010; Wiernasz *et al.*, 2014). The intronic SNP, rs4659128 in TBX15 gene is an important transcription factor regulating a variety of developmental processes crucial for normal brain functioning (Ayata *et al.*, 2018). For VPA poor response, another intronic variant (rs11569839) located in TNFRSF8 gene regulates very important DMEs for AEDs- MTHFR & mTOR. A recent study suggest selective inhibition of TNF-TNFRSF signalling may decrease acute seizures and potentially suppress the development of epilepsy (Patel DC, 2017). Thus, considering the nature of GWAS findings for a complex trait, these findings should not be considered causal, rather regions of top genomic loci can be important for drug action in different brain tissues in epilepsy. Interestingly, several of the top associated loci belong to different brain physiology or neurological development, none of them were ADME genes. But some of these SNPs have evidence of regulating important drug targets or DMEs of AEDs. Additionally, GO analysis results showed the highest fold enrichment for functions like regulation of potassium ion transmembrane transport, neuron development, Ca^{2+} ion transport, regulation of ion transmembrane transport, brain development, voltage-gated Ca^{2+} channel complex, voltage-gated K^+ channel complex, neuron recognition, post synaptic density membrane and so on. These functions are crucial molecular processes carried out during the action of AEDs thereby reducing seizure occurrence. Our findings suggesting that although the top loci were not direct AED related genes, but further functional validation may shed light into the molecular mechanistic insights related to AED response.

In spite of being the first GWAS study to explore the distribution of genetic variants associated with poor response to AEDs specific for Indian population, our study has some inherent limitations. Due to limited sample size, our study could not detect genetic variants

with small effect sizes, though we identified several suggestive loci. Specifically for the drug-wise stratified analysis, the sample size was too small. Further, we choose a definition of poor response to be ≥2 seizures in ten month, had we considered one seizure at the end of a year follow up compared to baseline data, we would have obtained larger sample size, but we assume that a less rigorous definition would have blurred potential genetic association. Apart from genetic factors, other clinical factors should also be considered to identify the best fit prediction model for poor response. Our study lacks validation in an independent cohort to gain power of statistical findings.

4.5 5. Conclusion

This is the first GWAS study on AED response in an Indian cohort for individual AED response. While our study did not reveal any genome-wide significant association for drug response in any of the groups, we identified several suggestive loci. Future similar studies should attempt to reproduce our findings, apart from the GWAS threshold ($p < 5 \times 10^{-8}$). Our study mainly explored the common variants, future hypothesis-driven research focusing on rare or structural variants, similar to such studies in epilepsy disease risk loci may provide clues towards improving AED treatment outcome. More studies with similar study design are required to replicate the findings and functional assays to elucidate the role of common variants in future analysis.

Chapter 5

Development of PGx panel with efficacy/ toxicity marker for pharmacogenetic testing towards pilot implementation.

5.1 Introduction

Clinical validity is indicated by measures of discriminative accuracy and predictive value. The diagnostic accuracy refers to the ability of a test to discriminate between the presence and absence of poor response and is indicated by the sensitivity and specificity. Sensitivity is the probability that the genetic variant associated with a higher risk of poor response to an administered drug (from here referred to as the genetic variant) is present in individuals with the adverse event while specificity is the probability that the genetic variant is absent in individuals with favourable response. Contingency tables can be constructed using empirical data or using hypothetical data calculated from summary statistics and association measures, such as odds ratios derived from observational studies with a case–control design in combination with the frequencies of the genetic variant and the adverse event. To indicate the diagnostic accuracy both sensitivity and specificity need to be reported. Optimizing the predictability of the efficiency of the PGx marker, to determine its accuracy, specificity and sensitivity in a population specific cohort thereby minimizing the chances of false positive prediction. A pharmacogenetic test that has high sensitivity (80%), but low specificity (10%) will be able to predict 80% of the individuals who will develop have a poor outcome of drug but it will misclassify 90% of the individuals who will not have a poor outcome. Measures of clinical validity can be calculated from a 2 × 2 contingency table for each variant identified. Predictive value measures are sensitive to the prevalence of the poor response and PPV remains generally low for rare drug outcomes even if the pharmacogenetic associations (ORs) are high.

The current work aims to identify if the associated variants overlap with that of the evidence from available literature including the 80 pharmacogenetic variants identified from objective 1. And also estimate if these variants associated with drug response phenotype can be used formulate clinically actionable pharmacogenetic tests for targeted therapy specific to Indian patients. The clinical validity of a pharmacogenetic markers is essential to indicate the ability of the associated genetic variant to predict the occurrence of poor response on prescription of a specific drug, in our study, an anti-seizure drug. It is determined by the strength of association between the genetic variant and the phenotype. In a case control study design, this is calculated by odd ratio along with 95% confidence interval with a statistical significance. A strong association is essential but not always sufficient condition to ensure the accuracy of estimating the accuracy of diagnosis. Thus, it is crucial to evaluate the diagnostic ability to correctly identify or predict an outcome of interest, which, in pharmacogenetics, indicates the ability of the test to predict poor response or lack of treatment efficacy.

5.2. Material & method

We performed a replication analysis where we obtained pharmacogenomic data predicting poor response to anti-epileptic drugs from four different data sources- PharmGKB, GWAS Catalog (https://www.ebi.ac.uk/gwas/), EpiGAD and our previous published article from objective 1 (Guin D, 2019b). From PharmGKB, using 'epilepsy' disease keyword, all the curated variant annotations were retrieved. A total of 522 variant information was obtained, of which 230 were significant associations. These were retained for our analysis, and the star allele variants were annotated using the standard nomenclature system for respective genes/ alleles and removing the duplicate variants, the remaining 113 variants across 17 genes were retained. Likewise, from GWAS Catalog, using the keyword, 'response to anti-convulsant', a total of 151 variants (in 134 genes) from 13 GWAS were obtained. A unique epilepsy genetic association database, EpiGAD enlists all the disease associated genetic variants known so far as well as that for drug response. We extracted the data for drug response from this database, which resulted in 211 associated variants. Retaining only the significant associations and annotating the star variants, we were left with 62 SNPs ranging in 30 genes. And finally, from our previous published literature 80 SNPs were obtained. We compared our results obtained from our high throughput genome analysis for overall poor response predicting to prescribed AEDs (**figure 5.2**), we found an overlap of 19 SNPs as enlisted in **Table 5.1**. We then estimated the diagnostic accuracy of these markers to identify if they qualify to be used for clinical applications to predict poor response to AED. The diagnostic predictability was calculated using the formula (Tonk ECM, 2017) as shown in **figure 5.1**. If the patient carrying the alternate allele associated with poor response are poor responders as recruited in our cohort, they are regarded as true positive and if the patient do not carry the allele but are poor responders, they are false positive. Likewise, true positive and true negatives are defined in this case. Specificity, sensitivity and diagnostic accuracy was calculated for predicting poor response from our GWAS summary statistics for overall poor response. These markers which sufficient efficiency may be beneficial for population specific diagnostic applications although it requires replication in independent cohort as well. Additionally, we identified 88 PGx variants from the literature which are approved by FDA, CPIC, SwissMedic, EMA and others and are currently used for commercial purposes. These are overlapping with the data obtained from the different sources in literature. We observed of the 19 SNPs, 8 are already in commercial use (**figure 5.2**). We also estimated the area under the curve for true predictability using ROC curve analysis performed in STATA 16.0.

Parameters	Formula
Sensitivity	$\frac{TP}{TP+TN}$ %
Specificity	$\frac{TN}{TN+FP}$ %
Efficiency	$\frac{TP+TN}{TP+TN+FP+FN}$ %
Precision	$\frac{TP}{TP+FP}$
Recall	$\frac{TP}{TP+FN}$
Accuracy	$\frac{TP+TN}{TP+TN+FP+FN}$ %
F-measure	$2X \frac{Precision \times Recall}{Precision + Recall}$

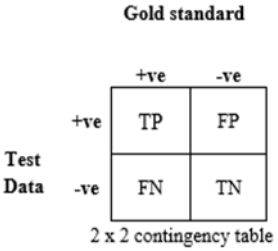


Figure 5.1: Formulae used for calculation of diagnostic accuracy using 2 × 2 contingency tables.

5.3 Results

We observed a gene and SNP level overlap between the four datasets and our summary statistics. Quite obviously, we observed a narrow SNP overlap like in most published GWAS. Only 19 SNPs overlapped from a total of ~4 lakh SNPs associated with poor response to AED. However, a substantial overlap of genes were observed. A total of 144 genes overlapped from around 34,000 genes. Most of these genes were ADME genes and commonly studied in previous candidate associations with AED outcomes. Interestingly, among the 19 overlapping variants, 8 of them are already used for commercial purpose to diagnose/ predict poor response in epilepsy treatment with common first line drugs.

The diagnostic accuracy of the 19 SNPs are as shown in **table 5.1**. Most these overlapping variants show moderate to low effect size in our study cohort suggesting either genetic variants cannot be considered solely responsible for predicting drug outcomes, suggesting that PGx traits are complex and polygenic basis may be involved**.** The statistical significance of these variants are also low $p<0.05$. This indicate may be functional validation of the associated variant or the genes involved may be crucial to decipher the biological mechanism by which they show association. Further substantiating that statistical association might not always hold relevance in a GWAS finding to direct a true biological association. Such future studies may strengthen PGx GWAS.

The specificity of the variants ranges from 55% to 98% suggesting a moderate to high specificity of these variants in predicting poor response. However, the sensitivity ranges from 3% to 48%. This shows low to moderate sensitivity of these variants with larger false negative results prediction rate. Although, it is critical to note that all these predictability analysis is based on our GWAS data, its cohort specific genotype calling. Identifying these associations in an independent, larger cohort would validation these diagnostic tests and that would help us identify true markers which can be taken forward for applications in precision medicine.

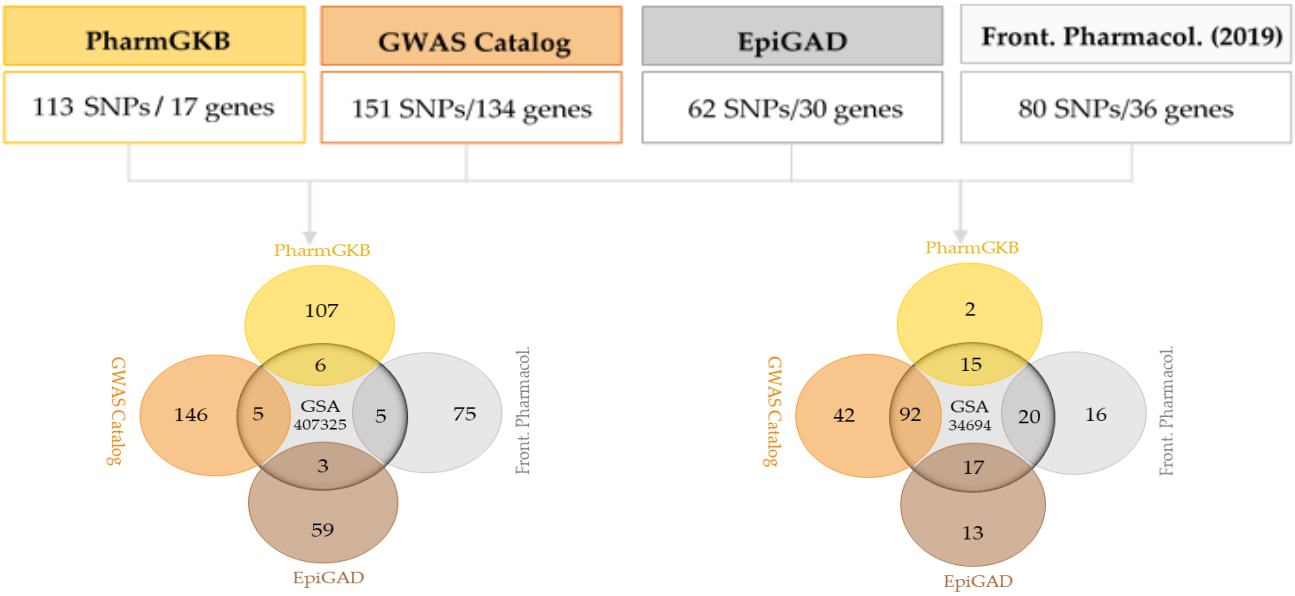


Figure 5.2: The overlap of GWAS findings (at SNP and gene level) with PharmGKB, GWAS Catalog, EpiGAD and our previous publication(Guin D, 2019a)

Table 5.1 tabulates the diagnostic accuracy and the predictive outcome of each of the 19 variants. We also performed receiver operating characteristics (ROC) analyses using STATA 16.0 (Support) for PGx variants that qualifies a marginal threshold for specificity and sensitivity (>30%). The area under the ROC curve (AUC_{ROC}) was used as a model quality, for the marker with highest AUC_{ROC} is the best performing PGx variant. **Figure 5.3** shows the ROC curve for the 19 SNPs overlapping with the four datasets. The ROC analysis results showed a low AUC for most of the overlapping variants which suggest that there can be other variables confounding with AED response. In PWE.

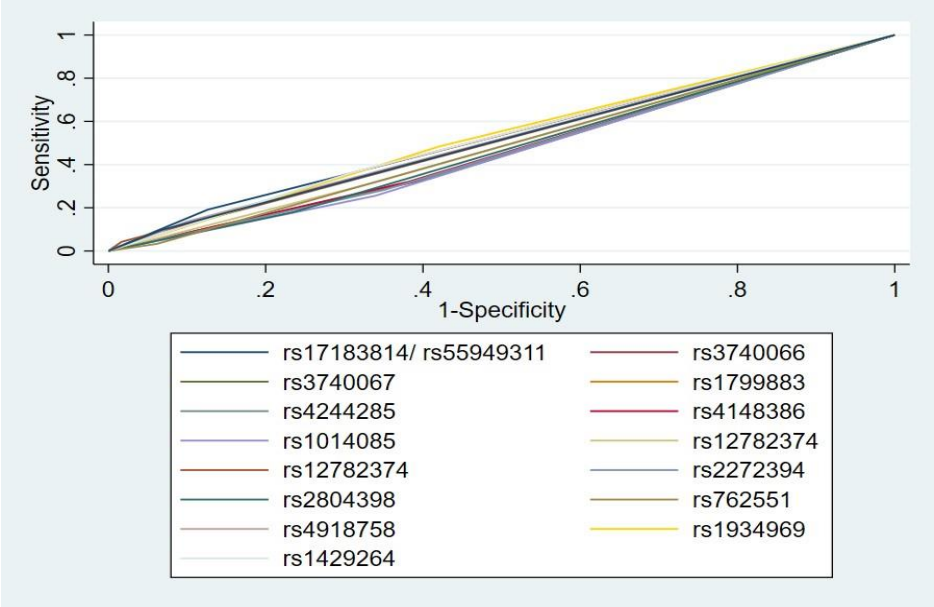


Figure 5.2: The ROC for risk allele count on pharmacogenetic SNPs used to measure AUC in PWE.

5.4 Discussion

Understanding the genetic variants in inter-individual drug response, in terms of drug efficacy, and safety profiles, may help us in determining the global spectrum of PGx diversity across populations. The pharmacological treatment of epilepsy has been largely empirical based on trial-and-error altering the dose of administered AED or changing the drug or prescribing in combination until seizure remission is achieved (Schmidt, 2014). Therapeutic response is a phenotype with continuous distribution. Patients genotyped at the extreme ends of this distribution either show full response or no response, while genotyping patients with 'in-between' response cannot be discretely divided into response or non-response group. Thus, accurate estimation of effect size comparable to sample size is still challenging. However, correlating the relationship between serum drug concentration, seizure control frequency, and presence of risk allele, may be useful in determining therapeutic recommendations of AED for each patient, thereby individualising treatment.

This study demonstrate how clinical validity and effect of pharmacogenetic tests may vary with population and setting in which test are used. The effect size, commonly estimated by ORs with 95% CI range, variant frequencies and drug non-response frequencies often differ across populations, for example, according to ethnic background and gender. Also, changes in the definition and measurement of the drug related phenotype may impact the observed

performance of genetic tests, as the different classification of individuals with and without the drug related event may lead to different adverse event frequencies and ORs (Gurwitz D, 2010).

In order to translate genomic findings for clinical utility, we characterised all the PGx evidence available so far with respect to poor response to AED, i.e. recurrent seizures even after AED administration to assess which markers have higher potential for clinical consideration. This directive analysis highlights that although eight of the nineteen variants (42%) are already in clinical use the other variants are promising candidates for future clinical applications with further validation. Although it is not yet possible to make general recommendations for incorporating this genetic data into decision- making process for AED therapy, recent studies are beginning to provide a foundation for future establishment of treatment guidelines(Glauser T, 2013).

While the AUC_{ROC} is an important measure for clinical validity it does not tell the whole story as it does not differentiate between the accuracy with which the genomic profile predicts the true genetic risk of individuals and the accuracy with which true genetic risk predicts drug-related outcomes, which is not under our control. We believe that the ability to differentiate between these components, prediction of genotype and phenotype, is important for interpretation of the value of genomic profile, particularly as the use of genomic profiles is very much in its infancy at present. Thus, genomic profiles should judge on the basis of their analytic validity as predictors of genetic rather than absolute risk. Other factors combined with genotypes, like environmental risk factors may be essential in predicting absolute. In this work, we provide insight into the genetic interpretation of AUC. Therefore, ROC curves for genomic profiles cannot be considered as a sole parameter to estimate diagnostic predictability without prior knowledge of the phenotype.

5.5 Conclusion

This study attempts to describe the landscape of clinically relevant PGx interactions in ASM response, contemplating previous efforts to provide useful information for optimization of population-specific PGx applications. In conclusion, 88 commercial PGx marker are known related to AED response. Among these nineteen SNPs overlapped with our GWAS findings for overall poor response. Assessing the diagnostic predictability of these nineteen markers showed moderate accuracy (50-60%). These markers are promising candidates for PGx application after appropriate validation and replication. Eight out of these nineteen markers are

already in use for drug labelling approved by the FDA. Strengthening the fact that genome-based markets can be exploited for application in precision medicine in epilepsy treatment.

Table 5.1: Diagnostic predictability estimate of the 19 replicated variants with the available literature.

Chr	Base position	SNP	Effect Allele	Non-Effect Allele	OR (95%CI)	P value	Function	Gene	Sensitivity	Specificity	PPV	NPV	Commercial Use
2	166152389	rs17183814	A	G	1.63(1.21-2.19)	0.0006515	exonic	*SCN2A*	0.19	0.87	0.50	0.63	✓
10	101604207	rs3740066	T	C	0.66(0.52-0.84)	0.0009844	exonic	*ABCC2*	0.34	0.59	0.35	0.58	✓
10	101603781	rs3740067	G	C	0.66(0.52-0.84)	0.001088	intronic	*ABCC2*	0.32	0.61	0.35	0.58	
7	87160618	rs2032582	T	A	2.60(1.32-5.10)	0.00441	exonic	*ABCB1*	0.51	0.55	0.42	0.63	✓
4	120241902	rs1799883	T	C	0.72(0.57-0.92)	0.01003	exonic	*FABP2*	0.37	0.69	0.43	0.63	
10	96541616	rs4244285	A	G	0.73(0.58-0.91)	0.01256	exonic	*CYP2C19*	0.34	0.59	0.35	0.58	✓
10	101548468	rs4148386	G	A	0.74(0.59-0.93)	0.01479	intronic	*ABCC2*	0.32	0.62	0.35	0.58	✓
8	57643998	rs1014085	C	T	0.71(0.54-0.93)	0.01975	intergenic	*LINC00968; IMPAD1*	0.26	0.66	0.33	0.58	
1	226026406	rs2234922	G	A	1.36(1.05-1.76)	0.02106	exonic	*EPHX1*	0.26	0.66	0.33	0.58	
10	96695351	rs12782374	A	G	0.75(0.59-0.94)	0.02335	intergenic	*CYP2C19; CYP2C9*	0.32	0.66	0.38	0.60	✓
3	10967712	rs2272394	A	G	0.51(0.30-0.89)	0.02587	exonic	*SLC6A11*	0.04	0.98	0.62	0.61	
10	101558634	rs2804398	T	A	0.76(0.60-0.95)	0.0298	intronic	*ABCC2*	0.28	0.65	0.34	0.58	
16	16123048	rs875740	A	C	1.29(1.03-1.60)	0.03049	intronic	*ABCC1*	0.18	0.76	0.33	0.59	
15	75041917	rs762551	C	A	0.79(0.64-0.99)	0.04502	intronic	*CYP1A2*	0.25	0.80	0.45	0.62	✓
10	96697252	rs4918758	T	C	1.26(1.02-1.57)	0.04721	intergenic	*CYP2C19; CYP2C9*	0.03	0.94	0.26	0.60	
10	96748495	rs1934969	A	T	1.25(1.00-1.58)	0.04735	intronic	*CYP2C9*	0.14	0.90	0.47	0.62	✓
16	84623484	rs1429264	C	T	1.26(1.01-1.59)	0.04737	intronic	*COTL1*	0.48	0.58	0.43	0.63	

The overlapping genetic variants from our GWAS overall GR-PR data with that of PharmGKB, GWAS Catalog, EpiGAD database as well as the published article (Guin D, 2019a). The markers presently in clinical use are also indicated.

Chapter 6

Conclusions & Future directions

6.1 Summary

Evidence continues to accumulate suggesting that genetic factors are responsible for inter-individual variability in clinical response to largely all classes of drugs, including those used in epilepsy treatment. Most of the studies performed to date investigated candidate genes and their relevance to drug outcomes. GWAS technologies empowers hypothesis-free comprehensive screening of genetic variations across the genome. The findings from previous GWAS also have had little impact on clinical practice so far. With this, the prime aim of this study was framed to elucidate PGx markers has potential to prognose therapeutic phenotypes in patients with epilepsy and thus can be used for evidence-based testing for clinical applications in predicting drug-specific outcomes prior treatment. Hence, we aim to identify such markers specific for our population which can be used for epilepsy patient management in India. While majority of the GWAS published comprises the Western population, our study was an attempt to identify genetic variants across the genome specific to Indian population. Therefore, screening the genome of PWE specific to Indian population with PGx relevance in epilepsy, considering all the clinical and demographic variables may signal if true genetic associations are possible.

PGx literature publication is growing at an exponential pace. But we are still far to achieve clinical application which emphasises the need to tailor therapeutic options to individualize and optimize drug therapy, this merger of genomic-evidence based therapeutic administration is rarely used in clinical practice today. For example, considering the US-FDA approved genome-based evidence for drug label warnings for toxic ADR or drug-interactions or poor drug metabolism, only 133 such drugs are available in the market with 363 gene/variant drug labelling information. After approximately six lakh publications on pharmacogenomics, we have only 5491 successful RCTs completion. Our first objective in this study was to screen the global literature to build a resource of PGx markers of drug response genes for clinical implementation. For this we developed a semi-automated approach using and in-house build R package called pubmed.mineR, for test-mining the PGx literature. From this literature corpus, we aim to extract PGx relationship which is defined as a ‘Disease-Drug-Gene/Variant’ co-occurrence where a particular drug is prescribed for the treatment of a disease, and if this drug related outcomes are studied with any gene or its variants. Such PGx relationships were extracted, screened and manually curated based on certain inclusion and exclusion criteria followed by their performance evaluation comparing the output of text-mining pipeline with other robust databases like OMIM, CTD and PharmGKB to assess the sensitivity and

specificity of the disease-drug–gene– polymorphism relationships. We also calculated the accuracy of each relationship obtained and compared their occurrence within the three datasets. We conducted a validation study by comparing our result with commercially used FDA-approved drug labelling biomarkers. Finally curating PGx relationships pertaining to 1,753 disease types, and 666 drugs, comprising 4,132 genes and their 33,942 polymorphisms collated from 180,088 publications. A total of 2304 PGx relationships were obtained from our pipeline. With the performance (precision = 0.806) with benchmark datasets like PharmGKB (0.904), OMIM (0.600), and CTD (0.729), 127 PGx relationships (among the 2304) belonged to the FDA list of 362 approved markers, indicating that our semi-automated text mining approach may reveal significant PGx information with markers for drug response prediction. In conclusion we can state that this pipeline can strengthen retrieval of additional PGx markers with robust evidence that can be validated for future PGx-based clinical utility thereby narrowing the translational gap from publications to clinical translation. Additionally, from the 2304 PGx relationships we identified 458 relationships among 11 neurological disease classes with 67 drugs prescribed across 235 genes and 443 genetic variants. Keeping in mind the focus of this study in epilepsy, we extracted 80 genetic variants across 36 genes associated with 11 different drugs for epilepsy (**Table 6.1**). These markers were then validated in the following part of the study in north Indian patients with epilepsy prescribed with common first line AEDs.

Table 6.1: PGx relationships obtained from semi-automated approach developed in objective 1 of this study

	PGx relationships	Diseases	Drugs	Genes	PGx variants
Total	2304	84	383	926	2226
Neurological	458	11	67	235	443
Epilepsy/ Seizure	72	-	11	36	80

Table 6.2: 80 PGx variants identified using the semi-automated approach developed in objective 1

Genetic variant	Gene	Drug	Disease
HLA-B*59:01:01:01	HLA-B	acetazolamide	Seizures, Epilepsy
rs3789243	ABCB1	antiepileptics	Seizures, Epilepsy
rs717620	ABCC2	antiepileptics	Seizures, Epilepsy
rs3740066	ABCC2	antiepileptics	Seizures, Epilepsy
rs17183814	SCN2A	antiepileptics	Seizures, Epilepsy
rs2304016	SCN2A	antiepileptics	Seizures, Epilepsy
rs2804398	ABCC2	antiepileptics	Seizures, Epilepsy

rs487750	KCNT1	antiepileptics	Seizures, Epilepsy
rs717620	ABCC2	antiepileptics	Seizures, Epilepsy
rs1047891	CPS1	antiepileptics	Seizures, Epilepsy
rs2606345	CYP1A1	antiepileptics	Seizures, Epilepsy
rs1186745	ABCB1	antiepileptics	Seizures, Epilepsy
rs717620	ABCC2	antiepileptics	Seizures, Epilepsy
rs2279020	GABRA1	carbamazepine	Seizures, Epilepsy
rs211037	GABRG2	carbamazepine	Seizures, Epilepsy
HLA-A*02:01:01:01	HLA-A	carbamazepine	Seizures, Epilepsy
rs2290732	GABRA1	carbamazepine	Seizures, Epilepsy
rs1051740	EPHX1	carbamazepine	Seizures, Epilepsy
HLA-B*15:11:01	HLA-B	carbamazepine	Seizures, Epilepsy
rs2234922	EPHX1	carbamazepine	Seizures, Epilepsy
rs211037	GABRG2	carbamazepine	Seizures, Epilepsy
rs2071197	HNF4A	carbamazepine	Seizures, Epilepsy
HLA-B*15:21	HLA-B	carbamazepine	Seizures, Epilepsy
rs28365062	UGT2B7	carbamazepine	Seizures, Epilepsy
rs4688040	NR1I2	carbamazepine	Seizures, Epilepsy
rs4828696	GABRA3	carbamazepine	Seizures, Epilepsy
HLA-A*74:01	HLA-A	carbamazepine	Seizures, Epilepsy
HLA-DRB1*03:01:01:01	HLA-DRB1	carbamazepine	Seizures, Epilepsy
rs506770	HSPA1A	carbamazepine	Seizures, Epilepsy
rs1043620	HSPA1A	carbamazepine	Seizures, Epilepsy
rs3130690	HLA-B	carbamazepine	Seizures, Epilepsy
rs2687116	CYP3A4	carbamazepine	Seizures, Epilepsy
rs3738046	EPHX1	carbamazepine	Seizures, Epilepsy
rs4646440	CYP3A4	carbamazepine	Seizures, Epilepsy
rs3219151	GABRA6	carbamazepine	Seizures, Epilepsy
rs3740067	ABCC2	carbamazepine	Seizures, Epilepsy
rs2290732	GABRA1	carbamazepine	Seizures, Epilepsy
rs1633021	-	carbamazepine	Seizures, Epilepsy
HLA-C*07:04:01	HLA-C	carbamazepine	Seizures, Epilepsy
rs2298771	SCN1A	clobazam	Seizures, Epilepsy
rs2298771	SCN1A	clobazam	Seizures, Epilepsy
rs1057868	POR	clobazam	Seizures, Epilepsy
HLA-C*07:18	HLA-C	lamotrigine	Seizures, Epilepsy
rs6755571	UGT1A4	lamotrigine	Seizures, Epilepsy
HLA-A*68:01:01:01	HLA-A	lamotrigine	Seizures, Epilepsy
HLA-A*23:01:01	HLA-A	lamotrigine	Seizures, Epilepsy
rs6755571	UGT1A4	lamotrigine	Seizures, Epilepsy
rs2011425	UGT1A4	lamotrigine	Seizures, Epilepsy
rs41291556	CYP2C19	mephenytoin	Seizures, Epilepsy
rs370803989	CYP2C19	mephenytoin	Seizures, Epilepsy
rs3758581	CYP2C19	mephenytoin	Seizures, Epilepsy
rs55948420	CYP2C19	mephenytoin	Seizures, Epilepsy
HLA-B*27:09	HLA-B	oxcarbazepine	Seizures, Epilepsy
HLA-DRB1*04:03:01	HLA-DRB1	oxcarbazepine	Seizures, Epilepsy
HLA-A*24:20	HLA-A	phenobarbital	Seizures, Epilepsy
HLA-B*56:02	HLA-B	phenytoin	Seizures, Epilepsy
rs12248560	CYP2C9	phenytoin	Seizures, Epilepsy
rs3758581	CYP2C9	phenytoin	Seizures, Epilepsy

rs774607211	CYP2C9	phenytoin	Seizures, Epilepsy
rs71486745	CYP2C9	phenytoin	Seizures, Epilepsy
rs7900194	CYP2C9	phenytoin	Seizures, Epilepsy
rs544027339	CYP2C9	phenytoin	Seizures, Epilepsy
HLA-B*15:13:01	HLA-B	phenytoin	Seizures, Epilepsy
HLA-DRB1*16:02:01	HLA-DRB1	phenytoin	Seizures, Epilepsy
rs2832407	GRIK1	topiramate	Seizures, Epilepsy
rs2396185	INSR	topiramate	Seizures, Epilepsy
rs2832407	GRIK1	topiramate	Seizures, Epilepsy
rs4984241	CA12	topiramate	Seizures, Epilepsy
rs1731017	ABAT	valproic acid	Seizures, Epilepsy
rs10445704	UGT1A6	valproic acid	Seizures, Epilepsy
rs6759892	UGT1A6	valproic acid	Seizures, Epilepsy
rs13015720	UGT1A6	valproic acid	Seizures, Epilepsy
rs12623271	UGT1A6	valproic acid	Seizures, Epilepsy
rs2307441	POLG	valproic acid	Seizures, Epilepsy
rs3816877	APEH	valproic acid	Seizures, Epilepsy
rs226957	XBP1	valproic acid	Seizures, Epilepsy
rs28898617	UGT1A6	valproic acid	Seizures, Epilepsy
rs6731242	UGT1A10	valproic acid	Seizures, Epilepsy
rs1799883	FABP2	valproic acid	Seizures, Epilepsy
rs1731017	ABAT	valproic acid	Seizures, Epilepsy

Pharmacogenomics bear the potential to guide the choice of most suitable AED for the treatment of epilepsy subtype considering the other factors like age, sex, seizure type, onset age, co-morbidities, imaging patterns and others [Löscher et al., 2009]. However, for epilepsies, reproducible PGx findings are limited to cutaneous ADR caused by aromatic AEDs [(Chung et al., 2004; McCormack et al., 2011; McCormack et al., 2018)]. Neurologists and clinicians are still keen and striving for robust genetic markers which can help them make better choices prior AED administration, and also if such markers can prognose patients with intractable epilepsy prior treatment initiation. The primary a-priori hypothesis of this study was that common polymorphisms would be associated with poor response to AED. Therefore, assessed the role of common genetic variants for drug response to commonly prescribed AEDs monotherapy using a GWAS approach in a cohort of 789 individuals from North India with detailed clinical and demographic profiling. The patients were followed-up at different time interval till 12^{th} month to evaluate their change in seizure frequency after respective AED therapy. All the patients were broadly classified into- “No-seizure or Good responder” if they remained seizure free in the previous 10 months of the study duration, and “Recurrent-seizures or Poor responder” groups if they had one or more seizures during the same period. In our

cohort, we obtained the highest poor response rate to be in patients on PHT (40.99%) and lowest in that on VPA (35.12%).

For the GWAS analysis we used a commercial genome-wide genotyping chip called Illumina Infinium GSA- 34-v2.0 with psych customization. After following several QC steps to exclude poor quality samples and SNPs and performing genome-wide imputation considering 1000G SAS population as reference, we performed genetic association assuming additive genetic model using logistic regression with age, sex and PC1, PC2 as covariates. Likewise, we performed logistic regression using the same covariates to evaluate the association of each SNP for each drug type, Phenytoin, Carbamazepine, and Valproic acid. Functionally annotation of the summary statistics was additionally performed to deduce biological relevance of the SNPs/ genomic loci with respect to the phenotype. Annotations were done for SNP-based and gene-based analysis, with annotations including the ANNOVAR categories, CADD, and RegulomeDB scores. Tissue specific gene set enrichment and GO analysis were performed to identify top functions for each group. Other biological evidence for genotype specific tissue-wise expression was obtained from GTEx v8 and 15-core chromatin state for regulatory potential of non-coding variants. After quality control 691 samples and 2174963 imputed genetic variants remained for statistical analysis. We only considered autosomal SNPs in our analyses. Although we did not observe any GWAS significant ($p < 5 \times 10^{-8}$) loci for any of the groups, several crossed the suggestive loci ($p < 5 \times 10^{-5}$). In the GWAS for overall response, we observed five SNPs (rs6033642, rs970635, rs4891466, rs76487654, rs1037515723) that crossed the suggestive p value threshold (**Table 6.3**) with various eQTL evidence in different brain. The most significant function GO analysis revealed regulation of potassium ion transmembrane transport with highest fold enrichment ($p < 1.0 \times 10^{-3}$). In PHT poor response group, one SNP crossed the suggestive threshold, rs10134329, which is an intronic variant in TTYH3 gene (**Table 6.3**). This gene family are known to form Ca2+ and regulating anion channels with potential role in cell adhesion, migration, and developmental signalling. According to BRAINEAC data, this SNP show differential expression in brain tissues. This variant is predicted to be present in the strong transcription site (Tx) in most of the brain tissues. The tissue expression analysis supports highest differential expression in Brain cerebellar hemisphere and cerebellum. The top GO term was neuron recognition, with 60 fold enrichment ($p< 0.01$). For CBZ poor response, the highest GWAS signal for rs4659128 which is an intronic variant in TBX15 gene (**Table 6.3**). These are a class of transcription factors that are known to regulate a variety of developmental processes. The most significant

curated GO biological process in this group is intracellular signal transduction (p=0.001). The top genomic loci associated with VPA poor response is an intronic SNP (rs11569839/ TNFRSF8) (**Table 6.3**). This gene belongs to the TNF receptor superfamily, predicted to enable transmembrane signalling receptor activity by positive regulation of NF-kappa β transcription factor activity, differentially regulating gene expression in brain issues. Interestingly, this variant is correlated with regulation of one of the most important DME or AED target, MTHFR (p=0.0079) and MTOR (p= 0.043) in temporal cortex and MTHFR in cerebellum (p=0.039). This variant is putatively lying in Tx/Wk or Enhancer region in heart and muscle tissues. The most important function carried out by the associated genes of this group is involved in layer formation in cerebral cortex ($p= 3.74 \times 10^{-5}$).

Summarising the GWAS findings, our study is the first GWAS investigating poor response to AEDs in South Asian population, particularly India. We can say that though our study did not reveal strong genetic association with large effect size contributing to genetic variance of poor response to AED treatment possibly due to limited sample size or suggesting that drug response is a complex trait where multiple loci with small effect size may be associated with it. Thus, our findings should not be considered causal, rather regions of top genomic loci can be important for drug action in different brain tissues in epilepsy. However, interestingly, the top associated loci either regulate other genes that are involved in ADME of AEDs or carry out crucial brain functions or they are differentially expressed in during seizure recurrence. Additionally, GO analysis results showed the highest fold enrichment for functions like regulation of K^+ ion transmembrane transport, neuron development, brain development, voltage-gated Ca^{2+} channel complex, neuron recognition, and so on. These functions are crucial molecular processes carried out during the action of AEDs thereby reducing seizure occurrence. Functional validation of such genes in future may shed light into the molecular mechanistic insights related to AED response.

Table 6.3: Genome-wide association study lead SNPs ($p \leq 1x10^{-5}$) associated with response to respective AED or overall response

rsID	Chr	Pos	Non effect allele	Effect allele	MAF	OR (95%CI)	Function	Adjusted p value	Genes
GR_PR_Overall									
rs6033642	20	13199755	G	C	0.208	1.98(1.50-2.60)	intergenic	$1.185x\ 10^{-6}$	*SPTLC3;ISM1*
rs970635	14	40123072	T	C	0.309	1.73(1.37-2.18)	intergenic	3.975×10^{-6}	*FBXO33;LINC02315*
rs4891466	18	70017821	T	C	0.263	1.78(1.38-2.29)	intergenic	4.072×10^{-6}	*LINC01899;CBLN2*

rs76487654	10	97262755	A	AACAC	0.2955	1.74(1.37-2.2)	intronic	4.829×10^{-6}	*SORBS1*
rs1037515723	6	164059731	C	CTT	0.4724	1.67(1.33-2.07)	intergenic	4.94×10^{-5}	*QKI;LOC102724152*
GR_PR_PHT									
rs6461508	7	2696940	C	T	0.3078	0.23(0.17-0.46)	intronic	1.00×10^{-5}	*TTYH3*
GR_PR_CBZ									
rs4659128	1	119464710	C	T	0.1442	4.82(2.64-8.79)	intronic	2.033×10^{-7}	*TBX15*
rs543063	2	85175660	TA	T	0.4131	0.40(0.27-0.58)	intergenic	3.25×10^{-6}	*KCMF1*
rs73085150	7	22532638	G	C	0.1851	3.50(2.05-5.96)	intronic	4.48×10^{-6}	*STEAP1B*
rs201566762	11	74068812	AG	A	0.4284	0.40(0.27-0.60)	intronic	5.106×10^{-6}	*PGM2L1*
rs6584298	10	101364049	C	T	0.3395	0.36(0.23-0.55)	intergenic	6.848×10^{-6}	*NKX2-3;SLC25A28*
GR_PR_VPA									
rs933406127	1	12157881	G	A	0.1534	3.72(2.04-6.79)	intronic	0.000009498	*TNFRSF8*

Chr: chromosome, Pos: position, MAF: minor allele frequency, OR: odds ratio, CI: confidence interval, Top loci and their annotations including SNP position (GRCh37/hg19 assembly) and gene for genic markers. For SNPs in linkage disequilibrium, only the SNP with the lowest p-value are depicted. Nearby genes are annotated by ANNOVAR. OR is calculated with respect to effect allele. Adjusted p value is calculated by logistic regression using age, sex PC1, PC2 as covariates for binary response trait.

Lastly, in this study we aimed to identify if the associated variants overlap with that of the evidence from available literature including the 80 pharmacogenetic variants identified from objective 1. A strong association is essential but not always sufficient condition to ensure the accuracy of estimating the accuracy of diagnosis. Thus, it is crucial to evaluate the diagnostic ability to correctly identify or predict an outcome of interest, which, in pharmacogenetics, indicates the ability of the test to predict poor response or lack of treatment efficacy. We performed a replication analysis where we obtained pharmacogenomic data predicting poor response to anti-epileptic drugs from four different data sources- PharmGKB, GWAS Catalog, EpiGAD and our previous published article from objective 1(Guin D, 2019a). We compared our results obtained from our high throughput genome analysis for overall poor response predicting to prescribed AEDSs, we found an overlap of 19 SNPs as enlisted in Table 5. We then estimated the diagnostic accuracy of these markers to identify if they qualify to be used for clinical applications to predict poor response to AEDS. These markers which sufficient efficiency may be beneficial for population specific diagnostic applications although it requires replication in independent cohort as well. Additionally, we identified 88 PGx variants from the literature which are approved by FDA, CPIC, SwissMedic, EMA and others and are currently used for commercial purposes. These are overlapping with the data obtained from the different sources in literature. We observed of the 19 SNPs, 8 are already in commercial use. Assessing the diagnostic predictability of these 19 markers showed moderate accuracy (50-60%). Although, these markers are promising candidates for PGx application after appropriate validation and replication, suggesting that although they cannot be solely considered for

predicting response outcomes, other clinical or biochemical variables can improve the diagnostic accuracy. Eight out of these 19 markers are already in use for drug labelling approved by the FDA. Strengthening the fact that genome-based markets can be exploited for application in precision medicine in epilepsy treatment.

6.2 Future Direction

GWAS findings are no endpoint results which can be used readily at clinical settings. Several future perspectives can strengthen the results. Firstly, GWAS findings from our study require replication in an independent patient cohort as well as functional characterization of the genetic variations identified are still required to assess the biological relevance of the associated genes in modulating the risk of poor response to AED therapy. If these genes are regulating other genes which are involved in the ADME or mode of action of AEDs or if other secondary signalling molecules are triggered by these genes and how the differential regulation of these genes in specific brain tissues needs to be addressed to decipher the exact biological mechanisms behind the observed associations. Secondly, combining studies or homogenous larger data from publically available biobanks are powerful statistical approach to gain power in statistical analysis. Thirdly, identifying the polygenic basis of drug response from GWAS data in one of the new avenue to be explored in PGx studies. And lastly, association studies targeted to identify rare variants and structural variants can be crucial to assess the true biological function in PGx response.

6.3 Conclusion

Recent advances hold promise that pharmacogenomics will positively impact treatment for any epilepsy patient in the near future, towards implementation of an evidenced-based strategy for improving the use of AEDs, thereby providing a cornerstone for precision medicine. Thus, we identified 2304 PGx relationships pertaining to 1753 disease types and 666 drugs. Our semi-automated text mining pipeline can be exploited to generate PGx relationships published for medications administered among different types of diseases. Thus, apply broadly to a variety of diseases and their respective drugs administered. On comparative analysis with currently used FDA approved PGx drug label biomarkers commercially available, a 68% overlap (127 markers) of our approach confirms the accuracy of our approach and demonstrates that these text-mined results are potentially useful for clinical value addition and widening the spectrum of clinical curation and improving therapeutic services. Further our GWAS study on AEDs response in an Indian cohort for individual AEDs response did not reveal any genome-

wide significant association for drug response in any of the groups, we identified several suggestive loci. Future similar studies should attempt to reproduce our findings, apart from the GWAS threshold ($p < 5 \times 10^{-8}$). Our study mainly explored the common variants, future hypothesis-driven research focusing on rare or structural variants, similar to such studies may provide clues towards improving AEDs treatment outcome. More studies with similar study design are required to replicate the findings and functional assays to elucidate the role of common variants in future analysis. Lastly, on overlapping findings from objective 1 and 2, 88 commercial PGx marker are known related to AED response. Among these 19 SNPs overlapped with our GWAS findings for overall poor response. Assessing the diagnostic predictability of these 19 markers showed moderate accuracy (50-60%). These markers are promising candidates for PGx application after appropriate validation and replication. Eight out of these 19 markers are already in use for drug labelling approved by the FDA. Strengthening the fact that genome-based markets can be exploited for application in precision medicine in epilepsy treatment.

References

1. A., S. (2022). Cenobamate for treatment-resistant focal seizures: current evidence and place in therapy. . J Cent Nerv Syst Dis., 14, 11795735211070209. . doi:doi: 10.1177/11795735211070209.

2. Abe, T., et al. (2008). Association between SCN1A polymorphism and carbamazepine-resistant epilepsy. Br J Clin Pharmacol, 66(2), 304-307. doi:10.1111/j.1365-2125.2008.03203.x

3. Addis L, R. R., Valentin A, Makoff A, Robinson R, Everett KV, Nashef L, Pal DK. . (2016). Analysis of rare copy number variation in absence epilepsies. . Neurol Genet., 2(2), e56. doi:doi: 10.1212/NXG.0000000000000056.

4. Aldenkamp AP, B. G. (1997). The Neurotoxicity Scale-II: results of a patient-based scale assessing neurotoxicity in patients with epilepsy. . Epilepsy Res., 27, 165-173.165.

5. Allabi, A. C., et al. (2003). Genetic polymorphisms of CYP2C9 and CYP2C19 in the Beninese and Belgian populations. Br J Clin Pharmacol, 56(6), 653-657.

6. Alshabeeb, M. A., Deneer, V.H., and Asselbergs, F.W. (2019). Use of pharmacogenetic drugs by the Dutch population. Front. Genet., 10, 567.

7. Amstutz, U., et al. (2018). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. Clin Pharmacol Ther, 103(2), 210-216. doi:10.1002/cpt.911

8. Amstutz U, H. L., Offer SM, et al. . (2018a). Clinical Pharmacogenetics implementation Consortium (CPIC) guideline for Dihydropyrimidine dehydrogenase genotype and Fluoropyrimidine dosing: 2017 update. . Clin Pharmacol Ther., 103(2), 210-216.

9. Amstutz U, H. L., Offer SM, et al. . (2018b). Clinical Pharmacogenetics implementation Consortium (CPIC) guideline for Dihydropyrimidine dehydrogenase genotype and Fluoropyrimidine dosing: 2017 update. . Clin Pharmacol Ther, 103(2), 210-216.

10. Anderson, G. D. (1998). A mechanistic approach to antiepileptic drug interactions. Ann Pharmacother, 32(5), 554-563.

11. Anne T., J.-M. D., Claudiu T.S., Bernard M. . (2007). Carbonic Anhydrase Inhibitors as Anticonvulsant Agents. Curr. Top. Med. Chem., 7, 855-864. .

12. Ayata, P., et al. (2018). Epigenetic regulation of brain region-specific microglia clearance activity. Nat Neurosci, 21(8), 1049-1060. doi:10.1038/s41593-018-0192-3

13. B., A.-K. (2008). Levetiracetam in the treatment of epilepsy. . Neuropsychiatr Dis Treat., 4(3), 507-523. doi:doi: 10.2147/ndt.s2937.

14. Baker GA, F. P., Middleton E. . (1994). Initial development, reliability, and validity of a patient-based adverse events scale. . Epilepsia, 35 (suppl 7), 80.

15. Balestrini S, S. S. (2018). Pharmacogenomics in epilepsy. Neurosci. Lett., 667, 27-39.

16. Banerjee PN, F. D., Allen Hauser W. . (2009). The descriptive epidemiology of epilepsy -a review. . Epilepsy Res., 85(1), 31-45.

17. Bauer J, S. B., Lenders T, et al. . (1995). Efficacy and tolerabilty of tiagabine: Results of an add-on study in patients with refractory partial seizures. . J Epilepsy, 8, 83-86. .

18. Bauer S, W. L., Paule E, Petschow C, Zöllner JP, Rosenow F, Strzelczyk A. . (2017). The efficacy of lacosamide as monotherapy and adjunctive therapy in focal epilepsy and its use in status epilepticus: clinical trial evidence and experience. . Ther Adv Neurol Disord, 10(2). doi:doi: 10.1177/1756285616675777.

19. Berghuis, B., et al. (2019). A genome-wide association study of sodium levels and drug metabolism in an epilepsy cohort treated with carbamazepine and oxcarbazepine. Epilepsia Open, 4(1), 102-109. doi:10.1002/epi4.12297

20. Bialer, M., et al. (2010). Key factors in the discovery and development of new antiepileptic drugs. Nat Rev Drug Discov, 9(1), 68-82. doi:10.1038/nrd2997

21. BIOCODEX. (2018). DIACOMIT (stiripentol) [package insert]. U.S. Food and Drug Administration.

22. Biton V, S. J., Vuong A, Hammer AE, Barrett PS, Messenheimer JA. . (2005). Double-blind, placebo-controlled study of lamotrigine in primary generalized tonic-clonic seizures. Neurology, 65(11), 1737-1743. . doi:doi: 10.1212/01.wnl.0000187118.19221.e4.

23. Bourgeois, S. e. a. (2016). A multi-factorial analysis of response to warfarin in a UK prospective cohort. . Genome Med., 8(2).

24. Brodie, M. J., et al. (2000). Management of epilepsy in adolescents and adults. Lancet, 356(9226), 323-329. doi:10.1016/S0140-6736(00)02515-0

25. Brodie MJ, L. H., Gil-Nagel A, et al. . Efficacy and safety of adjunctive ezogabine (retigabine) in refractory partial epilepsy. . Neurology., 75, 1817-1824. .

26. Byun E, C. S., Montalban X et al. (2008). Genome-wide pharmacogenomic analysis of the response to interferon β therapy in multiple sclerosis. . Arch. Neurol., 65(3), 337-344.

27. Callaghan N, K. R., O'Neill B, Crowley M, Goggin T. . (1985). A prospective study between carbamazepine, phenytoin and sodium valproate as monotherapy in previously untreated and recently diagnosed patients with epilepsy. . J Neurol Neurosurg Psychiatry., 48(7), 639-644. doi:doi: 10.1136/jnnp.48.7.639.

28. Carr DF, T. R., Pirmohamed M. . (2021). Pharmacogenomics of anticancer drugs: Personalising the choice and dose to manage drug response. . Br J Clin Pharmacol., 87(2), 237-255. doi:doi: 10.1111/bcp.14407

29. Caudle, K. E., et al. (2013). Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther, 94(6), 640-645. doi:10.1038/clpt.2013.172

30. Cavalleri, G. L., et al. (2011). Pharmacogenomics and epilepsy: the road ahead. Pharmacogenomics, 12(10), 1429-1447. doi:10.2217/pgs.11.85

31. Cavalleri GL, M. M., Alhusaini S, Chaila E, Delanty N. . (2011). Pharmacogenomics and epilepsy: the road ahead. . Pharmacogenomics., 12(10), 1429-1447. . doi:doi: 10.2217/pgs.11.85.

32. Cephalon, I. (2009). GABITRIL®(tiagabine hydrochloride) [package insert]. U.S. Food and Drug Administration.

33. Cereghino JJ, B. V., Abou-Khalil B, Dreifuss F, Gauer LJ, Leppik I. (2000). Levetiracetam for partial seizures: results of a double-blind, randomized clinical trial. . Neurology, 55(2), 236-242. doi:doi: 10.1212/wnl.55.2.236.

34. Chan, A., et al. (2011). Pharmacogenomics in neurology: current state and future steps. Ann Neurol, 70(5), 684-697. doi:10.1002/ana.22502

35. Chanfreau-Coffinier, C., Hull, L.E., Lynch, J.A., DuVall, S.L., Damrauer, S.M., Cunningham, F.E., Voight, B.F., Matheny, M.E., Oslin, D.W., Icardi, M.S., et al. (2019). Projected prevalence of actionable pharmacogenetic variants and level a drugs prescribed among US veterans health administration pharmacy users. . JAMA Netw. Open, 2, e195345.

36. Chappell, A. S., et al. (2002). A crossover, add-on trial of talampanel in patients with refractory partial seizures. Neurology, 58(11), 1680-1682. doi:10.1212/wnl.58.11.1680

37. Chaudhry, A. S., et al. (2010). CYP2C9*1B promoter polymorphisms, in linkage with CYP2C19*2, affect phenytoin autoinduction of clearance and maintenance dose. J Pharmacol Exp Ther, 332(2), 599-611. doi:10.1124/jpet.109.161026

38. Chu, X. M., et al. (2012). Influence of UDP-glucuronosyltransferase polymorphisms on valproic acid pharmacokinetics in Chinese epilepsy patients. Eur J Clin Pharmacol, 68(10), 1395-1401. doi:10.1007/s00228-012-1277-7

39. Chun-Yu Wei, M.-T. M. L., Yuan-Tsong Chen, . (2012). Pharmacogenomics of adverse drug reactions: implementing personalized medicine. Hum Mol Genet, 21(R1), R58-R65, . doi:https://doi.org/10.1093/hmg/dds341

40. Ciccone L, C. C., Nencetti S, Orlandini E. . (2021). Carbonic Anhydrase Inhibitors and Epilepsy: State of the Art and Future Perspectives. . Molecules, 26(21), 6380. doi:doi: 10.3390/molecules26216380.

41. ClinCalc. The Top 200 for 2020. .

42. Co, E. R. D. M. (2012). FYCOMPA® (perampanel) [package insert]. U.S. Food and Drug Administration.

43. Co., S. B. XCOPRI® (cenobamate tablets) [package insert] U.S. Food and Drug Administration.

44. Connor, S. (2003). Glaxo chief: Our drugs do not work on most patients. Independent (Lond.).

45. Cooper-DeHoff, R. M., et al. (2022). The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1, ABCG2, and CYP2C9 genotypes and Statin-Associated Musculoskeletal Symptoms. Clin Pharmacol Ther, 111(5), 1007-1021. doi:10.1002/cpt.2557

46. Corporation, N. P. (2000). TRILEPTAL® (oxcarbazepine) [package insert]. U.S. Food and Drug Administration.

47. Corporation, N. P. (2009a). AFINITOR (everolimus) [package insert]. U.S. Food and Drug Administration.

48. Corporation, N. P. (2009b). Tegretol® (carbamazepine) [package insert]. U.S. Food and Drug Administration.

49. Cunningham S, G. C., Hutchinson M et al. (2005). Pharmacogenomics of responsiveness to interferon IFN-β treatment in multiple sclerosis: a genetic screen of 100 type I interferon-inducible genes. . Clin. Pharmacol. Ther., 78(6), 635-646.

50. de Leon, J. (2009). Pharmacogenomics: the promise of personalized medicine for CNS disorders. . Neuropsychopharmacology, 34, 159-172.

51. de Leon, J. (2016). Pharmacogenetic tests in psychiatry: From fear to failure to hype. . J. Clin. Psychopharmacol., 36, 299-304.

52. Dlugos, D. J., et al. (2006). Defining the clinical role of pharmacogenetics in antiepileptic drug therapy. Pharmacogenomics J, 6(6), 357-359. doi:10.1038/sj.tpj.6500379

53. Dong, Z. L., et al. (2012). Effect of CYP3A4*1G on the fentanyl consumption for intravenous patient-controlled analgesia after total abdominal hysterectomy in Chinese Han population. J Clin Pharm Ther, 37(2), 153-156. doi:10.1111/j.1365-2710.2011.01268.x

54. Dunnenberger, H. M., Crews, K.R.,Hoffman, J.M.,Caudle,K.E., Broeckel, U., Howard, S.C., Hunkler, R.J., Klein, T.E., Evans, W.E., and Relling, M.V. (2015). . (2015). Preemptive clinical pharmacogenetics

implementation: current programs in fiveUSmedical centers. . Annu. Rev. Pharmacol. Toxicol., 55, 89-106. .

55. Eisai, I. (2008). BANZEL® (rufinamide) [package insert]. U.S. Food and Drug Administration.

56. Fakhoury T, C. A., Bensalem-Owen M. . (2007). Effectiveness of diazepam rectal gel in adults with acute repetitive seizures and prolonged seizures: a single-center experience. . Epilepsy Behav, 11(3), 357-360. doi:doi: 10.1016/j.yebeh.2007.05.014.

57. FDA, U. S. (2018). Pharmacogenomics: Overview of the Genomics and Targeted Therapy Group. Pharmacogenomic Biomarkers in Drug Labels from the FDA Web site.

58. FDA. (2022a). Table of Pharmacogenetic Associations. .

59. FDA. (2022b). Table of Pharmacogenomic Biomarkers in Drug Labeling. .

60. Fejerman N, C. R., Cersósimo R, Ferraro SM, Galicchio S, Amartino H. (2012). Sulthiame add-on therapy in children with focal epilepsies associated with encephalopathy related to electrical status epilepticus during slow sleep (ESES). Epilepsia, 53(7), 1156-1161. doi:doi: 10.1111/j.1528-1167.2012.03458.x.

61. Ferrell, P. B., Jr, & McLeod, H. L. (2008). Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. . Pharmacogenomics, 9(10), 1543-1546. . doi:https://doi.org/10.2217/14622416.9.10.1543

62. Flood, W. D., et al. (2004). Nxf and Fbxo33: novel seizure-responsive genes in mice. Eur J Neurosci, 20(7), 1819-1826. doi:10.1111/j.1460-9568.2004.03646.x

63. Franco V, P. E. (2015). CYP2C9 polymorphisms and phenytoin metabolism: implications for adverse effects. Expert Opin Drug Metab Toxicol., 11(8), 1269-1279. . doi:doi: 10.1517/17425255.2015.1053463.

64. Franz DN, L. J., Yapici Z, Ikeda H, Polster T, Nabbout R, Curatolo P, de Vries PJ, Dlugos DJ, Herbst F, Peyrard S, Pelov D, French JA. . (2021). Adjunctive everolimus therapy for tuberous sclerosis complex-associated refractory seizures: Results from the postextension phase of EXIST-3. . Epilepsia., 62(12), 3029-3041. doi:doi: 10.1111/epi.17099.

65. French JA, K. M., Bibbiani MD, et al. . (2015). Perampanel for tonic-clonic seizures in idiopathic generalized epilepsy. . Neurology., 85(11), 950-957. doi:DOI: 10.1212/WNL.0000000000001930

66. G., B. (1984). Psychoneuroendocrinology. . Pharmacology of the GABAergic system: effects of progabide, a GABA receptor agonist., 9(2), 135-140. doi:doi: 10.1016/0306-4530(84)90032-5.

67. G.L., H. (1990). The use of benzodiazepines in epilepsy and febrile seizures. CLEVELAND CLINIC JOURNAL OF MEDICINE, 57(1 Suppl 1), S31-38. doi:https://www.ccjm.org/content/57/1_suppl_1/S-31

68. Gammal, R. S., et al. (2023). Expanded Clinical Pharmacogenetics Implementation Consortium Guideline for Medication Use in the Context of G6PD Genotype. Clin Pharmacol Ther, 113(5), 973-985. doi:10.1002/cpt.2735

69. Gauthier AC, M. R. (2015). Clobazam: A Safe, Efficacious, and Newly Rediscovered Therapeutic for Epilepsy. . CNS Neurosci Ther., 21(7), 543-548. . doi:doi: 10.1111/cns.12399.

70. Gervasini G, B. J., Carrillo JA. . (2010). Pharmacogenetic testing and therapeutic drug monitoring are complementary tools for optimal individualization of drug therapy. . Eur J Clin Pharmacol 2010;, 66(8), 755-774.

71. Gil-Nagel A., L.-L. J., Almeida L., Maia J., Soares-Da-Silva P. (2009). Efficacy and safety of 800 and 1200 mg eslicarbazepine acetate as adjunctive treatment in adults with refractory partial-onset seizures. Acta Neurol Scand, 120, 281–287. doi:doi: 10.1111/j.1600-0404.2009.01218.x.

72. Glauser T, B.-M. E., Bourgeois B et al. . (2013). Updated ILAE evidence review of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. . Epilepsia, 54(3), 551-563.

73. Glauser T, K. G., Sachdeo R, Krauss G, Perdomo C, Arroyo S. . (2008). Rufinamide for generalized seizures associated with Lennox-Gastaut syndrome. . Neurology, 70(21), 1950-1958. doi:doi: 10.1212/01.wnl.0000303813.95800.0d.

74. GlaxoSmithKline. (2015). LAMICTAL (lamotrigine) [package insert]. U.S. Food and Drug Administration.

75. GlaxoSmithKline, V. P. a. (2012). POTIGA (ezogabine) [paxkage insert]. U.S. Food and Drug Administration.

76. Goetz, M. P., et al. (2018). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. Clin Pharmacol Ther, 103(5), 770-777. doi:10.1002/cpt.1007

77. Goetz MP, S. K., Guchelaar HJ, et al. . (2018). Clinical Pharmacogenetics implementation Consortium (CPIC) guideline for CYP2D6 and tamoxifen therapy. . Clin Pharmacol Ther., 103, 770-777.

78. Gong, L., Whirl-Carrillo, M., & Klein, T. E. . (2021). PharmGKB, an Integrated Resource of Pharmacogenomic Knowledge. . Current protocols, 1(8), e226. doi:https://doi.org/10.1002/cpz1.226

79. Goto, S., et al. (2007). Population estimation of the effects of cytochrome P450 2C9 and 2C19 polymorphisms on phenobarbital clearance in Japanese. Ther Drug Monit, 29(1), 118-121. doi:10.1097/FTD.0b013e318030def0

80. Gottesman O, K. H., Tromp G, et al. . (2013). The electronic medical records and genomics (eMERGE) Network: past, present, and future. . Genetics in Medicine, 15, 761-771.

81. Grover, S., et al. (2012). Genetic association analysis of transporters identifies ABCC2 loci for seizure control in women with epilepsy on first-line antiepileptic drugs. Pharmacogenet Genomics, 22(6), 447-465. doi:10.1097/FPC.0b013e3283528217

82. Grover, S., et al. (2013). A systematic review and meta-analysis of the role of ABCC2 variants on drug response in patients with epilepsy. Epilepsia, 54(5), 936-945. doi:10.1111/epi.12132

83. Grover, S., et al. (2010). Genetic polymorphisms in sex hormone metabolizing genes and drug response in women with epilepsy. Pharmacogenomics, 11(11), 1525-1534. doi:10.2217/pgs.10.120

84. Guin D, H. Y., Kukreti R. (2023). Assessment of clinically actionable pharmacogenetic markers to stratify anti-seizure medications. The Pharmacogenomic Journal, 10, 1038. doi:doi.org/10.1038/s41397-023-00313-y

85. Guin, D., Mishra, M.K., Talwar, P. et al. . (2017). A systematic review and integrative approach to decode the common molecular link between levodopa response and Parkinson's disease. . BMC Med Genomics, 10, 56. doi:https://doi.org/10.1186/s12920-017-0291-0

86. Guin D, R. J., Singh P, Grover S, Bora S, Talwar P, Karthikeyan M, Satyamoorthy K, Adithan C, Ramachandran S, Saso L, Hasija Y, Kukreti R. . (2019a). Global Text Mining and Development of Pharmacogenomic Knowledge Resource for Precision Medicine. . Front Pharmacol., 10, 839. doi:doi: 10.3389/fphar.2019.00839.

87. Guin D, R. J., Singh P, Grover S, Bora S, Talwar P, Karthikeyan M, Satyamoorthy K, Adithan C, Ramachandran S, Saso L, Hasija Y, Kukreti R. . (2019b). Global Text Mining and Development of

Pharmacogenomic Knowledge Resource for Precision Medicine. . Front Pharmacol., 2019(10), 839. doi:doi: 10.3389/fphar.2019.00839.

88. Gurwitz D, P. M. (2010). Pharmacogenomics: the importance of accurate phenotypes. . Pharmacogenomics, 11, 469-470.

89. Haerian, B. S., et al. (2011). Association of ABCB1 gene polymorphisms and their haplotypes with response to antiepileptic drugs: a systematic review and meta-analysis. Pharmacogenomics, 12(5), 713-725. doi:10.2217/pgs.10.212

90. Halford JJ, B.-M. E., Kwan P, Ness S, Schmitt J, Eerdekens M, Novak G. . (2011). A randomized, double-blind, placebo-controlled study of the efficacy, safety, and tolerability of adjunctive carisbamate treatment in patients with partial-onset seizures. . Epilepsia, 52(4), 816-825. doi:doi: 10.1111/j.1528-1167.2010.02960.x.

91. Hanrahan B, C. R. (2022). Ethosuximide. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing;.

92. Haroon A, T. M., Khanam R, Vohora D. . (2012). Antiepileptic drugs prescription utilization behavior and direct costs of treatment in a national hospital of India. . Ann Indian Acad Neurol., 15(4), 289-293. doi:doi: 10.4103/0972-2327.104338.

93. Heaney DC, M. B., Everitt A et al. . (2002). Socioeconomic variation in incidence of epilepsy: prospective community based studyin south east England. . BMJ, 325(7371), 1013-1016.

94. Hilger, E., et al. (2012). Lack of association between ABCC2 gene variants and treatment response in epilepsy. Pharmacogenomics, 13(2), 185-190. doi:10.2217/pgs.11.143

95. Hoffman, J. M. e. a. (2014). PG4KDS: a model for the clinical implementation of pre-emptive pharmacogenetics. . Am. J. Med. Genet. C. Semin. Med. Genet., 166c, , 45–55.

96. Hoffmann-La Roche, I. (2016). VALIUM® (Diazepam) [package insert]. U.S. Food and Drug Administration.

97. Howes, J. F., et al. (2007). Talampanel. Neurotherapeutics, 4(1), 126-129. doi:10.1016/j.nurt.2006.11.001

98. Hung, C. C., et al. (2013). Gene-wide tagging study of the effects of common genetic polymorphisms in the alpha subunits of the GABA(A) receptor on epilepsy treatment response. Pharmacogenomics, 14(15), 1849-1856. doi:10.2217/pgs.13.158

99. Inc, G. (2013). KLONOPIN TABLETS [package insert]. U.S. Food and Drug Administration.

100. Inc, L. (2010). Peganone® (Ethotoin) [package insert]. U.S. Food and Drug Administration.

101. Inc, M. P. (1993). FELBATOL® (felbamate) [package insert]. U.S. Food and Drug Administration.

102. Inc., A. (1978). DEPAKENE (valproic acid) [package insert]. U.S. Food and Drug Administration.

103. Innocenti F, U. S., Iyer L, et al. . (2004). Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. . J Clin Oncol, 22, 1382-1388.

104. Inoue Y, O. Y. S. G. (2014). Effectiveness of add-on stiripentol to clobazam and valproate in Japanese patients with Dravet syndrome: Additional supportive evidence. . Epilepsy Res., 108(4), 725-731.

105. investigators, T. A. o. U. r. p. (2019). The “All of Us” Research Program. N Engl J Med, 381, 668-676 doi:DOI: 10.1056/NEJMsr1809937

106. Iwamoto FM, K. T., Kim L,Duic JP,Butman JA, Albert PS,Fine HA. (2010). Phase 2 trial of talampanel, a glutamate receptor inhibitor, for adults with recurrent malignant gliomas. Cancer, 116(7), 1776-1782. doi:https://doi.org/10.1002/cncr.24957

107. J.A.P. Van Parys, H. M. (1994). Survey of 260 epileptic patients treated with oxcarbazepine on named-patient basis. Epilepsy Res, 19, 79-85. doi:https://www.sciencedirect.com/science/article/abs/pii/0920121194900914

108. Jang, H. W., et al. (2015). GWAS identifies two susceptibility loci for lamotrigine-induced skin rash in patients with epilepsy. Epilepsy Res, 115, 88-94. doi:10.1016/j.eplepsyres.2015.05.014

109. Janssen Pharmaceuticals, I. (1996). TOPAMAX® (topiramate) [package insert]. U.S. Food and Drug Administration.

110. Jaruthamsophon, K., Thomson, P. J., Sukasem, C., Naisbitt, D. J. & Pirmohamed, M. . (2021). HLA allele-restricted immune-mediated adverse drug reactions: framework for genetic prediction. . Annu. Rev. Pharmacol. Toxicol., 62, 509-529.

111. Jennum P, C. J., Ibsen R, Kjellberg J. . (2016). Long-term socioeconomic sonsequences and health care costs of childhood and adolescent-onset epilepsy. . Epilepsia., 57, 1078-1085.

112. Jiang, D., et al. (2009). Effects of CYP2C19 and CYP2C9 genotypes on pharmacokinetic variability of valproic acid in Chinese epileptic patients: nonlinear mixed-effect modeling. Eur J Clin Pharmacol, 65(12), 1187-1193. doi:10.1007/s00228-009-0712-x

113. Johnson JA, G. L., Whirl-Carrillo M, et al. . (2011). Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. . Clin Pharmacol Ther, 90, 625-629.

114. JW., S. (2003). The epidemiology of epilepsy revisited. . Curr. Opin. Neurol., 16(2), 165-170.

115. Kerr, B. M., et al. (1994). Human liver carbamazepine metabolism. Role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. Biochem Pharmacol, 47(11), 1969-1979.

116. Kim, D. W., et al. (2009). Lack of association between ABCB1, ABCG2, and ABCC2 genetic polymorphisms and multidrug resistance in partial epilepsy. Epilepsy Res, 84(1), 86-90. doi:10.1016/j.eplepsyres.2008.12.001

117. Kirchner GI, M.-W. I., Manns MP. . (2004). Clinical pharmacokinetics of everolimus. . Clin Pharmacokinet., 43(2), 83-95. . doi:doi: 10.2165/00003088-200443020-00002.

118. Knight EMP, A. S., Bahi-Buisson N, Benke TA, Cross JH, Demarest ST, Olson HE, Specchio N, Fleming TR, Aimetti AA, Gasior M, Devinsky O; Marigold Trial Group. (2022). Safety and efficacy of ganaxolone in patients with CDKL5 deficiency disorder: results from the double-blind phase of a randomised, placebo-controlled, phase 3 trial. . Lancet Neurol., 21(5), 417-427. doi:https://doi.org/10.1016/S1474-4422(22)00077-1

119. Knupp KG, S. I., Ceulemans B, Sullivan JE, Nickels KC, Lagae L, Guerrini R, Zuberi SM, Nabbout R, Riney K, Shore S, Agarwal A, Lock M, Farfel GM, Galer BS, Gammaitoni AR, Davis R, Gil-Nagel A. . (2022). Efficacy and Safety of Fenfluramine for the Treatment of Seizures Associated With Lennox-Gastaut Syndrome: A Randomized Clinical Trial. JAMA Neurol, 79(6), 554-564. doi:doi: 10.1001/jamaneurol.2022.0829.

120. Korinthenberg R, B. P., Woelfle C, Moenting JS, Ernst JP. . (2007). Pharmacology, efficacy, and tolerability of potassium bromide in childhood epilepsy. J Child Neurol., 22(4), 414-418. doi:doi: 10.1177/0883073807302758.

121. Krall, R. L., et al. (1978). Antiepileptic drug development: I. History and a program for progress. Epilepsia, 19(4), 393-408.

122. Krebs, K., and Milani, L. (2019). Translating pharmacogenomics into clinical decisions: do not let the perfect be the enemy of the good. . Hum. Genomics, 13, 1–13.

123. Kulig K, M. B. (2007). Carisbamate, a new carbamate for the treatment of epilepsy. IDrugs, 10(10), 720-727.

124. Kumari, R., et al. (2011). Pharmacogenomic association study on the role of drug metabolizing, drug transporters and drug target gene polymorphisms in drug-resistant epilepsy in a north Indian population. Indian J Hum Genet, 17 Suppl 1, S32-40. doi:10.4103/0971-6866.80357

125. Kumari, R., et al. (2013). SCN1AIVS5-91G>A polymorphism is associated with susceptibility to epilepsy but not with drug responsiveness. Biochimie, 95(6), 1350-1353. doi:10.1016/j.biochi.2013.02.006

126. Kutt, H., et al. (1968). Management of epilepsy with diphenylhydantoin sodium. Dosage regulation for problem patients. JAMA, 203(11), 969-972.

127. Kwan, P., et al. (2007). Association between ABCB1 C3435T polymorphism and drug-resistant epilepsy in Han Chinese. Epilepsy Behav, 11(1), 112-117. doi:10.1016/j.yebeh.2007.04.013

128. Kwan, P., et al. (2011). Gene-wide tagging study of the association between ABCC2, ABCC5 and ABCG2 genetic polymorphisms and multidrug resistance in epilepsy. Pharmacogenomics, 12(3), 319-325. doi:10.2217/pgs.10.183

129. Laboratories, A. (1999). TRIDIONE® (Trimethadione) [package insert]. U.S. Food and Drug Administration.

130. Langan YM, L. R., Jewell H, Toublanc N, Schaefer H, Sander JW, Patsalos PN. . (2003). Talampanel, a new antiepileptic drug: single- and multiple-dose pharmacokinetics and initial 1-week experience in patients with chronic intractable epilepsy. . Epilepsia., 44, 46-53.

131. Lavertu, A. e. a. (2018). Pharmacogenomics and big genomic data: from lab to clinic and back again. . Hum. Mol. Genet. 27,, 27, R72-R78.

132. Lazarowski, A., et al. (2011). Potential role of multidrug resistant proteins in refractory epilepsy and antiepileptic drugs interactions. Drug Metabol Drug Interact, 26(1), 21-26. doi:10.1515/DMDI.2011.006

133. Leaman R, I. D. R., Lu Z. . (2013). DNorm: disease name normalization with pairwise learning to rank. Bioinformatics (Oxford, England). 29((22):), 2909±2917.

134. Lee, C. R., et al. (2022). Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update. Clin Pharmacol Ther, 112(5), 959-967. doi:10.1002/cpt.2526

135. Lenkapothula N, C. M. (2022). Primidone. . In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

136. Leschziner GD, A. T., Pirmohamed M, Johnson MR. . (2007). ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. Pharmacogenomics J, 7, 154–179.

137. Leyva L, F. O., Fedetz M et al. (2005). IFNAR1 and IFNAR2 polymorphisms confer susceptibility to multiple sclerosis but not to interferon-β treatment response. J. Neuroimmunol. , 163(1-2), 165-171.

138. Li B, H. C., Brohawn SG. . (2021). Structures of tweety homolog proteins TTYH2 and TTYH3 reveal a Ca2+-dependent switch from intra- to intermembrane dimerization. Nat Commun., 12(1), 6913. doi:doi: 10.1038/s41467-021-27283-8.

139. Li, B., et al. (2021). Structures of tweety homolog proteins TTYH2 and TTYH3 reveal a Ca(2+)-dependent switch from intra- to intermembrane dimerization. Nat Commun, 12(1), 6913. doi:10.1038/s41467-021-27283-8

140. LIVINGSTON S, P. L., KRAMER I, NAJMABADI A. . (1961). Clinical evaluation of 1-methyl-5,5-phenyl-ethyl-hydantoin in the treatment of epilepsy. N Engl J Med., 265, 418-421. doi:doi: 10.1056/NEJM196108312650904.

141. Löscher W, K. H., Twyman RE, Schmidt D. . (2013). New avenues for anti-epileptic drug discovery and development. . Nat Rev Drug Discov., 12(10), 757-776. doi:doi: 10.1038/nrd4126.

142. Löscher W, K. U., Zimprich F, Schmidt D. . (2009). The clinical impact of pharmacogenetics on the treatment of epilepsy. Epilepsia., 50(1), 1-23. . doi:doi: 10.1111/j.1528-1167.2008.01716.x.

143. Loscher, W., et al. (1994). Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? Epilepsy Res, 17(2), 95-134.

144. Luna-Tortos, C., et al. (2008). Several major antiepileptic drugs are substrates for human P-glycoprotein. Neuropharmacology, 55(8), 1364-1375. doi:10.1016/j.neuropharm.2008.08.032

145. Lundbeck. (2009). SABRIL (vigabatrin) [pckage insert]. U.S. Food and Drug Administration.

146. Lundbeck. (2011). ONFI® (clobazam) [package insert]. U.S. Food and Drug Administration.

147. Lunenburg CATC, v. d. W. C., Nijenhuis M, et al. . (2020). Dutch Pharmacogenetics working group (DPWG) guideline for the gene–drug interaction of DPYD and fluoropyrimidines. . Eur J Hum Genet, 28(4), 508-517.

148. M, C. (2008). Pharmacogenomics in multiple sclerosis: getting the right medicine to the right patient. Therapy, 5(5), 623-629.

149. M., P. (2010). Acceptance of biomarker-based tests for application in clinical practice: Criteria and obstacles. . Clin Pharmacol Ther, 88(6), 862-866.

150. M€uller, D. J., Kekin, I., Kao, A.C.C., and Brandl, E.J. (2013). . (2013). Towards the implementation of CYP2D6 and CYP2C19 genotypes in clinical practice: Update and report from a pharmacogenetic service clinic. . Int. Rev. Psychiatry, 25, 554-571.

151. Ma, C. L., et al. (2014). Association of SCN1A, SCN2A and ABCC2 gene polymorphisms with the response to antiepileptic drugs in Chinese Han patients with epilepsy. Pharmacogenomics, 15(10), 1323-1336. doi:10.2217/pgs.14.89

152. Madian AG, W. H., Jones RB, Dolan ME. . (2012). Relating human genetic variation to variation in drug responses. . Trends Genet., 28(487-95.).

153. Manna, I., et al. (2011). A functional polymorphism in the SCN1A gene does not influence antiepileptic drug responsiveness in Italian patients with focal epilepsy. Epilepsia, 52(5), e40-44. doi:10.1111/j.1528-1167.2011.03097.x

154. Maranville, J. C. C., N. J. . (2016). Pharmacogenomic variants have larger effect sizes than genetic variants associated with other dichotomous complex traits. . Pharmacogenomics J., 16, 388-392.

155. Marinus Pharmaceuticals, I. (2022). ZTALMY® (ganaxolone) [package insert]. U.S. Food and Drug Administration.

156. Marson AG, A.-K. A., Alwaidh M, et al. (2007). The SANAD study of effectiveness of carbamazepine, gabapentin, lamotrigine, oxcarbazepine, or topiramate for treatment of partial epilepsy: an unblinded randomised controlled trial. Lancet, 369(9566), 1000-1015.

157. Martin P, d. W. P., Maurice T, Gammaitoni A, Farfel G, Galer B. (2020). Fenfluramine acts as a positive modulator of sigma-1 receptors. . Epilepsy Behav. 2020 Apr;105:, 105, 106989. doi:doi: 10.1016/j.yebeh.2020.106989.

158. Matey, E. T. e. a. (2022). Nine-gene pharmacogenomics profile service: the Mayo Clinic experience. . Pharmacogenomics J., 22, 69-74.

159. McCluggage, L. K., et al. (2009). Phenytoin toxicity due to genetic polymorphism. Neurocrit Care, 10(2), 222-224. doi:10.1007/s12028-008-9165-9

160. McCormack, M., et al. (2011). HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. N Engl J Med, 364(12), 1134-1143. doi:10.1056/NEJMoa1013297

161. McCormack, M., et al. (2018). Genetic variation in CFH predicts phenytoin-induced maculopapular exanthema in European-descent patients. Neurology, 90(4), e332-e341. doi:10.1212/wnl.0000000000004853

162. McCormack, M., et al. (2012). Genome-wide mapping for clinically relevant predictors of lamotrigine- and phenytoin-induced hypersensitivity reactions. Pharmacogenomics, 13(4), 399-405. doi:10.2217/pgs.11.165

163. McInnes, G., Yee, S. W., Pershad, Y. & Altman, R. B. . (2021). Genomewide association studies in pharmacogenomics. . Clin. Pharmacol. Ther., 110, 637-648.

164. McInnes G, Y. S., Pershad Y, Altman RB. . (2021). Genomewide Association Studies in Pharmacogenomics. . Clin Pharmacol Ther., 110(3), 637-648. . doi:doi: 10.1002/cpt.2349. Epub 2021 Jul 18

165. McMahon, F., Degenhardt, F., Aitchison, K., Alliey, N., Amare, A.T., Aschauer, H., Austin, J., Barta, C., Bohlen, K., Bousman, C., et al. (2019). Genetic Testing and Psychiatric Disorders (International Society of Psychiatric Genetics).

166. Mega JL, H. W., Frelinger AL 3rd, et al. . (2011). Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. . Jama, 306, 2221-2228.

167. Mehendale, H. M. (1995). Toxicodynamics of low level toxicant interactions of biological significance: inhibition of tissue repair. Toxicology, 105(2-3), 251-266.

168. Millichap, J. G. (1989). Acetazolamide for Partial Seizures. . Pediatric Neurology Briefs, 3(1), 1–2. doi:http://doi.org/10.15844/pedneurbriefs-3-1-1

169. Milosavljevic F, B. N., Pavlovic Z, Miljevic C, Pešic V, Molden E, Ingelman-Sundberg M, Leucht S, Jukic MM. . (2021). Association of CYP2C19 and CYP2D6 Poor and Intermediate Metabolizer Status With Antidepressant and Antipsychotic Exposure: A Systematic Review and Meta-analysis. . JAMA Psychiatry, 78(3), 270-280. doi:doi: 10.1001/jamapsychiatry.2020.3643.

170. Miura, M., et al. (2011). Impact of the CYP3A4*1G polymorphism and its combination with CYP3A5 genotypes on tacrolimus pharmacokinetics in renal transplant patients. Pharmacogenomics, 12(7), 977-984. doi:10.2217/pgs.11.33

171. MJ, B. (2017). Outcomes in newly diagnosed epilepsy in adolescents and adults: Insights across a generation in Scotland. Seizure, 44, 206-210.

172. MJ., M. (1994). Clinical pharmacokinetics of gabapentin. Neurology, 44(6 Suppl 5), S17-22; discussion S31-12.

173. MM., G. (2010). Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. . P T, 35(7), 392-415.

174. Monaghan EP, N. L., Shum L, Ashbrook DW, Lee DA. (1997). Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity. . Epilepsia., 38(9), 1026-1031. . doi:doi: 10.1111/j.1528-1157.1997.tb01486.x

175. Morales-Corraliza J, G.-G. P., Sanz R, Díaz-Otero F, Gutiérrez-Delicado E, Serratosa JM. . (2010). Familial partial epilepsy with variable foci: a new family with suggestion of linkage to chromosome 22q12. Epilepsia., 51(9), 1910-1914. doi:doi: 10.1111/j.1528-1167.2010.02680.x

176. Mullan, K. A., et al. (2022). Potential role of regulatory DNA variants in modifying the risk of severe cutaneous reactions induced by aromatic anti-seizure medications. Epilepsia, 63(4), 936-949. doi:10.1111/epi.17182

177. Musch B, C. J., Loiseau P, Fournier V, Beaussart M, Benoit C, Broglin D, Cenraud M, Chatel M, Deville MC, et al. (1987). Long-term treatment of epilepsy: open multicenter trial with progabide in epileptic patients. . Eur Neurol., 26(2), 113-119. . doi:doi: 10.1159/000116320.

178. MY., S. (2013). Efficacy of retigabine in adjunctive treatment of partial onset seizures in adults. . J Cent Nerv Syst Dis., 5, 31-41. doi:doi: 10.4137/JCNSD.S9299.

179. Nicoletti, P., et al. (2019). Shared Genetic Risk Factors Across Carbamazepine-Induced Hypersensitivity Reactions. Clin Pharmacol Ther, 106(5), 1028-1036. doi:10.1002/cpt.1493

180. Nohria V, G. E. (2007). Ganaxolone. Neurotherapeutics., 4(1), 102-105. . doi:doi: 10.1016/j.nurt.2006.11.003.

181. Olsen ASB, F. N. (2017). Sphingolipids: membrane microdomains in brain development, function and neurological diseases. Open Biol., 7(5), 170069. doi:doi: 10.1098/rsob.170069.

182. OMIM. (2021). Cytochrome P450, subfamily IIc, polypeptide 19; CYP2C19. Online Mendelian Inheritance in Man database record *124020.

183. Organisation, W. h. (2010). ICD-10: international statistical classification of diseases and related health problems: tenth revision. . Pneumonia.

184. Organisation., W. H. (2004). ICD-10 : international statistical classification of diseases and related health problems : tenth revision. WHO.

185. Orlando, L. A., Voils, C., Horowitz, C. R., Myers, R. A., Arwood, M. J., Cicali, E. J., McDonough, C. W., Pollin, T. I., Guan, Y., Levy, K. D., Ramirez, A., Quittner, A., & Madden, E. B. (2019). IGNITE network: Response of patients to genomic medicine interventions. . Molecular genetics & genomic medicine, 7(5), e636. doi:https://doi.org/10.1002/mgg3.636

186. Osório L, W. X., Zhou Z. . (2014). Distinct spatiotemporal expression of ISM1 during mouse and chick development. . Cell Cycle., 13(10), 1571-1582. doi:doi: 10.4161/cc.28494. Epub 2014 Mar 17.

187. Ozeki T, M. T., Yowang A, Takahashi A, Kubo M, Shirakata Y, Ikezawa Z, Iijima M, Shiohara T, Hashimoto K, Kamatani N, Nakamura Y. . (2011). Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. . Hum Mol Genet., 20(5), 1034-1041. doi:doi: 10.1093/hmg/ddq537.

188. P Brent Ferrell, J. a. H. L. M. (2008). Carbamazepine, HLA-B*1502 and risk of Stevens–Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. Pharmacogenomics., 9(10), 1543–1546. doi:doi: 10.2217/14622416.9.10.1543

189. Pamplona FA, d. S. L., Coan AC. . (2018). Potential Clinical Benefits of CBD-Rich Cannabis Extracts Over Purified CBD in Treatment-Resistant Epilepsy: Observational Data Meta-analysis. . Front Neurol., 9, 759. doi:doi: 10.3389/fneur.2018.00759.

190. Pardiñas AF, O. M., Walters JTR. . (2021). Pharmacogenomics: A road ahead for precision medicine in psychiatry. . Neuron., 109(24), 3914-3929. doi:doi: 10.1016/j.neuron.2021.09.011

191. Park, P. W., et al. (2009). Effect of CYP3A5*3 genotype on serum carbamazepine concentrations at steady-state in Korean epileptic patients. J Clin Pharm Ther, 34(5), 569-574. doi:10.1111/j.1365-2710.2009.01057.x

192. Parke- Davis, P. I. (2009a). Dilantin®(Phenytoin) [Package insert]. U.S. Food and Drug Administration.

193. Parke- Davis, P. I. (2009b). Zarontin® (Ethosuximide) [Package insert]. U.S. Food and Drug Administration.

194. Pasanen MK, N. M., Neuvonen PJ, Niemi M. . (2006). Slco1b1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. . Pharmacogenet Genomics., 16, 873-879.

195. Patel DC, W. G., Dahle EJ, McElroy PB, Thomson KE, Tesi RJ, Szymkowski DE, West PJ, Smeal RM, Patel M, Fujinami RS, White HS, Wilcox KS. . (2017). Hippocampal TNFα Signaling Contributes to Seizure Generation in an Infection-Induced Mouse Model of Limbic Epilepsy. eNeuro., 4(2), 0105-0117. doi:doi: 10.1523/ENEURO.0105-17.2017.

196. Pereira, N. L. e. a. (2019). Clopidogrel pharmacogenetics. . Circ. Cardiovasc. Interv., 12, e007811.

197. Pfizer. (2004). LYRICA (pregabalin) [package insert]. U.S. Food and Drug Administration.

198. Pfizer, I. NEURONTIN® (gabapentin) [package insert]. U.S. Food and Drug Administration.

199. Pharmaceutical, D. (2011). ZONEGRAN® (zonisamide) [package insert]. U.S. Food and Drug Administration.

200. Pharmaceuticals, J. (2018). EPIDIOLEX® (cannabidiol) [package insert]. U.S. Food and Drug Administration.

201. Pharmaceuticals, V. (2009). Mysoline® (Primidone) [package insert]. U.S. Food and Drug Administration.

202. Piana, C., et al. (2014). Implications of pharmacogenetics for the therapeutic use of antiepileptic drugs. Expert Opin Drug Metab Toxicol, 10(3), 341-358. doi:10.1517/17425255.2014.872630

203. Pirmohamed, M. (2023). Pharmacogenomics: current status and future perspectives. Nat Rev Genet. doi:10.1038/s41576-022-00572-8

204. Pirmohamed., M. (2001). Pharmacogenetics and pharmacogenomics. . Br J Clin Pharmacol. 2001, 52, 345-347.

205. Pohlmann-Eden B, W. D. (2013). The puzzle(s) of pharmacoresistant epilepsy. Epilepsia., 54 (Suppl 2), 1-4. doi:doi: 10.1111/epi.12174.

206. Porter, R. J., et al. (1992). New antiepileptic drugs: from serendipity to rational discovery. Epilepsia, 33 Suppl 1, S1-6.

207. Potschka, H., et al. (2001). P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. Neuroreport, 12(16), 3557-3560.

208. Potschka, H., et al. (2003). Brain access and anticonvulsant efficacy of carbamazepine, lamotrigine, and felbamate in ABCC2/MRP2-deficient TR- rats. Epilepsia, 44(12), 1479-1486.

209. Program., A. A. P. C. N. A. P. G. (2018).

210. Purcell S, N. B., Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. (2007). PLINK: a tool set for whole genome association and population-based linkage analyses. Am J Hum Genet, 81(3), 559-575. doi:doi: 10.1086/519795

211. Qu, J., et al. (2012). ABCC2 polymorphisms and haplotype are associated with drug resistance in Chinese epileptic patients. CNS Neurosci Ther, 18(8), 647-651. doi:10.1111/j.1755-5949.2012.00336.x

212. Ramasamy, K., et al. (2007). Severe phenytoin toxicity in a CYP2C9*3*3 homozygous mutant from India. Neurol India, 55(4), 408-409.

213. Ramsey, L. B., et al. (2014). The clinical pharmacogenetics implementation consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. Clin Pharmacol Ther, 96(4), 423-428. doi:10.1038/clpt.2014.125

214. Ramsey LB, J. S., Caudle KE, et al. . (2014). The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1 and Simvastatin-Induced Myopathy: 2014 Update. . Clin Pharmacol Ther, 96, 423-428.

215. Rani J, S. A., Ramachandran S. (2015). pubmed.mineR: an R package with text-mining algorithms to analyse PubMed abstracts. J Biosci., 40((4):), 671-682.

216. Rating D, W. C., Bast T. . (2000). Sulthiame as monotherapy in children with benign childhood epilepsy with centrotemporal spikes: a 6-month randomized, double-blind, placebo-controlled study. Sulthiame Study Group. Epilepsia, 41(10), 1284-1288. doi:doi: 10.1111/j.1528-1157.2000.tb04606.x.

217. Raucci U, P. N., Paolino MC, Di Nardo G, Villa MP, Pavone P, Terrin G, Specchio N, Striano P, Parisi P. (2020). Cannabidiol Treatment for Refractory Epilepsies in Pediatrics. Front Pharmacol, 11, 586110. doi:doi: 10.3389/fphar.2020.586110.

218. Rawat, C., et al. (2018). Clinical predictors of treatment outcome in North Indian patients on antiepileptic drug therapy: A prospective observational study. Neurol India, 66(4), 1052-1059. doi:10.4103/0028-3886.237000

219. Rawlins, M. D. T., J. W. (1991). Textbook of Adverse Drug Reactions (ed. Davies, D. M.). Oxford University Press, Oxford,, 18-45.

220. Relling, M. V., et al. (2011). Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clin Pharmacol Ther, 89(3), 387-391. doi:10.1038/clpt.2010.320

221. Relling, M. V., Krauss, R. M., Roden, D. M., Klein, T. E., Fowler, D. M., Terada, N., Lin, L., Riel-Mehan, M., Do, T. P., Kubo, M., Yee, S. W., Johnson, G. T., & Giacomini, K. M. (2017). New Pharmacogenomics Research Network: An Open Community Catalyzing Research and Translation in Precision Medicine. . Clin Pharmacol Ther, 102(6), 897–902. . doi:https://doi.org/10.1002/cpt.755

222. Relling, M. V., et al. (2014). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for rasburicase therapy in the context of G6PD deficiency genotype. Clin Pharmacol Ther, 96(2), 169-174. doi:10.1038/clpt.2014.97

223. Relling MV, M. E., Chang T, et al. . (2014a). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for rasburicase therapy in the context of G6PD deficiency genotype. . Clin Pharmacol Ther, 96, 169-174.

224. Relling MV, M. E., Chang T, et al. . (2014b). Clinical Pharmacogenetics implementation Consortium (CPIC) guidelines for rasburicase therapy in the context of G6PD deficiency genotype. . Clin Pharmacol Ther., 96, 169-174.

225. Relling, M. V., et al. (2019). Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. Clin Pharmacol Ther, 105(5), 1095-1105. doi:10.1002/cpt.1304

226. Rettie, A. E., et al. (1994). Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. Pharmacogenetics, 4(1), 39-42.

227. Riva, R., et al. (1996). Pharmacokinetic interactions between antiepileptic drugs. Clinical considerations. Clin Pharmacokinet, 31(6), 470-493. doi:10.2165/00003088-199631060-00005

228. Rivera C., V. J., Kaila K. . (2005). Two Developmental Switches in GABAergic Signalling: The K+-Cl- cotransporter KCC2 and Carbonic Anhydrase CAVII. . J. Physiol., 562, 27-36. doi:doi: 10.1113/jphysiol.2004.077495.

229. Roden DM, V. D. S., Wells QS, Mosley JD, Denny JC, Peterson JF. . (2018). Opportunities and Challenges in Cardiovascular Pharmacogenomics: From Discovery to Implementation. . Circ Res. 2018 Apr 27;, 122(9), 1176-1190. doi:doi: 10.1161/CIRCRESAHA.117.310965

230. Rodriguez-Munoz M, S.-B. P., Garzon J. (2018). Fenfluramine diminishes NMDA receptor-mediated seizures via its mixed activity at serotonin 5HT2A and type 1 sigma receptors. . Oncotarget., 9(34), 23373-23389. doi: doi: 10.18632/oncotarget.25169.

231. Rogawski MA, L. W. (2013). The intrinsic severity hypothesis of pharmacoresistance to antiepileptic drugs. . Epilepsia, 54(33-40).

232. S., H. (2010). Intractable epilepsy: relapsing, remitting, or progressive? Epilepsy Curr., 10, 146-148.

233. S.L. Moshé, E. P., P. Ryvlin, T. Tomson, . (2015). Epilepsy: new advances. Lancet, 385 884-898 doi:http://dx.doi.org/10.1016/S0140-6736(14)60456-6.

234. SA, U. P. (2009). Double-blind, Randomized Study Evaluating the Efficacy and Safety of Brivaracetam in Adults With Partial Onset Seizures: https://ClinicalTrials.gov/show/NCT00490035.

235. Saito, Y. A., & Camilleri, M. (2006). Clinical application of pharmacogenetics in gastrointestinal diseases. . Expert Opinion on Pharmacotherapy,, 7(14), 1857-1869. doi:doi:10.1517/14656566.7.14.1857

236. Sánchez-Mora C, R.-Q. J., Bosch R, Corrales M, Garcia-Martínez I, Nogueira M, Pagerols M, Palomar G, Richarte V, Vidal R, Arias-Vasquez A, Bustamante M, Forns J, Gross-Lesch S, Guxens M, Hinney A, Hoogman M, Jacob C, Jacobsen KK, Kan CC, Kiemeney L, Kittel-Schneider S, Klein M, Onnink M, Rivero O, Zayats T, Buitelaar J, Faraone SV, Franke B, Haavik J, Johansson S, Lesch KP, Reif A, Sunyer J, Bayés M, Casas M, Cormand B, Ribasés M. . (2015). Case-control genome-wide association study of persistent attention-deficit hyperactivity disorder identifies FBXO33 as a novel susceptibility gene for the disorder. . Neuropsychopharmacology, 40(4), 915-926. doi:doi: 10.1038/npp.2014.267.

237. Sapin JI, R. J. J., Grover WD. . (1988). Efficacy of primidone for seizure control in neonates and young infants. . Pediatr Neurol., 4(5), 292-295. doi:doi: 10.1016/0887-8994(88)90068-9.

238. Schaid, D. J., Chen, W. & Larson, N.B. . (2018). From genome-wide associations to candidate causal variants by statistical fine-mapping. . Nat. Rev. Genet., 19, 491-504.

239. Scheffer IE, B. S., Capovilla G, et al. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. . Epilepsia., 58(4), 512-521.

240. Schmickl CN, O. R., Orr JE, Edwards BA, Malhotra A. . (2020). Side effects of acetazolamide: a systematic review and meta-analysis assessing overall risk and dose dependence. . BMJ Open Respir Res., 7(1), e000557. doi:doi: 10.1136/bmjresp-2020-000557.

241. Schmidt, D. S., S. C. . (2014). Drug treatment of epilepsy in adults. BMJ, 28(348g), 254.

242. Schwark R, A. R., Bykhovskaia M. . (2022). Synapsin II Directly Suppresses Epileptic Seizures In Vivo. . Brain Sci., 12(3), 325. doi:doi: 10.3390/brainsci12030325.

243. Scott, S. A., et al. (2011). Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther, 90(2), 328-332. doi:10.1038/clpt.2011.132

244. Scott, S. A., et al. (2013). Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin Pharmacol Ther, 94(3), 317-323. doi:10.1038/clpt.2013.105

245. Scott SA, S. K., Stein CM, et al. . (2013). Clinical pharmacogenetics implementation consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. . Clin Pharmacol Ther, 94, 317-323.

246. Seo, T., et al. (2008). ABCC2 haplotype is not associated with drug-resistant epilepsy. J Pharm Pharmacol, 60(5), 631-635. doi:10.1211/jpp.60.5.0009

247. Seo, T., et al. (2006). ABCB1 polymorphisms influence the response to antiepileptic drugs in Japanese epilepsy patients. Pharmacogenomics, 7(4), 551-561. doi:10.2217/14622416.7.4.551

248. Shorvon, S. D. (2009a). Drug treatment of epilepsy in the century of the ILAE: the first 50 years, 1909-1958. Epilepsia, 50 Suppl 3, 69-92. doi:10.1111/j.1528-1167.2009.02041.x

249. Shorvon, S. D. (2009b). Drug treatment of epilepsy in the century of the ILAE: the second 50 years, 1959-2009. Epilepsia, 50 Suppl 3, 93-130. doi:10.1111/j.1528-1167.2009.02042.x

250. Shuldiner, A. R. e. a. (2009a). Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. Jama, 302, 849-857.

251. Shuldiner, A. R. e. a. (2009b). Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. . Jama, 302, 849-857.

252. Sibbing D, K. W., Gebhard D, et al. . (2010). Cytochrome 2c19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. . Circulation, 121, 512-518.

253. Siddiqui, A., et al. (2003). Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. N Engl J Med, 348(15), 1442-1448. doi:10.1056/NEJMoa021986

254. Sirven, J. I., et al. (2012). Antiepileptic drugs 2012: recent advances and trends. Mayo Clin Proc, 87(9), 879-889. doi:10.1016/j.mayocp.2012.05.019

255. SK Life Science, I. (2022). A Double-Blind, Randomized, Placebo-Controlled, Phase 2 Trial of YKP3089 as Adjunctive Therapy in Subjects With Partial Onset Seizures: https://ClinicalTrials.gov/show/NCT01866111.

256. Smyrna, G. U., Inc. (2018). BRIVIACT® (brivaracetam) [package insert]. U.S. Food and Drug Administration.

257. Society., R. C. o. P. a. B. P. (2022). Personalised prescribing: using pharmacogenomics to improve patient outcomes. . Report of a working party. (RCP and BPS, London).

258. Spear, B. B. (2001). Pharmacogenetics and Antiepileptic Drugs. Epilepsia, 42(Suppl. 5), 31-34.

259. Spiegel, E. A. (1936). Quantitative determination of the convulsive reactivity by electric stimulation of the brain with the skull intact. The journal of Laboratory and Clinical Medicine, 22(12).

260. Stefaniuk, M., et al. (2010). Expression of Ttyh1, a member of the Tweety family in neurons in vitro and in vivo and its potential role in brain pathology. J Neurochem, 115(5), 1183-1194. doi:10.1111/j.1471-4159.2010.07023.x

261. Steinhoff, B. J. (2021). Cenobamate tablets as a treatment for focal-onset seizures in adults. Expert Rev Clin Pharmacol, 14(2), 161-172. doi:10.1080/17512433.2021.1879637

262. Striano P, M. B. (2020). From Genetic Testing to Precision Medicine in Epilepsy. . Neurotherapeutics., 17(2), 609-615. doi:doi: 10.1007/s13311-020-00835-4.

263. Sunovion. (2013). APTIOM® (eslicarbazepine acetate) [package insert]. U.S. Food and Drug Administration.

264. Support, S. T. Citing Stata software, documentation, and FAQs. StataCorp. 2019., Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.

265. Swen, J. J. e. a. (2023). A 12-gene pharmacogenetic panel to prevent adverse drug reactions: an open-label, multicentre, controlled, cluster-randomised crossover implementation study. . Lancet. doi:https://doi.org/10.1016/S0140-6736(22)01841-4

266. Szumlanski C, O. D., Her C, Lee D, Brandriff B, Kelsell D, Spurr N, Wieben E, Weinshilboum R. . (1996). Thiopurine Methyltransferase Pharmacogenetics: Human Gene Cloning and Characterization of a Common Polymorphism. . DNA Cell Biol., 15(1), 17-30.

267. T., P. (2019). Individualized treatment approaches: Fenfluramine, a novel antiepileptic medication for the treatment of seizures in Dravet syndrome. . Epilepsy Behav., 91, 99-102. doi:doi: 10.1016/j.yebeh.2018.08.021.

268. Tai HL, F. M., Bonten EJ, Yanishevsky Y, d'Azzo A, Krynetski EY, Evans WE. (1999). Enhanced proteasomal degradation of mutant human thiopurine S-methyltransferase (TPMT) in mammalian cells: mechanism for TPMT protein deficiency inherited by TPMT*2, TPMT*3A, TPMT*3B or TPMT*3C. . Pharmacogenetics, 9(5), 641-650.

269. Takanashi, K., et al. (2000). CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. Pharmacogenetics, 10(2), 95-104. doi:10.1097/00008571-200003000-00001

270. Talwar P, K. N., Mahendru S, Baghel R, Grover S, Arora G, Grewal GK, Parween S, Srivastava A, Singh M, Vig S, Kushwaha S, Sharma S, Bala K; Indian Genome Variation Consortium, Kukreti S, Kukreti R. (2017). Genetic contribution of CYP1A1 variant on treatment outcome in epilepsy patients: a functional and interethnic perspective. Pharmacogenomics J., 17(3), 242-251. . doi:doi: 10.1038/tpj.2016.1.

271. Tan EK, C. S., Fook-Chong S, et al. . (2005). Functional COMT variant predicts response to high dose pyridoxine in Parkinson's disease. . Am J Med Genet B Neuropsychiatr Genet 2005;, 137B, 1-4.

272. Tate, S. K., et al. (2005). Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. Proc Natl Acad Sci U S A, 102(15), 5507-5512. doi:10.1073/pnas.0407346102

273. Tate, S. K., et al. (2004). Will tomorrow's medicines work for everyone? Nat Genet, 36(11 Suppl), S34-42. doi:10.1038/ng1437

274. Theken, K. N., et al. (2020). Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for CYP2C9 and Nonsteroidal Anti-Inflammatory Drugs. Clin Pharmacol Ther, 108(2), 191-200. doi:10.1002/cpt.1830

275. Tomson, T., et al. (1983). Single-dose kinetics and metabolism of carbamazepine-10,11-epoxide. Clin Pharmacol Ther, 33(1), 58-65.

276. Tonk ECM, G. D., Maitland-van der Zee AH, Janssens ACJW. . (2017). Assessment of pharmacogenetic tests: presenting measures of clinical validity and potential population impact in association studies. Pharmacogenomics J., 17(4), 386-392. . doi:doi: 10.1038/tpj.2016.34.

277. A Trial of Two Fixed Doses of ZX008 (Fenfluramine HCl) as an Adjunctive Therapy in Children and Young Adults With Dravet Syndrome. https://ClinicalTrials.gov/show/NCT02826863.

278. UA, M. (2004). Pharmacogenetics – five decades of therapeutic lessons from genetic diversity. Nat Rev Genet., 5(9), 669-676. doi:doi: 10.1038/nrg1428.

279. UCB, I. (1999). KEPPRA (levetiracetam) [package insert]. U.S. Food and Drug Administration.

280. UCB, I. (2008). VIMPAT® (lacosamide) [package insert]. U.S. Food and Drug Administration.

281. van der Wouden CH, C.-T. A., Cecchin E, et al. (2017). Implementing pharmacogenomics in Europe: design and implementation strategy of the ubiquitous pharmacogenomics consortium. . Clin Pharmacol Ther, 101, 341–358.

282. van der Wouden CH, C.-T. A., Cecchin E, et al. . (2017). Implementing pharmacogenomics in Europe: design and implementation strategy of the ubiquitous pharmacogenomics Consortium. . Clin Pharmacol Ther., 101, 341-358.

283. van der Wouden, C. H. e. a. (2020). Generating evidence for precision medicine: considerations made by the Ubiquitous Pharmacogenomics Consortium when designing and operationalizing the PREPARE study. . Pharmacogenet. Genomics, 30, 131-144.

284. van der Wouden, C. H. e. a. I. (2017). mplementing pharmacogenomics in Europe: design and implementation strategy of the Ubiquitous Pharmacogenomics Consortium. Clin. Pharmacol. Ther, 101, 341-358.

285. Van Driest, S. L. e. a. (2014). Clinically actionable genotypes among 10,000 patients with preemptive pharmacogenomic testing. . Clin. Pharmacol. Ther., 95, 423–431.

286. van Schaik, R. H. N., M€uller, D.J., Serretti, A., and Ingelman-Sundberg, M. (2020). Pharmacogenetics in psychiatry: An update on clinical usability. . Front.Pharmacol., 11, 575540.

287. Veronese, M. E., et al. (1991). Tolbutamide and phenytoin hydroxylations by cDNA-expressed human liver cytochrome P4502C9. Biochem Biophys Res Commun, 175(3), 1112-1118.

288. Vijverberg SJ, P. T., Cornel MC. . (2010). Ethical and social issues in pharmacogenomics testing. . Curr Pharm Des, 16(2), 245-252.

289. Wang L, M. H., Weinshilboum RM. . (2011). Genomics and drug response. . N Engl J Med, 364(12), 1144-1153.

290. Wang L, N. T., McLaughlin RW, Sikkink LA, Ramirez-Alvarado M, Weinshilboum RM. (2005). Human thiopurine S-methyltransferase pharmacogenetics: Variant allozyme misfolding and aggresome formation. . Proc Natl Acad of Sci USA, 102(26), 9394-9399. .

291. Wang L, S. W., Toft D, Weinshilboum R. . (2003). Thiopurine S-methyltransferase pharmacogenetics: chaperone protein association and allozyme degradation. . Pharmacogenetics., 13(9), 555-564.

292. WE., E. (2004). Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. . Ther Drug Monit., 26, 186-191.

293. Wei CH, H. B., Kao HY, Lu Z. (2013). tmVar: a text mining approach for extracting sequence variants in biomedical literature. . Bioinformatics (Oxford, England). 29(11), 1433-1439.

294. Wei CH, K. H., Lu Z. (2013). PubTator: a web-based text mining tool for assisting biocuration. Nucleic Acids Res., 41((Web Server issue):), W518–W522.

295. Wei CH, K. H., Lu Z. (2015). GNormPlus: An Integrative Approach for Tagging Genes, Gene Families, and Protein Domains. Biomed Res Int, 2015. doi:http://dx.doi.org/10.1155/2015/918710

296. Weinstock-Guttman B, T.-B. M., Bhasi K, Zivadinov R, Ramanathan M. (2007). Pharmacogenetics of MXA SNPs in interferon-β treated multiple sclerosis patients. . J. Neuroimmunol., 182(1-2), 236-239.

297. Weiss, J., et al. (2003). Interaction of antiepileptic drugs with human P-glycoprotein in vitro. J Pharmacol Exp Ther, 307(1), 262-267. doi:10.1124/jpet.103.054197

298. Whalley BJ, S. G., Constanti A. . (2009). Investigation of the effects of the novel anticonvulsant compound carisbamate (RWJ-333369) on rat piriform cortical neurones in vitro. . Br J Pharmacol., 156(6), 994-1008. . doi:doi: 10.1111/j.1476-5381.2008.00110.x.

299. White, K. D., Chung, W. H., Hung, S. I., Mallal, S. & Phillips, E. J. . (2015). Evolving models of the immunopathogenesis of T cell-mediated drug allergy: the role of host, pathogens, and drug response. . J. Allergy Clin. Immunol., 136, , 219-234.

300. WHO, T. (2022). Epilepsy Factsheet. WHO website.

301. Wiernasz, E., et al. (2014). Ttyh1 protein is expressed in glia in vitro and shows elevated expression in activated astrocytes following status epilepticus. Neurochem Res, 39(12), 2516-2526. doi:10.1007/s11064-014-1455-3

302. Wilke, R. A., et al. (2012). The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. Clin Pharmacol Ther, 92(1), 112-117. doi:10.1038/clpt.2012.57

303. Wishart DS, K. C., Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. . (2008). DrugBank: a knowledgebase for drugs, drug actions and drug targets. . Nucleic Acids Res., 36(Database issue), D901-906.

304. Wolking, S., et al. (2021). Role of Common Genetic Variants for Drug-Resistance to Specific Anti-Seizure Medications. Front Pharmacol, 12, 688386. doi:10.3389/fphar.2021.688386

305. Wolking S, C. C., Stapleton C, McCormack M, Delanty N, Depondt C, Johnson MR, Koeleman BPC, Krause R, Kunz WS, Marson AG, Sander JW, Sills GJ, Striano P, Zara F, Sisodiya SM, Cavalleri GL, Lerche H. . (2021). Role of Common Genetic Variants for Drug-Resistance to Specific Anti-Seizure Medications. Front Pharmacol., 12, 688386. doi:doi: 10.3389/fphar.2021.688386.

306. Wolking, S., et al. (2020). Pharmacoresponse in genetic generalized epilepsy: a genome-wide association study. Pharmacogenomics, 21(5), 325-335. doi:10.2217/pgs-2019-0179

307. Wolking S, S. H., Nies AT, McCormack M, Schaeffeler E, Auce P, Avbersek A, Becker F, Klein KM, Krenn M, Møller RS, Nikanorova M, Weckhuysen S, Consortium E, Cavalleri GL, Delanty N, Depondt C, Johnson MR, Koeleman BP, Kunz WS, Marson AG, Sander JW, Sills GJ, Striano P, Zara F, Zimprich F, Weber YG, Krause R, Sisodiya S, Schwab M, Sander T, Lerche H. . (2020). Pharmacoresponse in genetic generalized epilepsy: a genome-wide association study. . Pharmacogenomics., 21(5), 325-335. doi:doi: 10.2217/pgs-2019-0179.

308. Xue-Ping W, H.-J. W., Li-Na Z, Xu D, Ling L. . (2019). Risk factors for drug-resistant epilepsy: A systematic review and meta-analysis. . Medicine (Baltimore), 98(30), e16402. doi:doi: 10.1097/MD.0000000000016402.

309. Yan, D., Yu, Y., Ni, Q. et al. . (2023). The overexpression and clinical significance of TBX15 in human gliomas. . Sci Rep, 13, 9771. doi:https://doi.org/10.1038/s41598-023-36410-y

310. Yang JJ, L. W., Yang W, et al. . (2015). Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. . J Clin Oncol., 33, 1235-1242.

311. Yasiry Z, S. S. (2014). The relative effectiveness of five antiepileptic drugs in treatment of benzodiazepine-resistant convulsive status epilepticus: A meta-analysis of published studies. Seizure., 23(3), 167-174. doi:https://doi.org/10.1016/j.seizure.2013.12.007

312. Yates B, B. B., Gray K, Seal R, Tweedie S, Bruford E. . (2017). Genenames.org: the HGNC and VGNC resources in 2017. . Nucleic Acids Res., 45(D1), D619-625.

313. Yukawa, E., et al. (2006). Effect of CYP2C19 genetic polymorphism on pharmacokinetics of phenytoin and phenobarbital in Japanese epileptic patients using Non-linear Mixed Effects Model approach. J Clin Pharm Ther, 31(3), 275-282. doi:10.1111/j.1365-2710.2006.00712.x

314. Yun, W., et al. (2013). Effects of EPHX1, SCN1A and CYP3A4 genetic polymorphisms on plasma carbamazepine concentrations and pharmacoresistance in Chinese patients with epilepsy. Epilepsy Res, 107(3), 231-237. doi:10.1016/j.eplepsyres.2013.09.011

315. Z., Z. (2020). Pharmacogenomics in cardiovascular precision medicine. . J Lab Precis Med, 5, 30. doi:doi: 10.21037/jlpm-2019-cpm-05

316. Zaccara G, L. S., Leo A, Russo E. . (2021). Critical Appraisal of Cenobamate as Adjunctive Treatment of Focal Seizures in Adults. . Neuropsychiatr Dis Treat., 17, 3447-3457. . doi:doi: 10.2147/NDT.S281490.

317. Zannikos P, N. G., Yao C,Verhaeghe T,Franc MA,Solanki B,Bialer M. (2009). Pharmacokinetics of carisbamate (RWJ-333369) in healthy Japanese and Western subjects. Epilepsia, 50(8), 1850-1859. doi:https://doi.org/10.1111/j.1528-1167.2009.02081.x

318. Zembutsu H, N. S., Akashi-Tanaka S, et al. . (2017). Significant effect of polymorphisms in CYP2D6 on response to tamoxifen therapy for breast cancer: a prospective multicenter study. . Clin Cancer Res, 23, 2019-2026.

319. Zeng, Z., Huang, S. Y., & Sun, T. (2020). Pharmacogenomic Studies of Current Antidiabetic Agents and Potential New Drug Targets for Precision Medicine of Diabetes. . Diabetes therapy : research, treatment and education of diabetes and related disorders,, 11(11), 2521-2538. . doi:https://doi.org/10.1007/s13300-020-00922-x

320. Zhou K, P. H., Dawed AY, Pearson ER. . (2016). Pharmacogenomics in diabetes mellitus: insights into drug action and drug discovery. . Nat Rev Endocrinol., 12(6), 337-346. doi:doi: 10.1038/nrendo.2016.51.

321. Zimprich, F., et al. (2008). A functional polymorphism in the SCN1A gene is not associated with carbamazepine dosages in Austrian patients with epilepsy. Epilepsia, 49(6), 1108-1109. doi:10.1111/j.1528-1167.2008.01549_4.x

322. Zimprich, F., et al. (2004). Association of an ABCB1 gene haplotype with pharmacoresistance in temporal lobe epilepsy. Neurology, 63(6), 1087-1089.

323. Zogenix, I. (2020). FINTEPLA® (fenfluramine) [package insert]. U.S. Food and Drug Administration.

324. Zogenix International Limited, I., a subsidiary of Zogenix, Inc., et al. (2016). A Trial of Two Fixed Doses of ZX008 (Fenfluramine HCl) in Children and Young Adults With Dravet Syndrome: https://ClinicalTrials.gov/show/NCT02682927.

List of Publications

Published articles

1. Guin D, Thakran S, Singh P, Singh P, Kushwaha S, Sharma S, Agarwal R, Srivastava AK, Hasija Y, Kukreti R. Genome-wide association study identifies common variants associated with pharmacoresponse of anti-seizure medications. (in preparation).
2. **Guin D**, Hasija Y, Kukreti R. Assessment of clinically actionable pharmacogenetic markers to stratify anti-epileptic treatment. Pharmacogenomics J. 2023 Aug. doi: 10.1038/s41397-023-00313-y. (IF=3.245, Cite:,)
3. Singh P, **Guin D**, Pattnaik BR, Kukreti R. Genetic landscape of idiopathic pulmonary fibrosis: A systematic review, meta-analysis and epidemiological evidence of case-control studies. **J. Gene Med** (under review). Gene. 2023.
4. Kanojia N, **Guin D**, Thakran S, Machahary N, Kukal S, Thakur J, Panda B, Singh P, Srivastava A, Singh P, Grover S, Singh A, Sardana V, Saso L, Kukreti S, Kukreti R. Effect of antiepileptic drug monotherapy on endogenous sex hormonal profile in men and women with epilepsy. Journal of endocrine society (Oxford Academic) (under review).
5. Singh P, Srivastava A, **Guin D**, Thakran S, Yadav J, Chandna P, Sood M, Chadda RK, Kukreti R. Genetic Landscape of Major Depressive Disorder: Assessment of Potential Diagnostic and Antidepressant Response Markers. **Int J Neuropsychopharmacol**. 2023 Jan 19:pyad001. doi: 10.1093/ijnp/pyad001. (**IF= 3.913, Cite:**)
6. Kukal S[#]; Thakran S[#]; Kanojia N[*]; Yadav S[*]; Mishra M K; **Guin D**; Singh P, Kukreti R. Genic-intergenic polymorphisms of CYP1A genes and their clinical impact. **Gene.** 2023 Mar 20;857:147171. doi: 10.1016/j.gene.2023.147171. [[#*]equal contributors] (**IF= 3.913, Cite:**)
7. **Guin D[#],** Yadav S[#], Singh P, Singh P, Thakran S, Kukal S, Kanojia N, Paul PR, Pattnaik B, Sardana V, Grover S, Hasija Y, Saso L, Agrawal A, Kukreti R*. Human Genetic Factors Associated with Pneumonia Susceptibility, a Cue for COVID-19 Susceptibility. **Infect Genet Evol.** 2022 Aug;102:105299. doi: 10.1016/j.meegid.2022.105299. [[#]equal contributors] (**IF= 3.342, Cite:1**)
8. Kukal S, **Guin D**, Rawat C, Bora S, Mishra MK, Sharma P, Paul PR, Kanojia N, Grewal GK, Kukreti S, Saso L, Kukreti R. Multidrug efflux transporter ABCG2: expression and regulation (2021) **Cell. Mol. Life Sci.** doi. https://doi.org/10.1007/s00018-021-03901-y **(IF=9.26, Cite:4)**
9. Thakran S, **Guin D**, Singh P, Singh P, Kukal S, Rawat C, Yadav S, Kushwaha SS, Srivastava AK, Hasija Y, Saso L, Ramachandran S, Kukreti R. Genetic landscape of common epilepsies: Advancing towards precision in treatment. **Int J Mol Sci.** 2020 Oct 21;21(20):7784. doi: 10.3390/ijms21207784. (**IF= 4.556, Cite:26**)

10. **Guin D**, Rani J, Singh P, Grover S, Bora S, Talwar P, Karthikeyan M, Satyamoorthy K, Adithan C, Ramachandran S, Saso L, Hasija Y, Kukreti R*. Global Text Mining and Development of Pharmacogenomic Knowledge Resource for Precision Medicine. **Front Pharmacol. (2019)** Aug 7; 10:839. doi: 10.3389/fphar.2019.00839. eCollection 2019. **(IF=3.845, Cite: 5)**

11. Srivastava A, Singh P, Gupta H, Kaur H, Kanojia N, **Guin D**, Sood M, Chadda RK, Yadav J, Vohora D*, Saso L*, Kukreti R*. Systems Approach to Identify Common Genes and Pathways Associated with Response to Selective Serotonin Reuptake Inhibitors and Major Depression Risk. **Int J Mol Sci. (2019)** Apr 23; 20 (8). pii: E1993. **(IF=4.18, Cite: 14)**

12. Rawat C, **Guin D**, Talwar P, Grover S, Baghel R, Kushwaha S, Sharma S, Agarwal R, Bala K, Srivastava A K, Kukreti R*. Clinical predictors of treatment outcome in North Indian patients on antiepileptic drug therapy: A prospective observational study. **Neurology India (2018)** Jul-Aug; 66(4):1052-1059. doi: 10.4103/0028-3886.237000. **(IF= 2.166, Cite: 8)**

13. Jajodia A, Kaur H, Srivastava A, Kumari K, Baghel R, **Guin D**, Sood M, Satyamoorthy K, Jain S, Chadda RK, Kukreti R*. Schizophrenia susceptibility and neuregulin signaling pathway genes: A rare haplotype combination based association study in Indian population. **Psychiatry Research** (**2018)** Apr; 262:628-630. **(IF=2.528)**

14. **Guin D**, Mishra MK, Talwar P, Rawat C, Kushwaha SS, Kukreti S, Kukreti R. A systematic review and integrative approach to decode the common molecular link between levodopa response and Parkinson's disease. **BMC Medical Genomics (2017)** Sep 19; 10(1):56. doi: 10.1186/s12920-017-0291-0. **(IF=2.848, Cite:24)**

15. Prasher B, Varma B, Kumar A, Khuntia BK, Pandey R, Narang A, Tiwari P, Kutum R, **Guin D**, Kukreti R, Dash D; TRISUTRA Ayurgenomics Consortium, Mukerji M. Ayurgenomics for stratified medicine: TRISUTRA consortium initiative across ethnically and geographically diverse Indian populations. **J Ethnopharmacol. 2017** Feb 2; 197: 274-293. **(IF= 3.055, Cite:25)**

Book Chapters

1. **Guin D** and Kukreti R. Chapter 17: Drug Hypersensitivity linked to Genetic Variations of Human Leukocyte Antigen (HLA). Therapeutic Drug Monitoring: Newer Drugs and Biomarkers 2nd edition (Elsevier)

2. **Guin D**, Thakran S[#], Singh P[#], S. Ramachandran, Hasija Y, Kukreti R. Translational biotechnology: a transition from basic biology to evidence-based research. Published: 1st January 2021. Translational Biotechnology: A Journey from Laboratory to Clinics. India First Edition. ISBN: 9780128219720. Elsevier. (2021) (# indicate equal contribution)

3. Singh P, **Guin D**, Jain R and Kukreti R. The Nature and Evolution of Therapies in Schizophrenia: From Classical Time to Clinical Trials. Top 10 Commentaries in Schizophrenia. Avid sciences (2018)

4. Srivastava A, **Guin D**, Kukreti R, Vohora D. "Pharmacogenomics: An evolution towards clinical practice", in "Pharmaceutical medicine and translational clinical research, 1st edition" of Elsevier publications. ISBN no. 9780128021033. 1st Oct 2017. In Press.