Chapter 5

Summary and Conclusions
Recall of the existing pharmacogenetic literature on epilepsy piles up a large amount of inconsistent findings. One reason for which could be the restricted approach towards appropriate gene selection. Therefore, prioritization of genes for pharmacogenetic studies needs to be more comprehensive with existence of scope for genes other than DME’s, transporters and known drug targets. As it is known that wide level of phenotypic variation in epilepsy is represented by, various different types of seizures, epilepsies, for which genetic predisposition also varies. This leads to the conclusion that genes responsible for development of different phenotypes will certainly be different and will also influence the drug response of the patient differently i.e., a specific phenotype may have a specific disease related gene/pathophysiology related gene, other than DME or drug transporter, for its variable drug response. Therefore, inclusion of disease related genes/pathophysiology related genes while conducting pharmacogenetic study will pave the way for a new gene set for response prediction.

The objectives of the present study were to explore the role of genetic variants from epilepsy pathophysiology genes i.e., ion channels & their functionally related genes (presynapse) and genes from SVC (postsynapse) in influencing seizure control, dose and drug levels of patients treated with first-line AEDs. Considering the relevance of DME’s and transporters in AED response, the study also aimed to understand their role as well. Additionally, the study also explored the role of epilepsy pathophysiology genes in epilepsy manifestation of patients.

Based on the first objective, significant associations of SCN1A and GABRA1 haplotypes with “recurrent seizures”, in epilepsy patients on PHT monotherapy has revealed the significant role of presynaptic ion channel genes. Diploype analysis of GABRA1 with the shortest two-marker diplotype rs12658835_rs7735530 (AG/AG) (P-value corrected = 0.0349, OR=3.75, 95% CI=1.36-11.05), the longest five-marker diplotype rs12658835_rs7735530_rs7732641_rs2279020 (AGCA/AGCA) (P-value corrected = 0.0359, OR=2.48, 95% CI=0.96-6.41) and SCN1A with rs6432860_rs3812718 AC/AC (P-value corrected = 0.0349, OR=6.42, 95% CI=1.10-65.76) further confirmed the significant findings. Additionally, marginal significant association of STX1A haplotype rs867500_rs4363087 (GT OR=1.97, 95% CI=1.24-3.13, P-value corrected = 0.050) with “recurrent seizures” in CBZ monotherapy patients revealed the significant role of
presynaptic SVC genes in influencing drug response of patients. The association of \textit{GABRA1} in the present study further supported the existing few reports of PHT mode of action through $\alpha_1$ subunit of \textit{GABRA1}. Detailed functional analysis of \textit{GABRA1} gene may highlight the exact mechanism by which \textit{GABRA1} involves in variable patient response. \textit{In silico} analysis supported by functional analysis may also highlight the possible binding sites and the affinity of PHT for \textit{GABRA1} receptor. \textit{SCN1A} association supports the role of long-standing significant epilepsy pathophysiology related ion channel gene in predicting poor response of PHT monotherapy patients. This time the association marks high significance as it is unlike multitherapy or monotherapy drug resistant epilepsy patient’s rather, monotherapy poor responders. Further dose and drug level analysis also revealed the possible significant role of \textit{SCN1A} diplotype rs6432860\_rs3812718 AC/AC in predicting variable “drug levels at maximum dose” of patients as well as marginally significant role on “maintenance dose” for PHT monotherapy patients (P-value=0.021, drug levels$_{\text{max\_dose}}$ P-value=0.058, maintenance dose). The genotype (GG, P-value=0.042) and dominant model (AG+GG, P-value=0.065) of \textit{SCN1A} rs6432860 was also found to influence “drug levels at maximum dose” of PHT monotherapy patients, where higher levels were observed in patients with GG genotype whereas lower levels were observed for dominant model of the same SNP.

As a result of the second objective of the study, significant association of \textit{CYP2C9} haplotype rs9332092\_rs1057910 with “no seizures” in VP monotherapy patients further substantiated the possible involvement of \textit{CYP2C9} in VP disposition, however its role on pharmacokinetic variability i.e variable maintenance dose requirement and steady state drug levels of patients could not be explained. It was observed that CC haplotype of rs9332092\_rs1057910 was associated with “recurrent–seizure” phenotype of patients whereas TA haplotype of rs9332092\_rs1057910 was associated with “no-seizure” phenotype of patients. Diplotype analysis whereas could only reveal significant association of TA/TA of rs9332092\_rs1057910. Further, the associated variants belonged to the \textit{CYP2C9}*3A haplotype of \textit{CYP2C9}*3 allele and contained rs1057910, the exonic non-synonymous (Ile359Leu, 1075A>G) polymorphism of \textit{CYP2C9} i.e., \textit{CYP2C9}*3 allele variant with the functional significance of reducing the enzyme activity. The functional relevance of the polymorphism also advocates the possible influence on VP response via lowering of \textit{CYP2C9} expression.
The finding holds the significance as other than the well-defined phase II enzymes, the unexplored phase I enzyme e.g., CYP2C9 may also play significant role in VP disposition. Literature evidences comprising genetic as well as in silico i.e., docking and molecular dynamics simulations based reports have also supported the role of CYP2C9 in VP disposition. As we did not performed but a systematic screening of genetic polymorphism of CYP2C9 gene in valproate monotherapy patients may help in exploring detailed role of various CYP2C9 haplotypes and known as well as novel variants in predicting variable patient response.

As per the third, last objective the role of epilepsy pathophysiology genes on epilepsy manifestation was identified. Significant association of haplotypes of SNAP25 and SCN2A in “all epilepsy” (SNAP25rs363014|SNAP25rs3787283, GC, P-value_corrected =0.036, AT P-value_corrected =0.023) and “focal” seizure type (SCN2A_rs2075703|SCN2A_rs1947114, (GC, AT), P-value_corrected =0.039) of patients revealed the significance of both presynapse and postsynapse genes in epilepsy pathophysiology. Diplootype analysis in both “all epilepsy” (rs363014_rs3787283, AT, P-value_corrected =0.023, OR=1.81, 95%CI=1.09-2.98) and “focal” seizure type (rs2075703_rsz1947114, GC, P-value_corrected =0.010, OR=5.71, 95%CI=1.31-34.06) of patients further retained the significance.

Further, considering epilepsy a complex disorder the role of polygenic inheritance could not be neglected. Therefore interactive analysis of genes was performed which identified the significant interactive role of genes. The presence of an interactive two-locus model of VAMP2 and STX1A SNPs (CVC=10/10, OR=4.59, 95% CI=2.57-8.22; P<0.0001, P_{1000perm}=0.012) in “cryptogenic epilepsy” patients thus highlighted the role of two presynaptic genes in understanding disease pathophysiology of epilepsy. The study reports for the first time the synergistic interactive effect of presynaptic genes on susceptibility to “cryptogenic epilepsies”. The present study may lay the foundation for future research involving interactive effect of presynaptic genes, thus providing better understanding of genetic aetiology of epilepsy.

Conclusively, present study identified the significant role of presynaptic as well as postsynaptic genes in predicting epilepsy drug response towards the monotherapy first-line AEDs. The evidence for presynapse is however mild but provides a novel lead
which may direct the future pharmacogenetic studies to go via the presynapse genes other than the classical DME’s and transporters, while studying variable patient response. Association of non-conventional genes CYP2C9 and GABRA1 with VP and PHT response supports the dynamicity of AED mode of action described earlier also, by few reports. The interactive influence of genes on epilepsy pathopysiology further supports the complex inheritance pattern of epilepsy alike other complex disorders, thus nullify the monogenic concept in epilepsy pathopysiology. The association of presynaptic genes marks the relevance of presynapse in epilepsy pathopysiology as well. Further replication in a larger cohort of same as well as different study may help in establishing the clinical relevance of the study.