OVERALL CONCLUSIONS AND FUTURE PROSPECTS
6.1 CONCLUSIONS

We have studied to analyze AD at molecular level. The overall study aimed for deciphering the biomolecules such as TFs, genes and proteins etc. and interactions among them at system level for better understanding of the bioprocesses involved in AD. The overall bottom up approach being utilized and their proposed outcomes to deal with AD as our system through various computational approaches for its system level understanding are given in Figure 6.1. Future prospects and the practical applications of our approach are also discussed briefly to provide new directions for the AD research.

![Figure 6.1](image)

**Figure 6.1.** Bottom up approach being utilized with their corresponding outcomes to deal with AD as our system

Important findings of this thesis are being summarized here:-

- No such Phylogenetic tree is available based on BACE1 for species included in our tree. Phylogenetic results gave new direction to the position of lineages based on BACE1 evolutionary tree. Based upon combination of phylogenetic and functional motif analysis we suggest that guinea pig has an advantage over traditional rat and
mouse as model organism, because it is more close to mammalian class, even though all three belongs to class rodentia. Additionally, identification of Cys-Arg mutation at C-terminal will explore plausible secondary cause of variations among human BACE1 isoforms.

- From the PPI studies we have found five additional important proteins closely associated with BACE1 which can be promising candidates for AD research studies. It will also provide a platform for elucidation of regulation mechanism of BACE1 and will also helpful in designing new drug targets and inhibitors for the prevention and cure of AD.

- The Differential Gene Expression (After multiple comparisons through \( t \) and ANOVA \( F \)-statistics) results showed the extensive links between AD and AG at molecular level, identifying core biological processes and genes they share.

- It has been found through ranked list of genes and gene enrichment analysis that not only AG and AD share common patho-physiological processes but also there is involvement of other important human disease with these biological processes. Interactions of TFBS, genes, and encoded proteins at molecular level for other disease such as diabetes, dominant optic atrophy, coronary heart disease, sudden cardiac arrest, Gaucher disease, myriad carcinomas, and cirrhosis signifies putative association among AG, AD and all the above mentioned disease.

- It has been observed that conserved TFs are potent gene regulators for AD. We also explored this conservation of TFs towards their regulation mechanism for gene involved in AG, AD and other diseases. We found shared nature of TFs of AD which was associated with other disease such as haematopoiesis, Epstein-Barr virus infection, viral carcinogenesis and other carcinomas, dominant optic atrophy, atrial fibrillation, coronary heart disease and sudden cardiac death. Major classes for secondary structure elements were dominated by helix-turn-helix for the families’ homeo, Arid, and Myb and its winged version for the families Forkhead, IRF, and Ets. Another major class found was Zinc-coordinating which belongs to two families’ hormone-nuclear receptor, and beta-beta-alpha-zinc finger. These structural level
constraints proposed plausible targets for neuronal tangles and plaques through normal and winged helix-turn-helix and beta-beta alpha structures. Linked TFBS for these physico-chemical elements could be manipulated to deal with involved complexities.

- Genes like C1orf115, DPF3, PSMD4, USP25, KCNA5, LZTS1, CSPG5, and SLC25A6 are found to be novel candidates associated with AD, and could be useful for targeting either different brain regions (conditioned to their presence) or various biomolecular entities for designing treatment strategies for AD and other diseases.

- Some genes are found to be involved in multiple processes such as PSME3 and PSME4 are involved in PPI, TTGS, and KEGG pathways. These two genes are also found to be involved in normal AG process. While cross checking the output of GO enrichment analysis the above mentioned two genes were found in nucleoplasm with high level of significance (p-value 5.43E-04; GOrilla). It indicates how a particular method might not capture all the information latent in biological data and similar analysis with other tools, or methods could provide insightful annotations. Based upon this analysis we proposed that Bioinformatics/Computational analysis has to be done using multiple tools/approaches.

- Similarly genes such as TBL1X (transducin (beta)-like 1X-linked) and KIAA0528 are found to be involved in TTGS, miRNA targets, and also involved in both AD and AG related metabolic processes.

- When the genes of the present study (Chapter 3) were compared with the results of Ray and Zhang (2010), interestingly many common genes were found involved in various brain regions. At least one common gene is found in almost all regions. Between EC and HIP region, KCNAB2 and SPF3 are found, between EC and PCC region GPR22 is found, between HIP and PCC region KCNAB2 is found. There are four genes from this study named TBL1X, EFNB2, RND2 and CDH10 which were found to be involved between HIP and MTG region and TBL1X, EFNB2 genes between MTG, EC and HIP region [Ray and Zhang, 2010]. Concerned pathways such as Wnt, axon guidance, and Akt have also been found associated. These genes and
their associated pathways could be treated as hotspots while planning experimental procedures for association studies.

- Novel information for network motifs such as BiFan, MIM, and SIM and their close variants has also been discovered and this implicit information will help to improve research into AD. Biologically significant network motifs identified for AD pathways.

- There are few resources available for AD but there is no such catalog for AD prime genes which provides all these essential quantitative genetic details which includes LD, haplotypes, nsSNPs, tag SNPs, disease related functional information, along with phosphorylation states on a common platform. In this regard, ADDGAP is first of its kind model where the users could easily retrieve and explore the quantitative genetic parameters and the phosphorylation states for AD prime genes.

- The molecules showed no indication for mutagenicity, tumorigenicity and also, no indications for irritating and reproductive effects were found which was determined by the TOPKAT. The top scoring molecule S1 have shown score of -14.34 kcal/mol in the AutoDock simulation studies and IFD score of -459.966. The results of molecular modelling show that the S1 molecule reaches at energetic equilibrium in the binding site and is stable there. Furthermore, the H-bonds accepted and donated by this molecule, along with the hydrophobic interactions, can be used to suggest the binding stability of this molecule.

- Poor ADME characteristics are one of the reasons why drug candidates don’t succeed in clinical trials. ADME properties of the top scoring docked compounds were predicted using QikProp module of Schrödinger 2012. The module predicts properties such as log P_{o/w}, IC50 values for blockage of HERG K^+ channels, log BB, overall CNS activity, Caco-2 and MDCK cell permeability, logKhsa for human serum albumin binding and human oral bioavailability. Prediction of these characteristics prove the suitable candidates as potent markers and could be taken further for experimental verifications and clinical trials.
6.1 FUTURE PROSPECTS

- Further *in vitro* and *in vivo* study is required on these lead molecules as the binding mode and simulation study provided hints for the future design of new derivatives with higher potency and specificity.

- The top scoring docked compound (lead molecule) showed no indication for mutagenicity, and tumorigenicity. Also, no indications for irritating and reproductive effects were found. It is anticipated that the proposed molecule would serve as a potential candidate for experimental studies and will be prolific for neurological disorders such as AD.

- All the projected bio-molecules (from all chapters) would serve as potential candidates for experimental studies and will be productive for neurological disorders such as AD.

- It is believed that generated information for nucleosomes, and linkers could proved to be biologically meaningful for future structure based studies associated with genes and proteins involved in AD.

- We applied supplie approach for the design of ADDGAP model to expand and update it on regular basis to provide state-of-the-art information to the scientific community.

- The biologically meaningful information generated through computational analyses would be of utmost use to scientific community after experimental verifications.