Transcriptional analysis of ASCL1 and the effects of neurogenesis in GABAergic neurons

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Introduction

Neurogenesis refers to the process by which undifferentiated neural progenitor stem cells into functional neurons.

Neurogenesis is can be controlled by bHLH proteins. One example of a bHLH protein is ASCL1 which generate inhibitory GABAergic interneurons which are derived from the ventral telencephalon from MGE and CGE regions.

GABAergic neurons prevent continuous excitatory firing of neurons.

Mutations in ASCL1 in GABAergic neurons are associated with Schizophrenia with strabismus and epileptic seizures
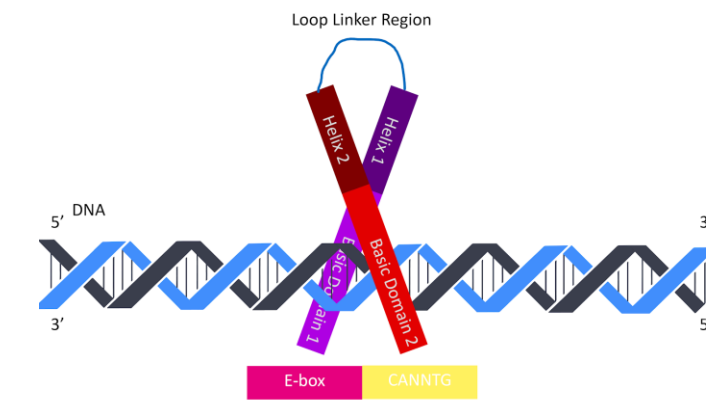

Figure 1:The bHLH structural motif which contains a basic region joined by the two α-helix, then a short linker loop region and binds to the DNA via E box motif sequence "CANNTG"
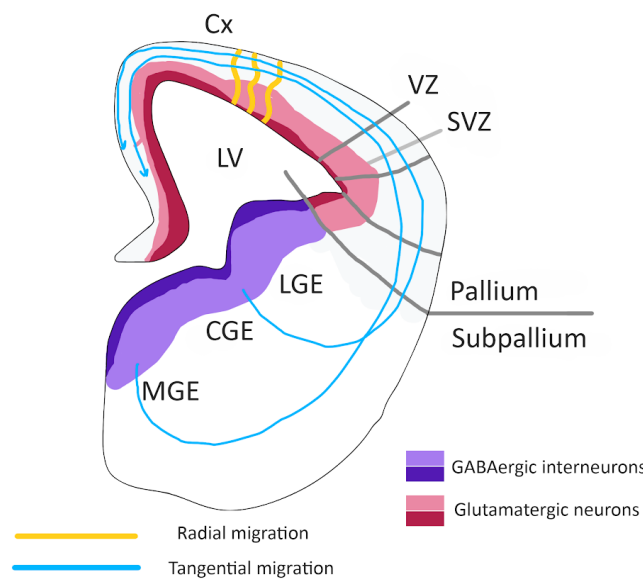

Figure 2: Diagram of a hemisection of a mouse embryo forebrain

Aims

- To understand the role of regulation of ASCL1 within GABAergic neurons
- Identify co-expression groups that occur alongside ASCL1
- To investigate the role of ASCL1 targets genes by gene ontology

Methods

Table I. Details of the datasets used in the present study and the biological function.

Database	Biological function and meaning
GSE29985	Observing the ectopic expression of Arx and comparing it to GABAergic neurons
GSE31635	Comparing to GABAergic precursors and compared interneuron precursors
E-MTAB-4840	Observing the expression of genes associated in human embryonic brain
GSE78949	The enrichment of Ascl1, Smad7 and Nr2f1 in ESC to observe GABAergic markers
GSE46791	Comparing the epigenetic status of NSCs and immature GABAergic neurons

Figure 3: Flow diagram of processing the RNASeq and microarray datasets into differential expressed gene list.
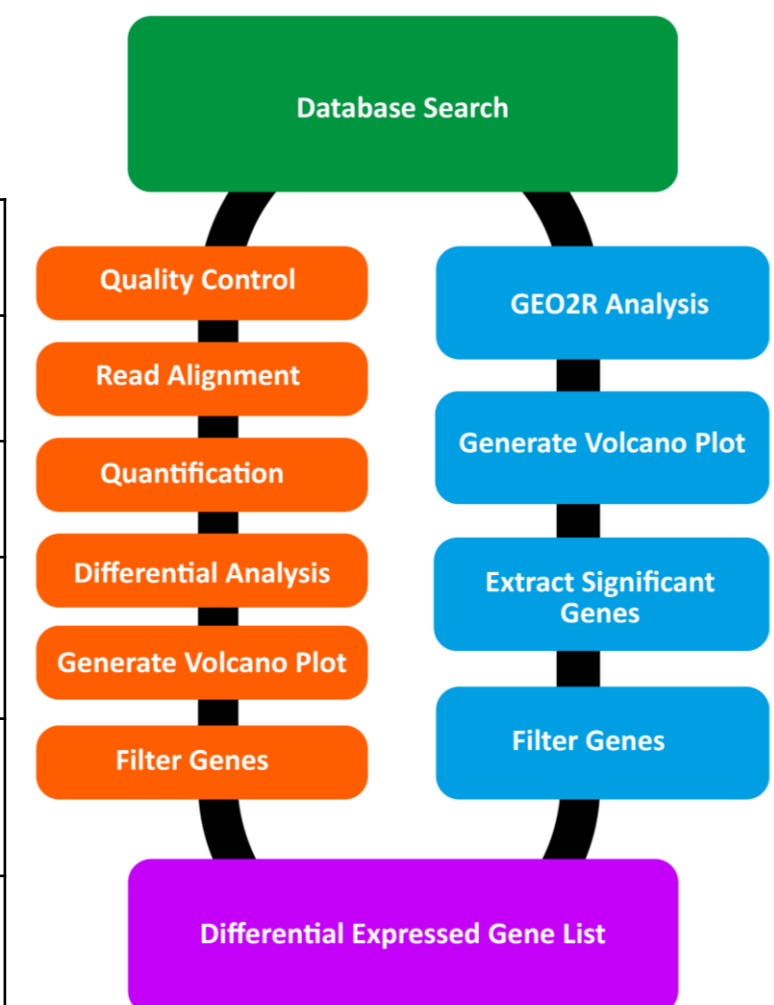
Results

In order to identify which genes from dataset overlap. The merged gene list from datasets: GSE29985, GSE31635, GSE46791.
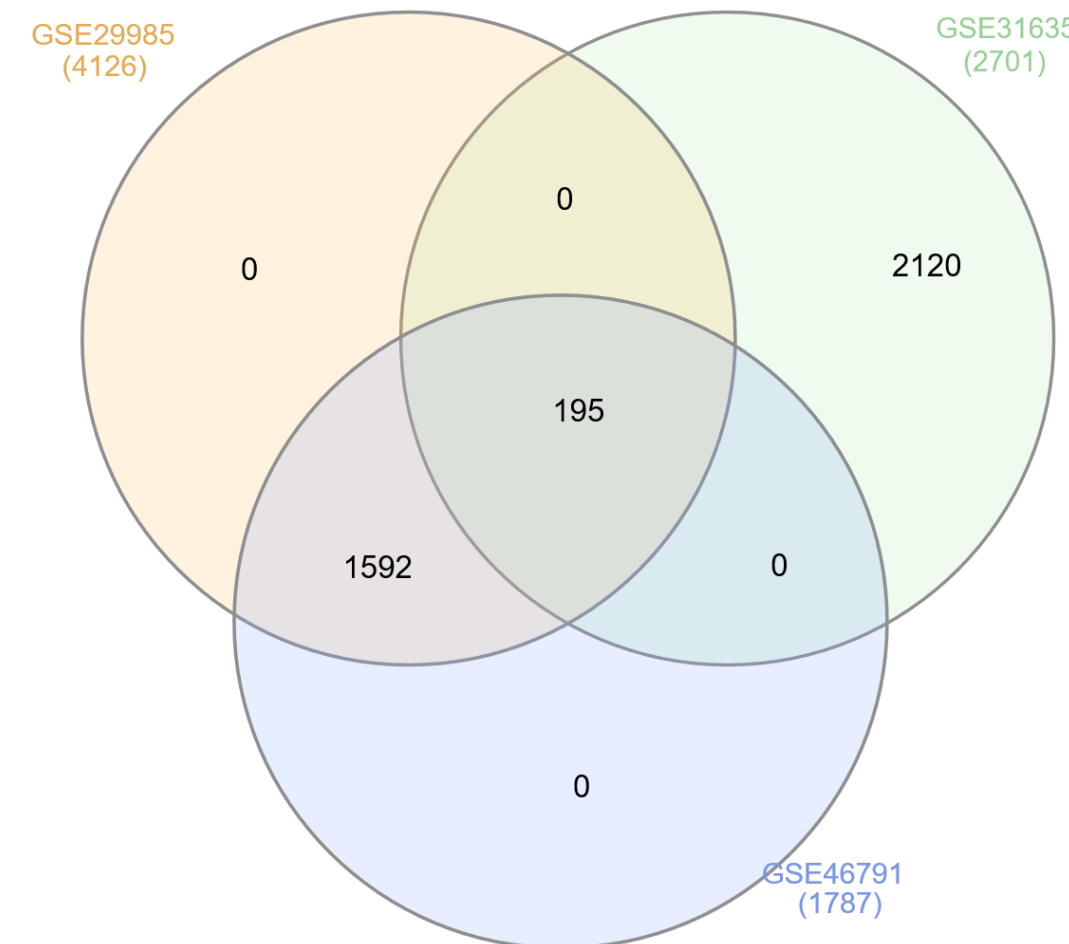

Figure 4: Venn diagrams generated by InteractiVenn. Showing the both all of the upregulated and downregulated genes merge. Displaying the comparison of 3 gene lists: GSE31635, GSE29985 and GSE46791.

To investigate which the relationship between ASCL1 target genes and gene ontology. A GO term analysis is performed using the same overlapping gene list generated from InteractiVenn.
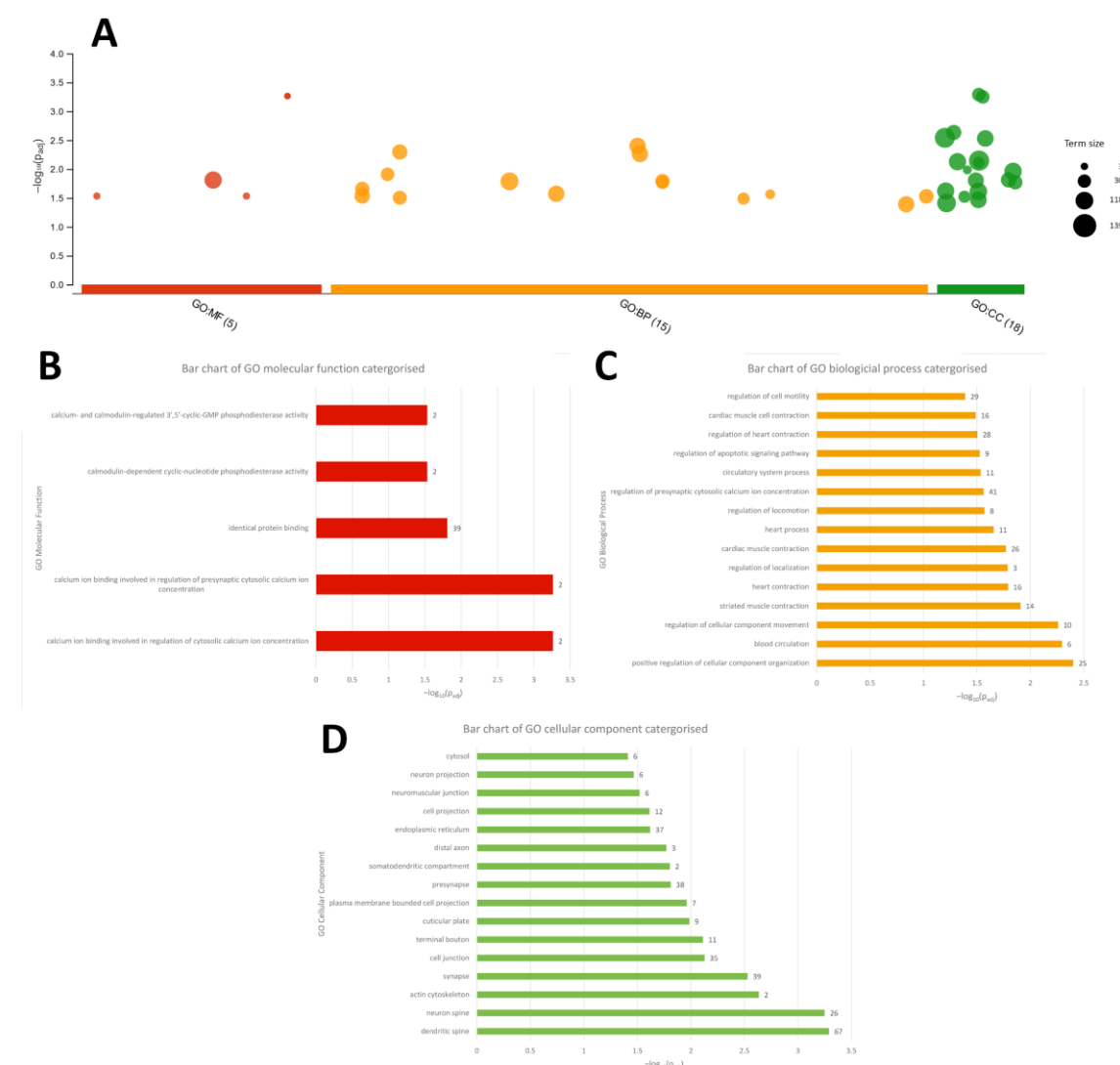

Figure 5: **A,** GO Term Enrichment analysis visualised as a Manhattan-like-bubble graph. Results of GO enrichment analysis for **B**, molecular function **C**, biological process and **D**, cellular component categories. The X-axis shows the -Log10 of the adjusted P-value. The Y axis represents the GO term associated with in each graph.

To observe what gene-gene interactions were present in the list generated from InteractiVenn, STRING was used.
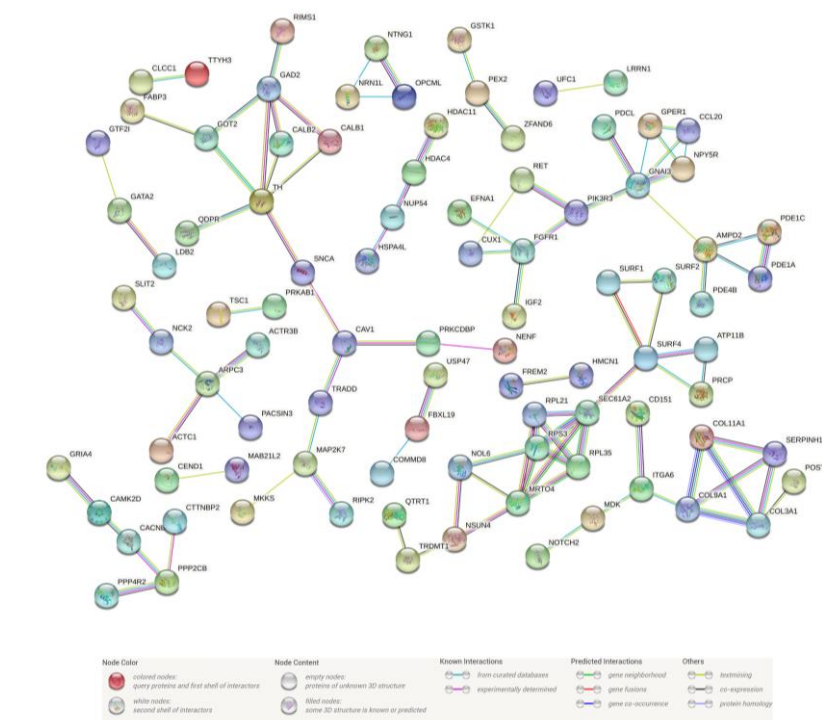

Figure 6: STRING Analysis of merged genes which contain upregulated and downregulated genes. The minimum required interaction confidence score was set at 0.65. The unconnected proteins are hidden in the network.

Discussion

- CALB1 and CALB2 is expressed in GABAergic neurons and from prenatal development to adulthood

- GAD2 is co-expressed with CALB1 (48%)and CALB2 (18%)in GABAergic neurons

- Slit2 is has a miniscule role in interneuron migration

Conclusion

- Databases *E-MTAB-4840, GSE78949* had little to no biological relevance

- Further research would investigate neurogenesis in adult samples

- Additionally explore why Slit2 is expressed weakly in VZ

References

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