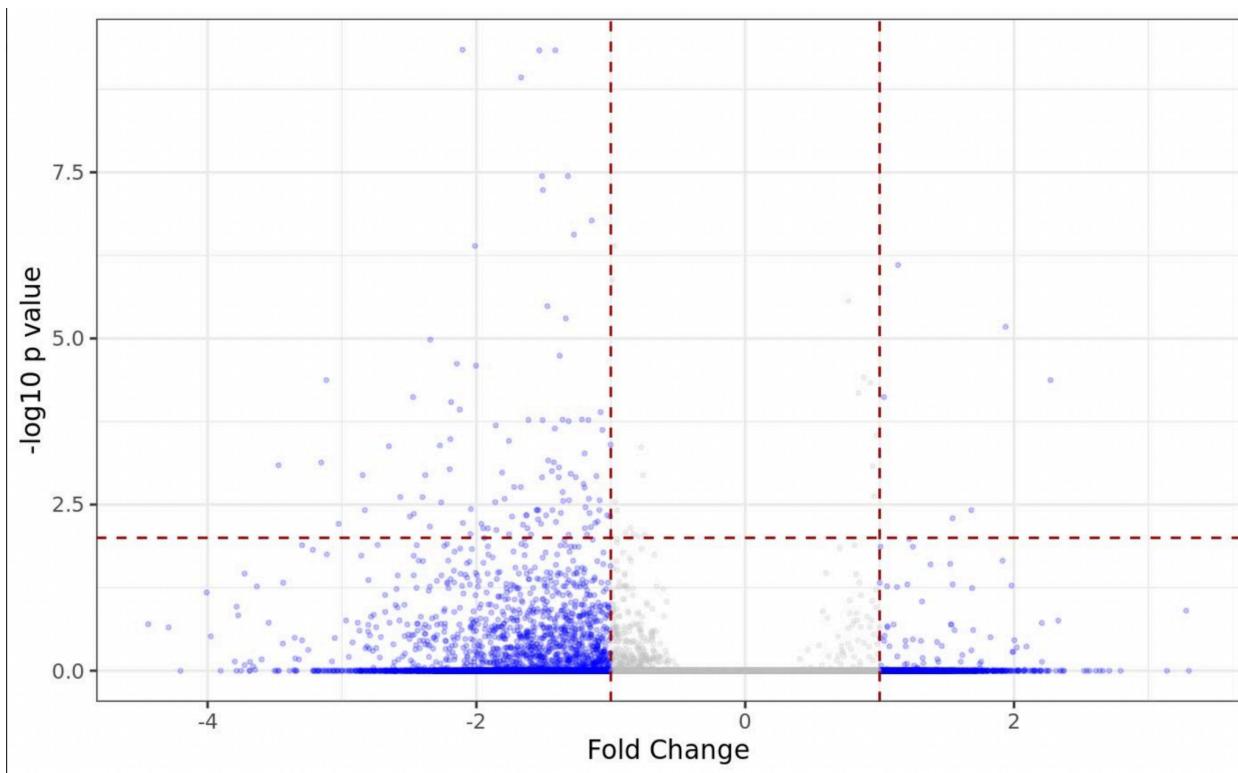


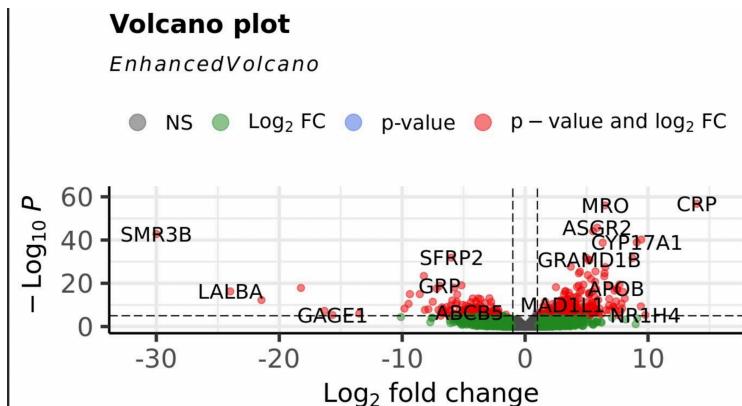
Zainab Neemuchwala  
QBIO 490  
3.23.2025

### Part 1: Naive Differential Methylation



The volcano plot visualizes differentially methylated CpG sites between younger and older TCGA BRCA patients. Most points cluster near a fold change of zero, indicating minimal widespread methylation differences between the two age groups. A few CpG sites exceed the significance threshold ( $-\log_{10} p\text{-value} > 2$ ), suggesting potential age-associated methylation changes. However, this analysis does not compare metastatic and non-metastatic patients, so no conclusions about methylation differences related to metastasis can be drawn from this plot.

### Part 2: Direct comparison of methylation status to transcriptional activity



### 1. Naive Differential Methylation (Volcano Plot) Analysis

The volcano plot illustrates differentially methylated CpG sites between metastatic and non-metastatic TCGA BRCA patients. Genes with significant changes in methylation (based on p-values and log<sub>2</sub> fold change) are highlighted, with hypermethylated and hypomethylated genes distributed across the x-axis. Genes such as MAD1L1, SFRP2, and CRP exhibit substantial methylation differences, suggesting potential roles in cancer progression. However, we cannot determine whether these changes directly influence gene expression or patient outcomes without integrating transcriptional activity.

### 2. Direct Comparison of Methylation Status to Transcriptional Activity

The second plot compares methylation and transcriptional changes, identifying genes that are both hypomethylated and upregulated. Genes such as MAD1L1 and SLC13A5 appear in this overlap, suggesting potential regulatory relationships. However, methylation is only one-factor influencing gene expression, and other epigenetic mechanism variations may also contribute. Therefore, while this analysis provides insights into epigenetic regulation, functional validation is needed to confirm causality.

### 3. CpG Site and Protein Domain Visualization with Literature Support

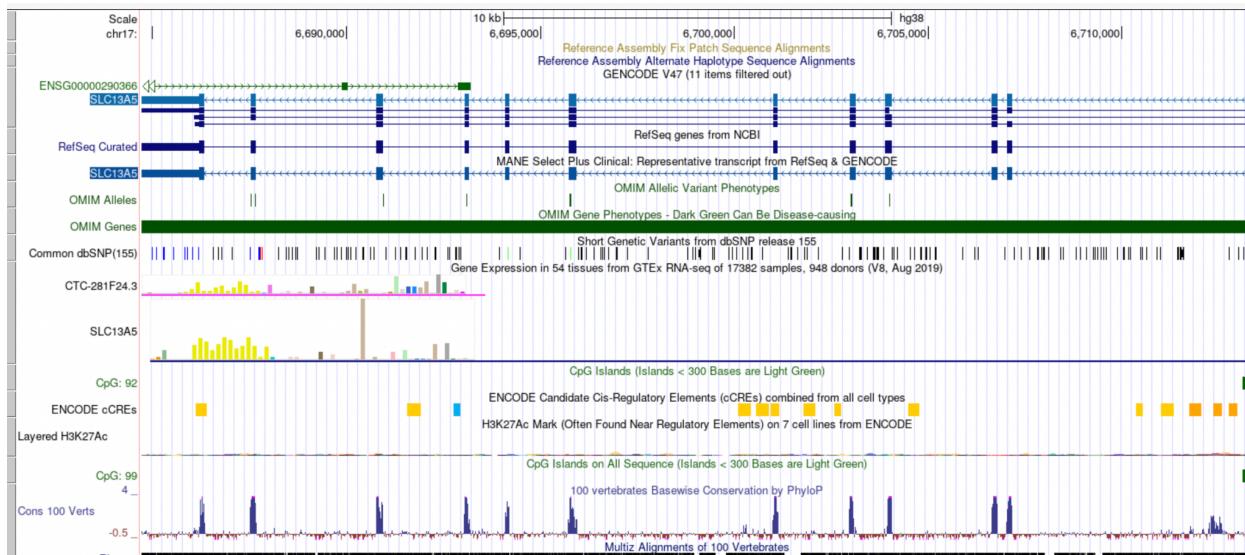
#### a) MAD1L1



From the UCSC Genome Browser, CpG islands (light green bars) are present in the MAD1L1 gene region. CpG islands are typically found in promoter regions and play a role in gene regulation via methylation. For MAD1L1, the CpG islands overlap with: the promoter region (potentially affecting gene transcription) and the gene body, which may influence alternative splicing or expression levels. Regulatory Features in the MAD1L1 Region include H3K27Ac marks (blue peaks) (indicate active enhancers or promoters) and ENCODE Candidate Cis-Regulatory Elements (cCREs) (suggest functional regulatory regions within or near the gene)

MAD1L1 is involved in mitotic checkpoint regulation and may play a role in cancer susceptibility and neuropsychiatric disorders. CpG island methylation could impact its expression, potentially influencing cell cycle regulation. Zheng et al. identified a CpG site in the promoter region of MAD1L1 where methylation was inversely associated with cancer incidence, suggesting a protective role [1]. In addition research on prostate cancer cells demonstrated that treatment with phytoestrogens led to altered methylation profiles of several genes, including MAD1L1, resulting in inhibited cell growth and induced apoptosis [2]. These findings align with the hypothesis that MAD1L1 functions as a tumor suppressor, where its promoter methylation status influences gene expression and cancer progression.

b) SLC13A5



The UCSC Genome Browser screenshot of SLC13A5, located on chromosome 17 shows that the SLC13A5 gene is transcribed with multiple exons, as indicated by the dark blue boxes, and the direction of transcription is shown by connecting arrows. CpG islands, which are known to play a role in gene regulation, are prominently located near the promoter region and within the gene body. The presence of these CpG islands suggests potential regulation through DNA methylation, which can impact gene expression. The dbSNP track shows several SNPs scattered throughout the gene, some of which overlap with CpG islands. If these SNPs alter CpG methylation sites, they could influence SLC13A5 expression, potentially contributing to disease phenotypes. The GTEx expression data shows that SLC13A5 is highly expressed in certain tissues. ENCODE identifies multiple candidate cis-regulatory elements (cCREs) in the region, with H3K27Ac histone marks near CpG islands, indicating possible enhancer activity.

Given its role in citrate transport, abnormal CpG methylation in SLC13A5 could lead to transcriptional silencing. Changes in epigenetic regulation could disrupt normal citrate metabolism, affecting neuronal function and contributing to disease. Recent studies have highlighted the significance of SLC13A5 in cancer epigenetics. In colorectal cancer, SLC13A5's promoter CpG islands have been identified as differentially methylated, suggesting a role in tumorigenesis [3]. Similarly, in renal cell carcinoma, hypermethylation of SLC13A5's promoter CpG islands has been associated with tumor aggressiveness and poorer patient outcomes [4]. These findings indicate that aberrant methylation of SLC13A5 may serve as a potential biomarker for cancer prognosis.

c) SCD5



The SCD5 gene, located on chromosome 4, is associated with lipid metabolism and has been implicated in various cancers. From the genomic visualization, there are multiple CpG islands, particularly around the promoter region, suggesting potential epigenetic regulation of SCD5 expression. The presence of H3K27Ac marks, which are often linked to active regulatory elements, indicates transcriptional activity near the CpG sites. Additionally, conserved sequence elements across multiple vertebrate species suggest that SCD5 has an evolutionarily conserved function.

Recent literature supports the role of SCD5 in cancer through epigenetic modifications. Hypermethylation of SCD5 CpG islands has been associated with melanoma progression, leading to transcriptional silencing and altered lipid metabolism [5]. Another study found that in breast cancer, SCD5 promoter methylation correlates with reduced expression and increased tumor aggressiveness [6]. These findings suggest that SCD5 may serve as a potential biomarker for cancer prognosis.

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