

# Correspondence of molecular and pathologic features between ductal carcinoma in situ (DCIS) biopsy and surgical excision specimens



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## ABSTRACT

**Background.** DNA methylation alterations are known to occur in ductal carcinoma in situ (DCIS) of the breast. Here, we aim to evaluate consistency of molecular features between biopsy and surgical specimens from the same DCIS subject.

**Methods.** We measured genome-wide DNA methylation and evaluated its consistency among 13 matched DCIS specimen pairs.

**Results.** Matched specimens exhibited consistent pathologic and DNA methylation patterning. Within-subject variability is substantially lower than between-subject variability ( $P_{median} = 3.69 \times 10^{-6}$ ). A limited number of differential CpGs indicate wound-healing gene activation.

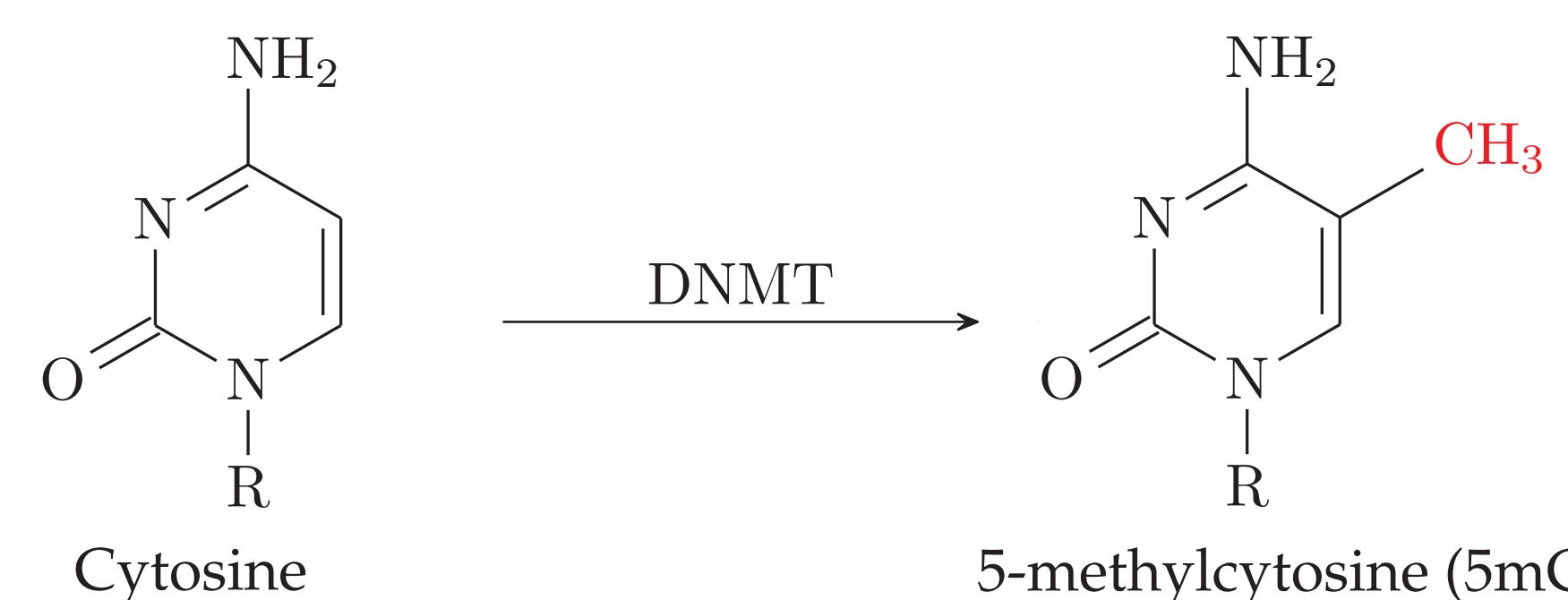
**Conclusion.** DNA methylation biomarkers measured in pre-operative specimens have utility for clinical decision-making.

## INTRODUCTION

Ductal carcinoma in situ (DCIS), a pre-invasive breast lesion, exhibits:

- 8 to 10-fold higher risks of invasive breast cancer
- consistent pathologic features and discrete biomarkers

Methylation at DNA cytosine position 5 in cytosine-guanine dinucleotides (CpGs) is an epigenetic mark commonly altered in cancer:



Our lab previously identified nearly 650 CpGs that are biomarkers for DCIS progression<sup>1</sup>. Whether DNA methylation pattern is consistent between biopsy and surgical specimens remains unclear.

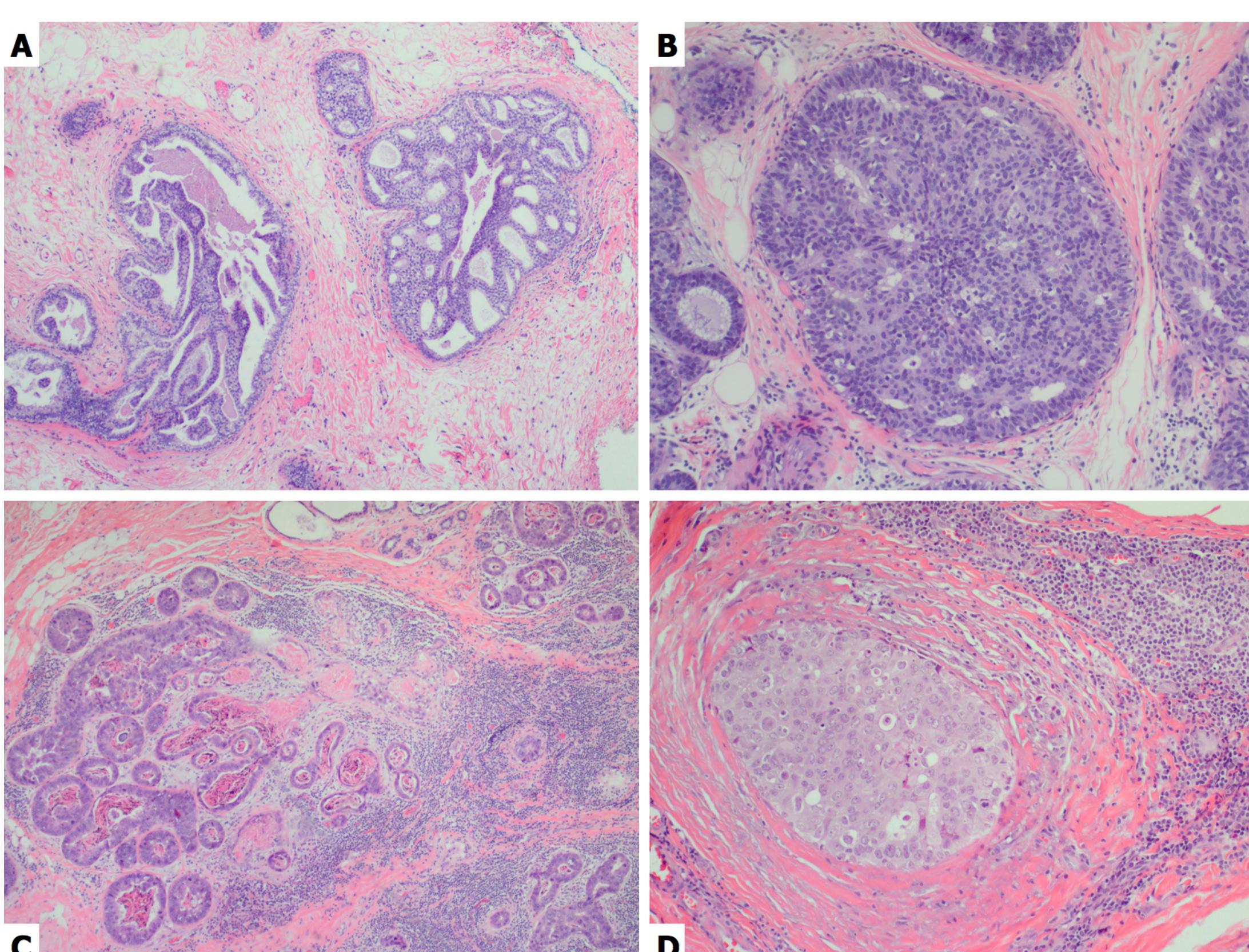


Figure 1: DCIS histopathologic features. (A) Low-grade DCIS with cibiform and micropapillary patterns. (B) Intermediate-grade DCIS with cibiform pattern. (C) High-grade DCIS with necrosis, prominent inflammation, and periductal fibrosis, or (D) periductal fibrosis and chronic inflammation.

## METHODS

Guided 2-mm DNA isolation, bisulfite modification from matched DCIS biopsy and surgical specimens (n=15)

Illumina HumanMethylation450 array and preprocessing (n=13)

Hierarchical clustering  
Pathway analysis  
Variability comparisons

## CONSISTENCY IN DNA METHYLATION PATTERNING

Unsupervised clustering revealed greatest similarity between matched specimens (Fig.1). We also observed striking similarities between matched specimens in the context of gene transcription (Fig.2).

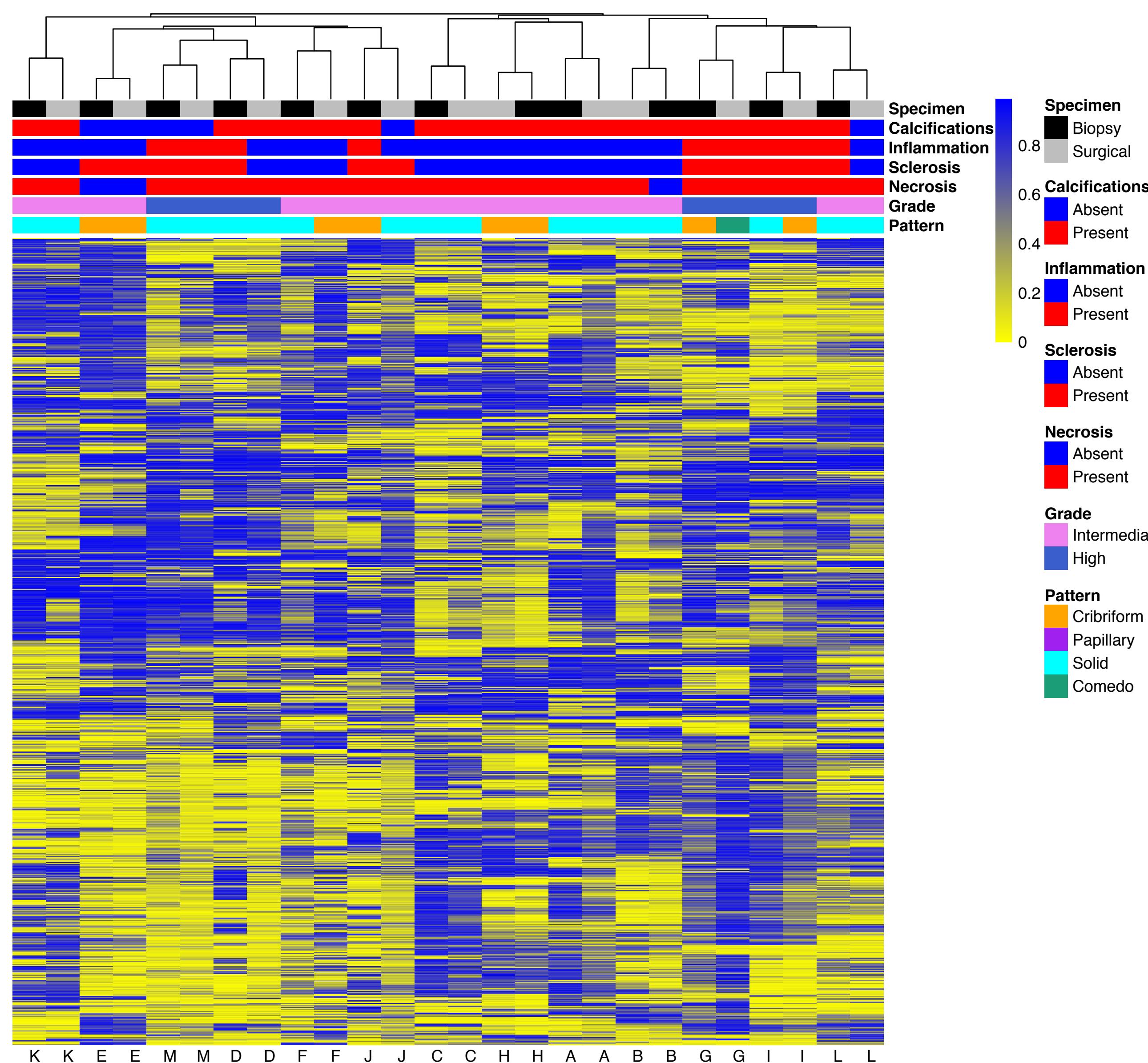


Figure 2: Hierarchical clustering with Manhattan distance and average linkage metric. Rows and columns represent CpGs and specimens, respectively.

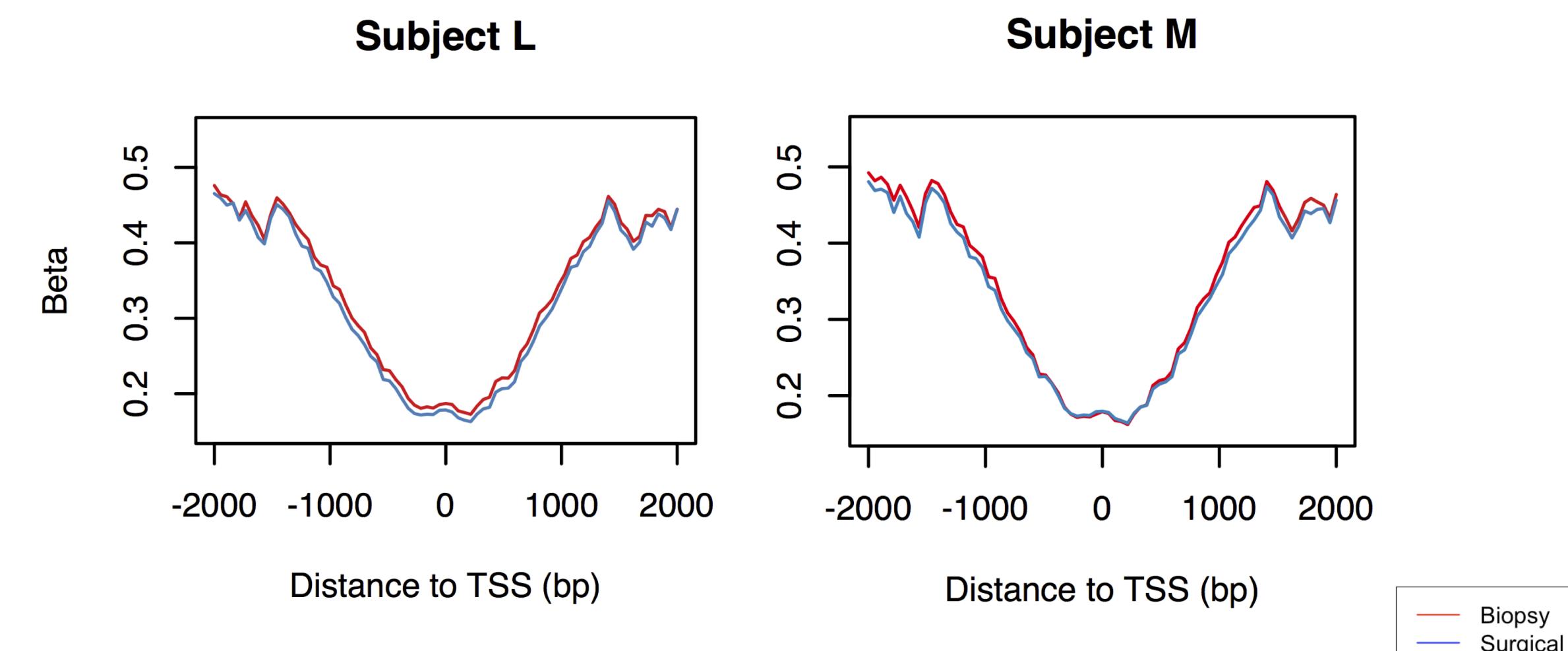


Figure 3: Representative examples of DNA methylation by genomic context between patient-matched specimens. Beta, fraction of methylated alleles.

## PATHWAY ANALYSIS AND SUBJECT VARIATIONS

Since surgical specimens were acquired after biopsy, we hypothesized that pathways related to wound healing are enriched in surgical excision specimens. Thus, we calculated:

$$\Delta\beta = \beta_{\text{biopsy}} - \beta_{\text{matched surgical}}$$

Pathway analysis suggests that limited CpGs (< 0.03%) below  $\Delta\beta \leq -0.15$  were related to wound healing (Table 1).

Table 1: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched in surgical specimens with false discovery rate (FDR) <0.01.

Pathway Name	Fraction	FDR
Ascorbate and aldarate metabolism	0.33	0.0002
Type I diabetes mellitus	0.23	0.0013
Cell adhesion molecules	0.13	0.0048
Pentose and glucuronate interconversions	0.21	0.0095
Porphyrin and chlorophyll metabolism	0.19	0.0095
Extracellular matrix receptor interaction	0.15	0.0095
Glutamatergic synapse	0.13	0.0095
Asthma	0.23	0.0095
Viral myocarditis	0.17	0.0095

We then compared matched- versus permuted  $\Delta\beta$  distributions, which represent within- and between-subject variability, respectively (Fig.4).

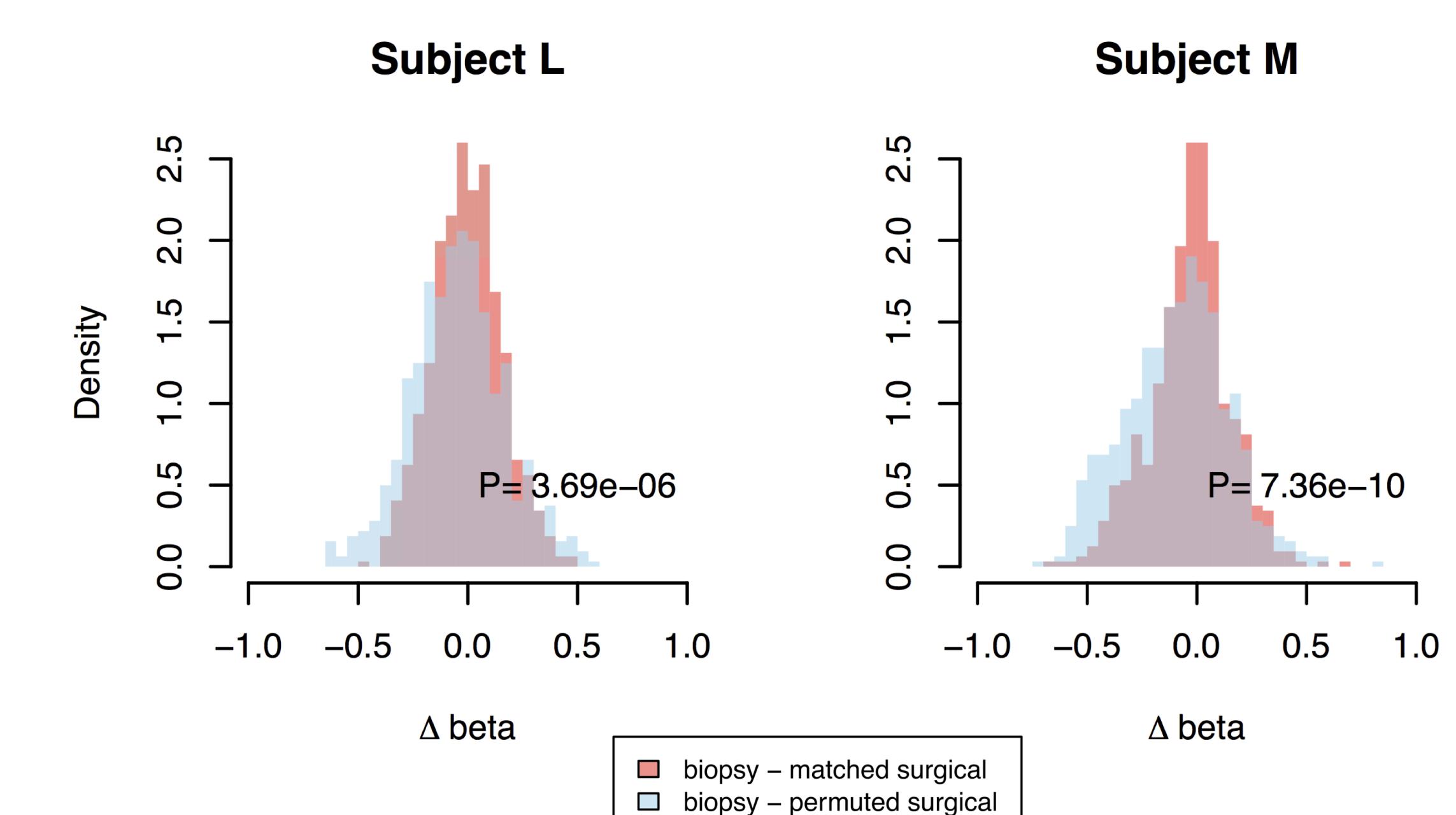


Figure 4: Within- vs. between-subject variation. Kolmogorov-Smirnov test shows 12/13 (92.3%) specimens exhibit differences in the distributions of within- and between-subject variations in 641 DCIS progression-related CpGs<sup>1</sup>.

## CONCLUSION

Our findings provide evidence supporting accurate epigenetic profiling in DCIS without the use of potentially invasive surgical procedures.

## ACKNOWLEDGMENTS & REFERENCES

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1. Johnson KC, et al. *Clin. Epigenet.* 7:75 (2015)