RNA-SEQ ANALYSIS OF GENE EXPRESSION IN FUSED SUTURES

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BACKGROUND

- Craniosynostosis is a **craniofacial malformation** defined as the premature fusion of one or more cranial sutures which results in skull deformities.
- Heterogeneous origin, affected by **genetic and environmental factors**.[1]
- However, the molecular mechanisms behind it remain unclear and require further research into epigenetic mechanisms, which are poorly understood.[2]

- **Aim:** To analyze gene expression in children with single suture synostosis by RNA sequencing (RNA-seq), comparing affected and normal sutures.

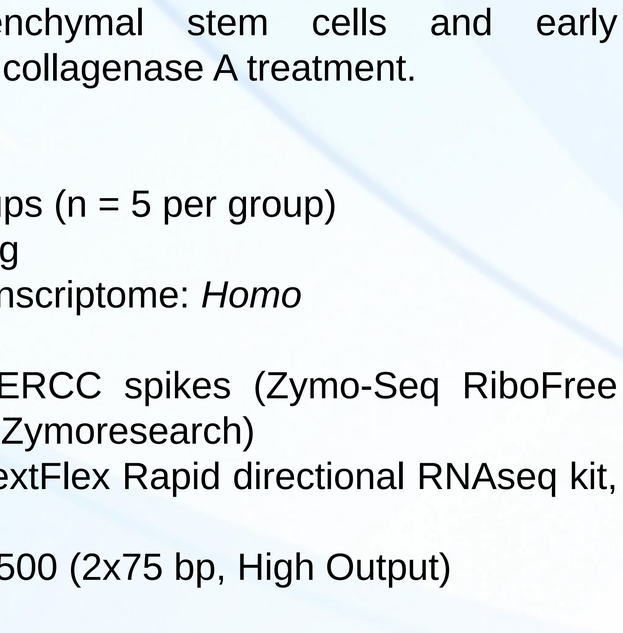
METHODS

Samples: Surgical bone fragments from fused and normal sutures, healthy or pathological bone in patients without known mutations in association with craniosynostosis.

	Suture/Bone	
Patient	Healthy	Pathologic
1	N1 Metopic	N2 Sagital
2	N3 Coronal	N4 Metopic
3	N5 Coronal	N6 Metopic
4	N7 Metopic	N8 Coronal
5	N9 Parietal	N10 Parietal

Embryonic origin: Neural crest / Para-axial

Samples distribution



Superior view of skull anatomy: Sutures, fontanelles and main bones

- **Cell Preparation**: Mesenchymal stem cells and early osteoblasts recovered after collagenase A treatment.

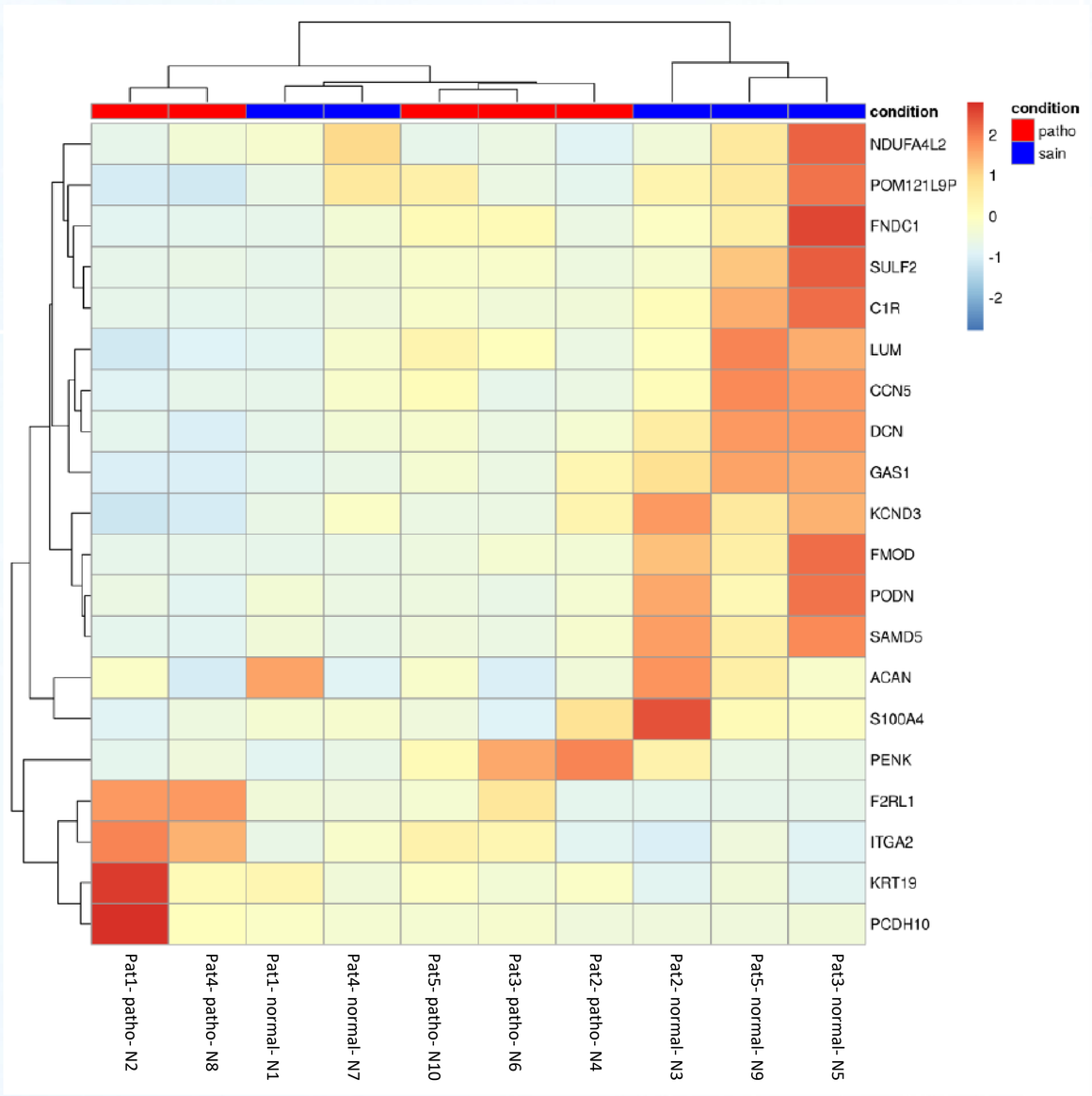
- **RNA-seq**:
  - 10 samples and 2 groups (n = 5 per group)
  - Whole-RNA sequencing
  - Reference genome/transcriptome: *Homo sapiens* (GRCh38)
  - rRNA depletion with ERCC spikes (Zymo-Seq RiboFree Total RNA Library Kit - Zymoresearch)
  - Library preparation (NextFlex Rapid directional RNAseq kit, PerkinElmer)
  - Sequencing : NextSeq500 (2x75 bp, High Output)

- **Bioinformatic Analysis**: Alignment on reference genome/ Transcriptome and QC, Tag counting Differential expression analysis. DESeq2 R package v1.26.0

RESULTS

- Sequencing quality control : The RNA sequencing run passed all Illumina quality thresholds : ≈ 95% of bases have a quality score above Q30.
  → **High-quality sequencing data**.

- Differential expression analysis identified **88 significantly deregulated genes** (adjusted p-value ≤ 0.05, |log2FC| ≥ log2(1.5)).
- Among them, **28 genes were upregulated** and **60 were downregulated** in pathological sutures compared to normal ones.
- The **heatmap of the top 20 significant genes** shows coherent variations between pathological and normal samples.



Differential analysis : Heatmap (20 most significant genes)

DISCUSSION

- Our results confirm that **transcriptional and epigenetic regulation have a crucial role in premature cranial suture fusion**.
- Overexpressed genes could be markers of abnormal activation of biological pathways such as cell hyperproliferation.
- Underexpressed genes may reflect a loss of function in the regulation of normal cellular differentiation.
- Further **functional validation**, including studies with a larger patient cohort and microRNA profile analysis, is needed to better understand the molecular interactions and their role in osteoblast differentiation.
- Identifying potential therapeutic targets could pave the way for **preventive or corrective strategies for bone anomalies in craniosynostosis malformations**.

References :

1. Johnson D, Wilkie AO. Craniosynostosis. Eur J Hum Genet. 2011 Apr;19(4):369-76. doi: 10.1038/ejhg.2010.235. Epub 2011 Jan 19. PMID: 21248745; PMCID: PMC3060331
2. Bin Alamer O, Jimenez AE, Azad TD. Single-suture craniosynostosis and the epigenome: current evidence and a review of epigenetic principles. Neurosurg Focus. 2021 Apr;50(4):E10. doi: 10.3171/2021.1.FOCUS201008. PMID: 33794485.

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