Abstract Text:

The genetics of non-syndromic craniosynostosis remain obscure. The molecular mechanisms of patency and fusion have not been fully elucidated. Calvarial and sutural morphogenesis involves a complex balance between cell proliferation, differentiation, and apoptosis. The developing osteogenic front is tightly regulated by precisely timed gene expression. Any disruption of expression results in abnormal development. MicroRNAs (miRNAs) are small endogenous noncoding RNAs which regulate gene expression post-transcriptionally by binding to target mRNAs and inhibiting translation. They have been implicated in the etiology of multiple diseases through dysregulation of gene expression. The purpose of this research was to measure miRNA expression in patent and synostotic human infant calvarial sutures and identify any differential expression.

The research protocol received IRB approval and written informed consent was obtained for 7 subjects. Bone fragments of fused and patent sutures were recovered during cranial remodeling surgery of patients with non-syndromic craniosynostosis less than one year of age. Total RNA was isolated from the patient paired cranial suture fragments and global miRNA expression was assayed for 14 samples (7 paired fused/patent) using the Illumina BeadArray platform. Expression levels for greater than 700 annotated miRNAs were compared pairwise and by group with patent miRNA expression as baseline. Raw data was processed using Illumina's GenomeStudio software. Background noise was subtracted and sample signals normalized using the Quantile method. Assay performance was assessed through internal controls and duplicate samples. All internal controls performed well and paired t-tests revealed no significant differences between duplicate samples. Preliminary differential expression was measured using Illumina's Custom Model and a Diff Score calculated for each probe set. Probe sets with a detection p-value greater than 0.05 were not included in further statistical analysis which was performed using R and excel. Log2 fold changes were calculated with values ≥ 2.0 and ≤ -2.0 considered significant.

We identified 31 overexpressed and 9 underexpressed miRNAs in fused sutures compared with patent sutures.

We conclude that miRNAs are differentially expressed in patent and synostotic calvarial sutures in human infants. miRNAs may play a role in the proximate molecular mechanisms involved in the maintenance suture patency.