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Functional characterization of the Bardet Biedl syndrome-associated gene 9 in nonsyndromic craniosynostosis

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Introduction: The molecular etiopathogenesis of nonsyndromic craniosynostosis (NSC) are still largely unknown. Recent evidence obtained thorugh GWAS on a large cohort of patients indicated the significant association of sagittal NCS to the Bardet Biedl Syndrome-associated gene 9 (BBS9). BBS9 is a structural protein located in the transition zone of the primary cilium, a cell membrane sensor, involved in multiple developmental processes. Preliminary data demonstrated that cells isolated from fused sutures of midline NCS patients display aberrant BBS9 expression and a differently shaped and developed primary cilium. The aim of this study was to investigate the functional role of BBS9 in the aberrant osteogenic phenotype of calvarial cells isolated from NSC patients.

Methods: Calvarial-derived mesenchymal stem cells (CMSC) were isolated in primary culture from surgically-collected fused and patent sutures of patients affected by NSC. The expression of BBS9 was analyzed through immunofluorescence using confocal microscopy, in presence and absence of in vitro osteogenic induction. The BBS9 gene was transiently silenced by siRNA in CMSC isolated from fused specimens and the effect of this gene modulation was analyzed using real time PCR.

Results: The expression and localization of BBS9 appeared aberrant in fused-sutures- derived cells compared to controls; and increased after 5 days of osteogenic induction. siRNA-mediated gene silencing allowed reducing by 90% BBS9 expression upon 48 hours of treatment and induced the downregulation of the ospecific transcription factor RUNX2. Furthermore, the expression of SMO, a key molecule of the hedgehog signaling pathway, was down-regulated by 50% upon BBS9 silencing.

Conclusion: These data seemed to indicated a previously unknown role of BBS9 in osteogenesis, enabling to confirm its functional implication in the aberrant osteogenic process occurring at the site of premature suture closure in NSC.