Introduction: In contrast to endochondral growth with ossification of a pre-existing cartilaginous matrix, cranial sutures act as intramembranous bone growth sites. New bone is produced at the sutural edges by proliferation of mesenchymal cells and subsequent differentiation into osteoblasts. The suture itself remains in an unossified stage in which maintenance of growth at the osteogenic fronts requires a balance between proliferation and differentiation. Disruption of any of these processes can result in premature fusion of calvarial sutures, known as craniosynostosis. Premature fusion of cranial sutures is a complex pathomechanism in which alterations of transcription factors, growth factors and their receptors may be the regulating items. In suture fusion type I collagen, TGF-beta, FGR and BSP-1 have been identified to be upregulated in the suture matrix, whereas CBFA1, FGF-2 and IGF-1 become expressed in the bone fronts. This process is most likely responding to external stimuli like the expanding brain. Dura mater and periosteum as well may have an influence on suture fusion and bone formation but exact signalling in normal and pathological suture fusion is still unknown. Studies on the bony structure and bony contents of normal and pathological sutures in humans were expected to give us further information about suture morphogenesis.

Material and Methods: 5 infants with true suture synostosis of the sagittal suture were operated on in which the fused segment was resected. Normal sagittal, coronal and lambdoid sutures were obtained from 5 infants at autopsy. The circumstances leading to death were sudden infant death syndrome or other causes not related to central nervous system (CNS) diseases or cranial vault pathological entities. Informed parental consent was obtained prior to investigations. Radiographical analyses of the sutures were performed routinely to exclude or to confirm true synostosis. Microarchitecture and suture content analyses from normal cranial sutures as well as from pathological sutures between the age of three and twelve months were carried out. Comparisons of the osseous structure of the adjacent bony plates of normal and pathological pathologies were performed by static 2D-histomorphometry, and bone mineral density distribution analyses (BMDD) by quantitative backscattered electron imaging (qBEI). Spectra of energy dispersive x-ray analyses (EDX) were utilized to investigate the unmineralized suture content.

Results: 2D-histomorphometric assessment of structure parameters in terms of bone volume per tissue volume (BV/TV, %), trabecular thickness (Tb.Th., μm), trabecular number (Tb.N., 1/mm) as well as trabecular separation (Tb.Sp., μm) in the adjacent bony plates alongside normal sagittal, coronal and lambdoid sutures showed no structural differences. Moreover, osteoid indices as osteoid thickness (O.Th., μm), osteoid volume/bone volume (OV/BV, %) and osteoid surface/bone surface (OS/BS, %) do not differ in the bony plates of normal sagittal, coronal and lambdoid sutures. In non-fused segments the peristeal surface of the bony plates exhibited an irregular resorptive surface and the bone adjacent to this surface demonstrates formation of woven bone. In fused segments the peristeal surface of the bony plates showed a smooth outline and the adjacent zone of bone contained numerous primary osteons. However, the adjacent trabecular architecture as determined by BV/TV, Tb.Th, Tb.N., and Tb.Sp. revealed no significant differences and thus showed comparable values in control specimen and cases with sagittal synostosis (Fig. 1 A,B). Histomorphometric assessment of osteoid parameter as O.Th., OV/BV, and OS/BS which indicate mineralization changes do not vary between control specimen and cases with sagittal synostosis. BMDD analyses in normal and synostotic sutures showed comparable values in the mean calcium content (Ca mean, Wt%) of the bony plates, whereas in synostotic sutures the heterogeneity in mineralization was significantly decreased (Fig. 2 A-D). In fused segments reduced Δ calcium width (Ca width, Wt%) is associated with a decrease of woven bone packets due to advanced remodeling in favor of the formation of primary osteons. EDX microanalyses of the fused and non-fused segments showed no differences in elemental compositions, whereas increased levels of hydroxyapatite component calcium (Ca) as well as sulphur (S) were found in normal and synostotic suture margins in comparison to their unmineralized suture centers. Continuously variable data was subjected to the Shapiro–Wilks test to define distribution. That distributed parametric were analysed by the t-test. Data distributed non-parametric were analysed by the paired Wilcoxon rank sum test. P-values of p ≤ 0.05 were considered to be significant.

Discussion: This study illustrates that synostotic sutures show a decreased heterogeneity in mineralization which came along with a decreased width of mineralized bone packets due to already remodeled bone initiated by completed fusion of segments. These investigations strengthen previous findings by adding new information about morphogenesis and especially bone biology in cranial sutures. In conclusion our data seems to emphasize the theory of a dynamic fusion process in suture synostosis just commencing too early for its age. Beyond the elaboration of histomorphometric, EDX microanalyses and BMDD analyses future research is needed giving us further new perceptions in craniosynostosis. Future studies should focus on the molecular mechanisms and its signaling on premature suture fusion probably discovering new therapeutic approaches.

Fig. 1 A,B: Undecalcified histological sections of non-fused (A) and fused segments (B) from sagittal sutures (von Kossa staining, 5x).

Fig. 2: Bone Mineral Density Distribution Analyses. Quantitative backscattered electron image of a non-fused segment at the sagittal suture. The intramembranous gap has a dimension of approximately 1.67 mm (A). In the fused segment of the sagittal suture the gap is approximately 0.45 mm (B). The overall mineral content (Ca mean, Wt%) of the bone in the non-fused segments showed no significant difference in comparison to the fused-segments (C). However, the mineralization’s heterogeneity (Ca width, Wt%) significantly decreased in the fused segments due to the formation of evenly mineralized primary osteons after suture synostosis (D).