Newly discovered bone stem cell causes premature skull fusion

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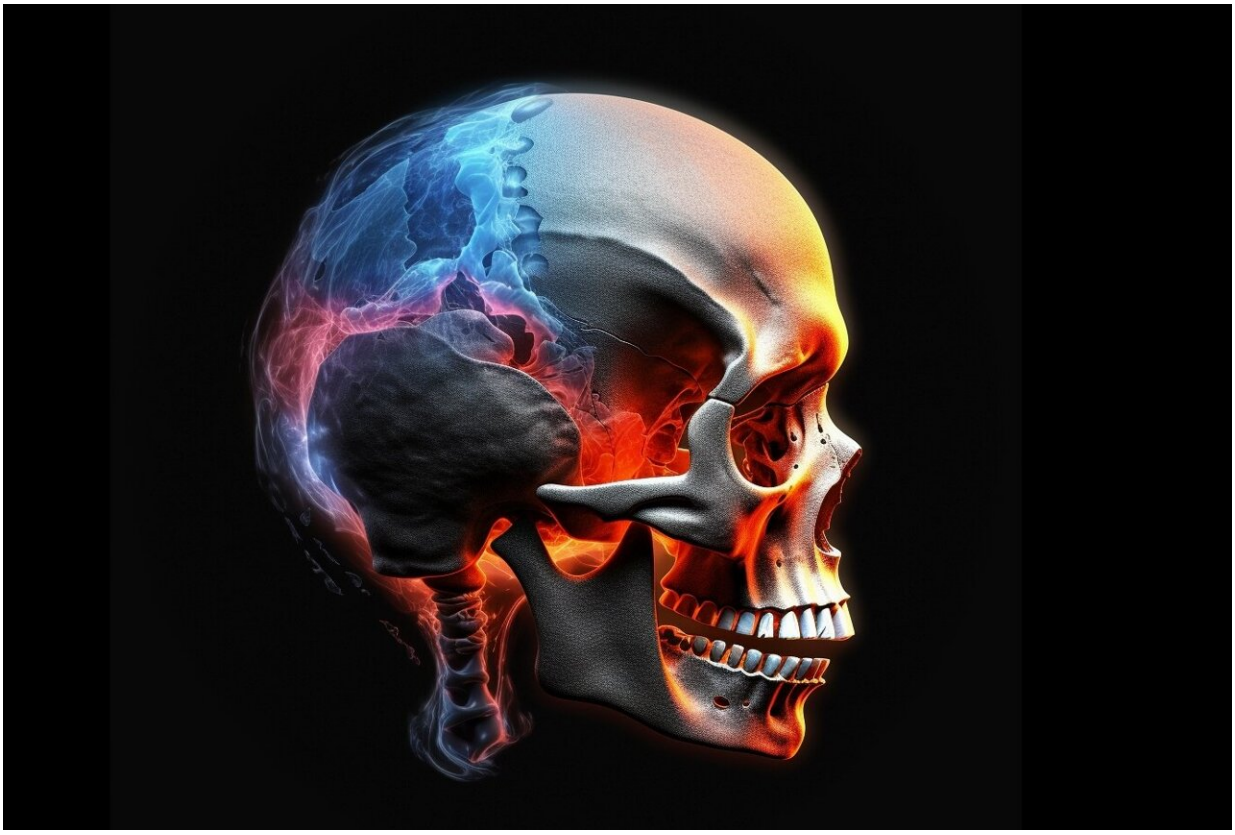


A conceptual rendering of how a new stem cell in the joints between the flat bones of the skull drives skull growth and fusion. Credit: AI image generated using Midjourney on May 24, 2023; provided by the Greenblatt lab.

Craniosynostosis, the premature fusion of the top of the skull in infants,

is caused by an abnormal excess of a previously unknown type of bone-forming stem cell, according to a preclinical study led by researchers at Weill Cornell Medicine.

Craniosynostosis arises from one of several possible gene mutations, and occurs in about one in 2,500 babies. By constricting brain growth, it can lead to abnormal brain development if not corrected surgically. In complex cases, multiple surgeries are needed.

In the study, which appears in *Nature*, the researchers examined in detail what happens in the skull of mice with one of the most common mutations found in human craniosynostosis. They found that the mutation drives premature skull fusion by inducing the abnormal proliferation of a type of bone-making stem cell—the DDR2+ stem cell—that had never been described before.

"We can now start to think about treating craniosynostosis not just with surgery but also by blocking this abnormal stem cell activity," said study co-senior author Dr. Matt Greenblatt, an associate professor of pathology and laboratory medicine at Weill Cornell Medicine and a pathologist at NewYork-Presbyterian/Weill Cornell Medical Center.

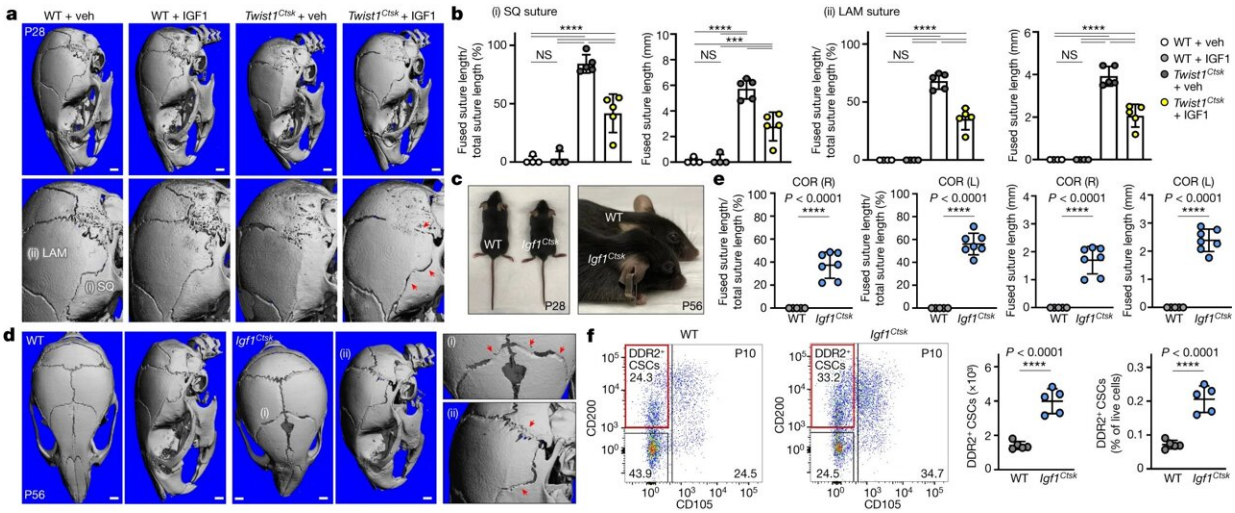
The other co-senior author of the study was Dr. Shawon Debnath, a research associate in the Greenblatt laboratory.

In a study published in *Nature* in 2018, Drs. Debnath and Greenblatt and their colleagues, described the discovery of a type of bone-forming stem cell they called the CTSK+ stem cell. Because this type of cell is present in the top of the skull, or "calvarium," in mice, they suspected that it has a role in causing craniosynostosis.

In the new study, they investigated that possibility by engineering mice in which CTSK+ stem cells lack one of the genes whose loss of function

causes craniosynostosis. They expected that the gene deletion somehow would induce these calvarial stem cells to go into bone-making overdrive. This new bone would fuse the flexible, fibrous material called sutures in the skull that normally allow it to expand in infants.

"We were surprised to find that, instead of the mutation in CTSK+ stem cells leading to these stem cells being activated to fuse the bony plates in the skull as we expected, mutations in the CTSK+ stem cells instead led to the depletion of these stem cells at the sutures—and the greater the depletion, the more complete the fusion of the sutures," Dr. Debnath said.



Identification of IGF1 as a CTSK^{+} CSC-derived factor regulating DDR2^{+} CSCs. **a**, μCT of the skulls of *Twist1*^{Ctsk} mice at P28 after IGF1 treatment, showing rescue of fusion. The experimental design is presented in Extended Data Fig. 9c. Arrows show sites of suture patency (WT + veh, $n = 4$; WT + IGF1, $n = 4$; *Twist1*^{Ctsk} + veh, $n = 5$; *Twist1*^{Ctsk} + IGF1, $n = 5$). Veh, vehicle. **b**, Fused suture length as a proportion of total suture length in SQ and LAM (WT + veh, $n = 4$; WT + IGF1, $n = 4$; *Twist1*^{Ctsk} + veh, $n = 5$; *Twist1*^{Ctsk} + IGF1, $n = 5$). **c**, Gross appearance of *Igf1*$^{fl/fl}$*;Ctsk-cre* (*Igf1*^{Ctsk}) mice at P28 and P56. **d**, μCT 3D scans of the skulls of wild-type and *Igf1*^{Ctsk} mice at P56 (WT, $n = 5$; *Igf1*^{Ctsk}, $n = 7$).

Red arrows show fusing sutures. **e**, Fused suture length as a proportion of total suture length in wild-type ($n = 5$) and $Igf1^{Ctsk}$ ($n = 7$) mice at P56. **f**, FACS analysis (left; representative of 5 biological replicates) and quantification (right; dot represents an individual mouse) of $DDR2^+$ CSCs in the sutures of wild-type and $Igf1^{Ctsk}$ mice at P10 ($n = 5$), showing expansion of sutural $DDR2^+$ CSCs. Data are mean ± s.d. Unpaired two-tailed Student's *t*-test (**e**,**f**); one-way ANOVA with Tukey's post-test (**b**). Images in **a**,**d** represent results from at least four biological replicates. Scale bars, 1.0 mm. Credit: *Nature* (2023). DOI: 10.1038/s41586-023-06526-2

The unexpected finding led the team to hypothesize that another type of bone-forming stem cell was driving the abnormal suture fusion. After further experiments, and a detailed analysis of the cells present at fusing sutures, they identified the culprit: the DDR2+ stem cell, whose daughter cells make bone using a different process than that utilized by CTSK+ cells.

The team found that CTSK+ stem cells normally suppress the production of the DDR2+ stem cells. But the craniosynostosis gene mutation causes the CTSK+ stem cells to die off, allowing the DDR2+ cells to proliferate abnormally.

To investigate these stem cells in human tissue, the team formed a collaboration with craniosynostosis surgeon Dr. Caitlin Hoffman, neurogeneticist Dr. Elizabeth Ross, and neuropathologist Dr. David Pisapia, all at Weill Cornell Medicine and NewYork-Presbyterian/Weill Cornell Medical Center; and craniosynostosis surgeon Dr. Thomas Imahiyerobo of Columbia University Vagelos College of Physicians and Surgeons and NewYork-Presbyterian/Columbia University Irving Medical Center.

The researchers found the human versions of DDR2+ stem cells and

CTSK+ stem cells in calvarial samples from craniosynostosis surgeries—underscoring the likely clinical relevance of their findings in mice.

The findings suggest that inappropriate DDR2+ stem cell proliferation in the calvarium, in infants with craniosynostosis-linked gene mutations, could be treated by suppressing this stem cell population, through mimicking the methods that CTSK+ stem cells normally use to prevent expansion of DDR2+stem cells. The researchers found that the CTSK+ stem cells achieve this suppression by secreting a growth factor protein called IGF-1, and possibly other regulatory proteins.

"We observed that we could partly prevent calvarial fusion by injecting IGF-1 over the calvarium," said study first author Dr. Seoyeon Bok, a postdoctoral researcher in the Greenblatt laboratory.

"I can imagine DDR2+ stem cell-suppressing drug treatments being used along with surgical management, essentially to limit the number of surgeries needed or enhance outcomes," Dr. Greenblatt said.

In addition to treatment-oriented research, he and his colleagues now are looking for other bone-forming stem cell populations in the skull.

"This work has uncovered much more complexity in the skull than we ever imagined, and we suspect the complexity doesn't end with these two stem cell types," Dr. Greenblatt said.

More information: Matthew Greenblatt, A multi-stem cell basis for craniosynostosis and calvarial mineralization, *Nature* (2023). DOI: 10.1038/s41586-023-06526-2.
www.nature.com/articles/s41586-023-06526-2