Evolutionary biology: Why cattle only have two toes

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During evolutionary diversification of vertebrate limbs, the number of toes in even-toed ungulates such as cattle and pigs was reduced and transformed into paired hooves. Scientists at the University of Basel have identified a gene regulatory switch that was key to evolutionary adaption of limbs in ungulates. The study provides fascinating insights into the molecular history of evolution and is published by *Nature* today.

The fossil record shows that the first primitive even-toed ungulates had legs with five toes (=digits), just like modern mice and humans. During their evolution, the basic limb skeletal structure was significantly modified such that today's hippopotami have four toes, while the second and fifth toe face backwards in pigs. In cattle, the distal skeleton consists of two rudimentary dew claws and two symmetrical and elongated middle digits that form the cloven hoof, which provides good traction for walking and running on different terrains.

Comparative analysis of embryonic development

A team led by Prof. Rolf Zeller from the Department of Biomedicine at the University of Basel has now investigated the molecular changes which could be responsible for the evolutionary adaptation of ungulate limbs. To this aim, they compared the activity of genes in mouse and cattle embryos which control the development of fingers and toes during embryonic development.

The development of limbs in both species is initially strikingly similar and molecular differences only become apparent during hand and foot plate development: in mouse embryos the so-called Hox gene transcription factors are distributed asymmetrically in the limb buds which is crucial to the correct patterning of the distal skeleton. In contrast, their distribution becomes symmetrical from early stages onward in limb buds of cattle embryos: "We think this early loss of molecular asymmetry triggered the evolutionary changes that ultimately resulted in development of cloven-hoofed distal limb skeleton in cattle and other even-toed ungulates", says Developmental Geneticist Prof. Rolf Zeller.

Loss of asymmetry preceded the reduction and loss of digits

The scientists in the Department of Biomedicine then focused their attention on the Sonic Hedgehog (SHH) signaling pathway, as it controls Hox gene expression and the development of five fingers and toes in mice and humans. They discovered that the gene expression in limb buds of cattle embryos is altered, such that the cells giving rise to the distal skeleton fail to express the Hedgehog receptor, called Patched1. Normally, this receptor serves as an antenna for SHH, but without Patched1 the SHH signal cannot be received and the development of five distinct digits is disrupted. The researchers could establish that the altered genomic region – a so-called cis-regulatory module – is linked to the observed loss of Patched1 receptors and digit asymmetry in cattle embryos.

"The identified genetic alterations affecting this regulatory switch offer unprecedented molecular insights into how the limbs of even-toed ungulates diverged from those of other mammals roughly 55 million years ago", explains Rolf Zeller. At this stage, it is unclear what triggered

inactivation of the Patched1 gene regulatory switch. "We assume that it is the result of progressive evolution, as this switch degenerated in cattle and other even-toed ungulates, while it remained fully functional in some vertebrates such as mice and humans".

More information: Javier Lopez-Rios, Amandine Duchesne, Dario Speziale, Guillaume Andrey, Kevin A. Peterson, Philipp Germann, Erkan Ünal, Jing Liu, Sandrine Floriot, Sarah Barbey, Yves Gallard, Magdalena Müller-Gerbl, Andrew D. Courtney, Christophe Klopp, Sabrina Rodriguez, Robert Ivanek, Christian Beisel, Carol Wicking, Dagmar Iber, Benoit Robert, Andrew P. McMahon, Denis Duboule and Rolf Zeller Attenuated sensing of SHH by Ptch1 underlies evolution of bovine limbs *Nature* (2014) | DOI: 10.1038/nature13289

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