Lineage tracing of cells from the sino-atrial node area to determine the fate of the chicken cardiac conduction system

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Introduction.
Knowledge about the developmental origin of the cardiac conduction system (CCS) is important to understand the pathophysiology of arrhythmias. During early development, the heart consists of two bilateral plates of splanchnic mesoderm, which fuse to form the primary heart tube (PHT) derived from the first heart field. A large number of cells at the venous pole of the PHT constitute a different cardiac progenitor population, called the second heart field (SHF). The contribution of this SHF population to the developing CCS remains the subject of discussion. We explored the feasibility of in ovo physical lineage tracing in chicken embryos, to perform long-term follow-up of SHF-derived cells contributing to the CCS.

Methods and Results.
Labeling was achieved by injecting a solution of two fluorescent dyes (DiI/5-TAMRA, Invitrogen) at HH stages 14-17, using a programmable microinjector (IM-300 Narishige, Japan). Right-sided labeling of the splanchnic mesoderm and the right cardinal vein was performed. Early embryos were sacrificed and analyzed 1-3 hours after injection (HH stages 14-17). Fluorescent labeling was found in the mesenchyme surrounding the sinus venosus and the right cardinal vein. In older stages of development (up to HH26), labeling of the developing sinus node and venous valves was found.

Conclusions.
Our results show that it is feasible to label the SHF and elements of the CCS in ovo, which makes it possible to trace the fate of these cells over a longer period of time. Follow-up after HH stage 26 is needed to determine whether the contributions of the SHF are not only found in the sinus node but also in the atrioventricular node, common bundle and bundle branches. Future plans include earlier labeling experiments as well as contribution of the left-sided SHF and determination of the differentiation capacities of these SHF derived cells.