The development of the venous pole of the heart
Mommersteeg, M.T.M.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

Introduction
The development of the venous pole of the heart
The recent success of treatment of congenital heart disease, which affects 0.7-1% of all children, has led to a marked increase in the number of children with congenital heart disease reaching adulthood. However, congenital heart disease is still the leading cause of death in children younger than one year and much research is still required to understand how these defects develop. Although environmental influences have been reported, the major causes underlying congenital heart defects have to be sought in genetic defects in genes necessary for heart development. Therefore, insight into how congenital heart defects develop requires knowledge of the genes and molecular processes involved in normal heart development.

The heart is the first functional organ to form during embryogenesis. Beating starts at 3 weeks of conception in humans, comparable with embryonic day 8.5 in the mouse, when the embryo becomes dependent on a functioning blood circulation for sufficient oxygenation and nutrition. At this stage, the heart is just a simple contractile heart tube. This initial heart tube undergoes a series of processes in which it elongates, loops, and forms chambers and septa, before it reaches the adult situation of the four-chambered heart. The septa are formed to separate the four chambered heart into two circulatory systems, a systemic and pulmonary circulation, the pulmonary circulation only becoming functional after birth. Oxygen-low blood returning from the systemic circulation of the body enters the right atrium through the superior and inferior caval veins. From the right atrium it enters the right ventricle, which pumps the blood through the pulmonary artery into the pulmonary circulation. After the blood is re-oxygenized in the lungs it returns to the left atrium via the pulmonary vein. From the left atrium the blood continues to the left ventricle and is returned to the body through the aorta to supply the body with oxygen and nutrients (Figure 1).

Figure 1. A, a ventral view on the inside of an adult human heart. The caval and pulmonary veins deliver the blood to the heart, whereas the blood leaves the heart through the pulmonary arteries and aorta. B, the dorsal view shows the sinus venosus myocardium in blue and the pulmonary myocardium in yellow.
In this thesis, we have focused on the development of the venous pole of the heart, in particular on the connection of the systemic caval veins and pulmonary veins to the atria (Figure 1). During the development of the heart both the systemic and pulmonary venous entrances to the atria become surrounded by myocardium. Sleeves of myocardium, called the sinus venosus myocardium, develop around the entrances of the superior and inferior caval veins to the right atrium and around the coronary sinus. This sinus venosus myocardium forms the smooth walled part of the right atrium. Within the sinus venosus myocardium, the pacemaker of the heart, the sinus node develops. The extent of formation of the myocardium surrounding the pulmonary venous return is different in human and mouse. During human development, the pulmonary vein starts with a single connection, but becomes incorporated along with its myocardium into the roof of the left atrium, causing four pulmonary veins with short myocardial sleeves to enter the left atrium. In mouse, in contrast, the pulmonary vein will not be incorporated into the left atrium, and retains a solitary atrial orifice with long myocardial sleeves.

The mechanisms of development of the venous myocardial structures, which form relatively late during heart development, have remained poorly understood. In contrast to the arterial side of the heart, the venous part has escaped most attention. However, a substantial part of all congenital heart defects concerns the venous side of the heart. Moreover, the venous myocardial structures are a frequent origin of ectopic electrical activity underlying atrial fibrillation, which is the most common cardiac arrhythmia encountered in clinical practice.

Opinions diverge on the definition of the sinus venosus and the extent of contribution of its myocardium to the heart. The entrances of the caval veins and pulmonary veins into the atria are located close to each other during development and adult life. Therefore, both a similar or distinct developmental origin for the sinus venosus and pulmonary myocardium have been proposed. The pulmonary myocardium itself, in turn, has been suggested to develop either by outgrowth of the atrial myocardium or by differentiation of the surrounding mesenchyme into myocardium. Furthermore, the finding that the pulmonary myocardium is a frequent origin of atrial fibrillation has created a longstanding debate on the presence of pacemaker cells in the pulmonary myocardium.

In this thesis we studied the development of the venous pole of the heart using gene expression analysis and three dimensional reconstructions of the area. The role of genes expressed at the venous pole was studied using knock-out embryos and embryos with hypomorphic gene expression levels. We extended this approach with lineage analyses to determine both the origin and mechanisms of development of the venous structures of the heart. Lineage analysis using the Cre-loxP system enabled to irreversibly label cells in vivo and to follow these cells and their daughter cells throughout time. The research in this thesis has made a contri-
bution to the understanding of the complicated mechanisms of development of the venous pole of the heart and the development of defects in this area.

The normal development of venous myocardial structures and the role of developmental aspects in the initiation of atrial fibrillation are outlined in the introduction, Chapter 2.

In Chapters 3, 4 and 5 the development of the sinus venosus myocardium is described. In Chapter 3 we identify the location of the sinus venosus progenitors in the cardiac mesoderm. Their spatial and genetic relation with the other cardiac progenitors during development has been investigated. In Chapter 4 the development of the sinus venosus myocardium is studied. The sinus venosus develops from a Tbx18-positive, Nkx2-5-negative progenitor population. Absence of Tbx18 causes defective formation of the sinus venosus. Chapter 5 continues on the development of the sinus venosus, but focuses now on the development of the sinus node. We describe a molecular pathway for the development of the sinus node with roles for Nkx2-5, Tbx3 and Pitx2c. In Chapter 6, we concentrate on the development of the myocardium surrounding the pulmonary vein. The pulmonary myocardium is shown to have a biphasic mechanism of development, which is affected in Pitx2c mutants. The level of Nkx2-5 expression is found to be crucial for the appearance of cells with a pacemaker-like gene program in the pulmonary myocardium. Chapter 7 focuses on the contribution of a cardiac progenitor population, which enters the heart through the dorsal mesocardium on the right side of the pulmonary vein and contributes to the atrioventricular septal complex.