

Development of the Cardiac Conduction System in Atrioventricular Septal Defect in Human Trisomy 21

NICO A. BLOM, JAAP OTTENKAMP, MARCO C. DERUITER, ARNOLD C.G. WENINK, AND ADRIANA C. GITTENBERGER-DE GROOT

Departments of Pediatric Cardiology [N.A.B., J.O.] and Anatomy and Embryology [M.C.D., A.C.G.W., A.C.G.-d.G], Leiden University Medical Center, 2300 RC Leiden, The Netherlands

ABSTRACT

In patients with atrioventricular septal defect (AVSD), the occurrence of nonsurgical AV block has been reported. We have looked for an explanation in the development of the AV conduction system. Human embryos with AVSD and trisomy 21 and normal embryos were examined (age 5–16 wk gestation). Antibodies to human natural killer cell-1 (HNK-1), muscle actin (HHF-35), and collagen VI were used to delineate the conduction system. As in normal hearts, HNK-1 transiently stains the AV conduction system, the sinoatrial node, and parts of the sinus venosus in AVSD. A large distance is present between the superior and inferior node-like part of the right AV ring bundle, comparable to 6-wk-old normal hearts. The definitive inferior AV node remains in dorsal position from 7 wk onward and does not appose to the superior node-like part as seen in normal hearts. Furthermore, in AVSD, a transient third HNK-1-positive “mid-

dle bundle” branch that is continuous with the retroaortic root branch and the superior node-like part can be identified, and thus the AV conduction system forms a figure-of-eight loop. At later stages, the AV node remains in dorsal position close to the coronary sinus ostium with a long nonbranching bundle that runs through thin fibrous tissue toward the ventricular septum. The formation of the AV node and the ventricular conduction system in AVSD and Down syndrome differs from normal development, which can be a causative factor in the development of AV conduction disturbances. (*Pediatr Res* 58: 516–520, 2005)

Abbreviations

AVSD, atrioventricular septal defect
HHF-35, muscle actin
HNK-1, human natural killer cell-1

In patients with atrioventricular septal defect (AVSD) with Down syndrome, both the occurrence of nonsurgical and late surgical AV block has been reported (1–4). The anatomy of the conduction tissue in AVSDs has been well documented and is characterized by a posterior displacement of the AV node and a long nonbranching bundle (5). It has been suggested that abnormal development of the conduction system plays a role in the onset of AV conduction disturbances in AVSD and human trisomy 21, but this has never been investigated (4). According to the “ring” theory, the cardiac conduction system is derived from four separate rings of specialized myocardium between the primitive segments of the heart that partially disappear during development (6). More recently, the use of immunohistochemical markers has resulted in a better understanding of the normal

development of the cardiac conduction system in various species, including humans (7–15). Studies in humans, using the neural tissue antigen GIN2 as marker for the conduction system, have suggested that the ventricular conduction system originates from one ring of specialized myocardium between the primitive ventricles (7). During looping and septation, this so-called primary ring forms the AV conduction system with the His bundle and bundle branches, the retroaortic root branch, and the right atrioventricular ring bundle, the last two structures of which disappear during normal development. With the use of another neural tissue antigen, human natural killer cell-1 (HNK-1), this picture has been broadened in both rats and humans, bringing into view the sinoatrial conduction system (8,10,11). The embryonic presence of three atrial internodal connections was demonstrated between the sinoatrial node and two AV node primordia (8,10). In the present study, we examined the developing cardiac conduction system in human embryos with AVSD and trisomy 21 in comparison with normal development using HNK-1 immunohistochemistry as an early conduction tissue marker.

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Correspondence: Adriana C. Gittenberger-de Groot, Ph.D., Department of Anatomy and Embryology, Leiden University Medical Center, P.O. Box 9602, 2300 RC Leiden, The Netherlands; e-mail: acgitten@lumc.nl.

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METHODS

Human embryos and fetuses with trisomy 21 and balanced AVSD were studied and compared with normal hearts. The embryos were obtained by legal abortion. The study was approved by the medical ethical committee of Leiden University Medical Center, and informed consent was obtained. The embryos were staged according to external landmarks (O'Rahilly and Muller). Gestational age of the embryos ranged from 5 to 16 wk. Eight hearts that showed an AVSD with common AV valvar orifice (7 to 16 wk gestation) and 10 normal hearts (5 to 16 wk gestation) were examined. Whole embryos or embryonic or fetal hearts were fixed at room temperature in 4% phosphate-buffered formalin solution and embedded in paraffin. The embryos were serially sectioned transversely (6 μm) and washed in phosphate-buffered solution. In eight embryos, only staining with hematoxylin-eosin was performed. In 10 embryos and fetuses (age 6–16 wk), MAb were used. The sections were washed for 15 min in phosphate-buffered solution with 0.3% hydrogen peroxide to block the endogenous peroxidase activity and washed again with phosphate-buffered solution. Sections were incubated alternately with the monoclonal anti-fibronectin antibody (Dako A245, Glostrup, Denmark), anti-collagen VI antibody (provided by Dr. Scott Klewer, Tucson, AZ), anti-HNK-1 antibody (Hybridomabank, Iowa City, IA) and anti-muscle actin (HHF-35) antibody (Dako M635), diluted in phosphate-buffered solution with 1% ovalbumin and 0.05% Tween-20. After overnight incubation, the sections were rinsed in phosphate-buffered solution. Rinsing was followed by incubation for 2 h with 1:200 diluted rabbit anti-mouse conjugated to horseradish peroxidase (Dako, P260), 2-h incubation with 1:50 goat anti-rabbit immunoglobulin (Nordic Tilburg, Netherlands), and 2 h with 1:500 rabbit peroxidase anti-peroxidase (Nordic) with washings in between. After washing, the staining reaction was performed with 0.04% diaminobenzidine tetrahydrochloride (D5637; Sigma Chemical Co., St. Louis, MO) in 0.05 M trismaleic acid (pH 7.6) and 0.006% hydrogen peroxide for 10 min, followed by washing. The slides were counterstained with Mayer's hematoxylin for 10 s. HNK-1 antigen expression was used to delineate the developing conduction system at early stages of development. At later stages, HNK-1 antigen expression disappears and anti-fibronectin, anti-collagen VI, and anti-HHF-35 antibodies were used as tissue markers to distinguish the different components of the conduction system from the working myocardium and the fibrous heart skeleton. Graphic 3D reconstructions were made to obtain a better insight into the relationship of the different components of the conduction system.

RESULTS

Seven to 9 wk of gestation: two hearts with complete AVSD stage 19 (crown-rump lengths 23 and 29 mm) and one heart with complete AVSD stage 21 (crown-rump length 35 mm). In the normal embryos, the ostium primum has closed and the inferior and superior AV endocardial cushions have fused. In the youngest embryos (stage 19) with complete AVSD, the aorta is not wedged between the atria. The primary atrial septum has normally developed, and the size and the histology of the nonfused superior and inferior AV endocardial cushions seem normal. An "ostium primum" is present between the free mesenchymal edge of the atrial septum primum and the non-fused cushions (future bridging leaflets). A large interventricular communication is present between a deficient ventricular muscular inlet septum and the cushions. The size of the ventricular inlet septum corresponds to 5–6 wk of normal cardiac development. Postero-inferiorly, the amount of extra-cardiac mesenchyme (spina vestibuli) is reduced. As a result of this, the primary atrial septum and the right pulmonary ridge are visible as two separate structures.

As in normal human embryos at these stages of development, the myocardium of the developing AV conduction system strongly expresses the HNK-1 antigen. In the 7-wk-old AVSD heart, HNK-1 staining is present in the ring of myocardium around the right part of the AV canal, the so-called right AV ring bundle. Both in AVSD and in normal hearts, this ring contains two node-like structures, one inferiorly and one su-

periorly. However, a very large distance remains present between these two node-like structures in the AVSD heart, whereas in normal hearts, they are in close apposition at 7–8 wk gestation. The prominent inferior node (future AV node) continues as a nonbranching HNK-1-positive bundle toward the crest of the deficient ventricular inlet septum. The size, oval shape, and HNK-1 staining pattern of the inferior AV node are similar to normal hearts, but its dorsal position corresponds to 6 wk of normal cardiac development. The HNK-1-positive nonbranching bundle divides into a left and right bundle branch on both sides of the septum. Unlike in normal development, the HNK-1-positive myocardium also continues as a third tract on the crest of the ventricular septum and joins superiorly the HNK-1-positive myocardium behind the unwedged aorta, the so-called retroaortic root branch and the superior node-like part of the right AV ring bundle. In normal human hearts, this anterior continuity of the ventricular conduction system cannot be demonstrated at any stage of development. The retroaortic root branch is continuous with the small mediosuperior node-like structure and the right AV ring bundle.

In normal human embryos of 6 to 8 wk gestation, HNK-1 expression is visible in parts of the myocardium of the sinus venosus in the right atrium and around the common pulmonary veins. Three HNK-1-positive connections are visible between the sinoatrial node and the developing AV conduction system: one anterior tract running through the fused venous valves (septum spurium) in the right atrial roof connecting the sinoatrial node and the superior right AV ring bundle and two posterior connections in left and right venous valve connecting the sinoatrial node and the inferior right AV ring bundle. In the youngest AVSD heart, sparse myocardial HNK-1 staining can be recognized in the sinoatrial node, the right venous valve, the base of the atrial septum, and the coronary sinus ostium. Although the staining pattern seems similar to normal hearts, the quality of the atrial and sinus venosus myocardium is too poor to identify tracts. The size and the position of the sinoatrial node are normal (Figs. 1 and 2).

Ten to 12 wk of gestation: three hearts with complete AVSD. In these three AVSD hearts, the atrial septum primum has formed a normal thin valvula foraminis ovalis, and the superior limbus of the septum secundum covers the ostium secundum. The thick muscular base of the atrial septum has a small mesenchymal edge, which is in continuity with the superior bridging leaflet.

Inferior to this attachment, the free rim of the atrial septum is now formed by a thick myocardial knob-like structure, the leading edge of which still consists of mesenchyme (future bridging tendon) and connects to the inferior bridging leaflet. Valve formation is almost complete, and the space between superior and inferior bridging leaflets has become smaller. The ventricular inlet septum is small and, a large interventricular communication is present.

As in normal hearts at this stage of development, the developing conduction system has lost its HNK-1 expression. With the use of the tissue markers anti-collagen VI and anti-HHF-35 antibodies, the sinoatrial node, the AV node, the nonbranching bundle, and bundle branches could be distinguished clearly from the working myocardium and fibrous

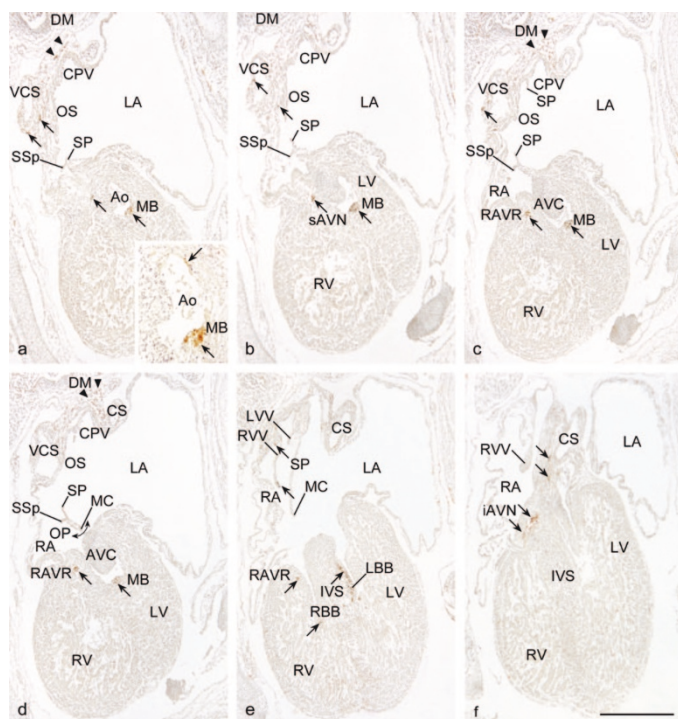


Figure 1. HNK-1 antigen expression (brown) in transverse sections (*a-f*) of a 7-wk-old human embryo with complete AVSD and trisomy 21. &U279D; HNK-1 positive myocardium; \blacktriangleright , HNK-1-positive dorsal mesocardium (DM). (*a*) HNK-1 expression anterior to the unwedged aorta in the “middle bundle” branch (MB) and sparse staining behind the aorta (Ao), also shown in detail. Note the sparse HNK-1 staining in the sinoatrial node region in front of vena cava superior (VCS). (*b* and *c*) HNK-1 expression is present in the right AV ring bundle (RARV), including a mediasuperior node-like part (sAVN), and on the crest of the deficient inlet septum (MB). Also, some HNK-1 staining is present near the VCS entrance. (*d*) Section showing the ostium primum (OP). A small mesenchymal cap forms the free rim of the atrial septum. The HNK-1-positive RAVR and the MB are visible. (*e*) A more dorsal section shows the HNK-1-positive left bundle branch (LBB) and part of the right bundle branch (RBB). (*f*) Prominent HNK-1-positive inferior AV node (iAVN) is located in dorsal position in close relation to the coronary sinus (CS) ostium. The iAVN is continuous with the RAVR and the future His bundle. HNK-1 staining is also present in the base of the atrial septum. AVC, endocardial AV cushions; CPV, common pulmonary vein; LA, left atrium; LV, left ventricle; RA, right atrium; VS, ventricular septum; RV, right ventricle; RVV, right venous valve; SP, atrial septum primum; SSp, septum spurium. Bar = 0.7 mm.

heart skeleton. The spindle-shaped sinoatrial node is normally located in front of the vena cava superior entering the right atrium. The retroaortic root branch and its anterior connection with the ventricular conduction system cannot be identified at this stage. Some remnants of the right AV ring bundle are still recognizable, but the superior AV node cannot be identified. The size and the shape of the inferior oval-shaped AV node do not significantly differ from normal hearts at this stage. Different from normal hearts, the AV node remains in its dorsal position, adjacent to the coronary sinus ostium, and has some extension to the left of the atrial septum. The right venous valve and sinus septum are closely related to the AV node. The AV node continues as the penetrating bundle at the point where the bridging tendon joins the attachment of the inferior bridging leaflet. The penetrating bundle continues as the nonbranching bundle and is remarkably long. This bundle runs through a

thin strand of fibrous tissue and is located very close to the endocardium. On top of the deficient ventricular inlet septum, the common bundle divides into a left and right bundle branch. The left bundle branch is also positioned in a more posterior position and has no relation with the outflow tract. The right bundle branch runs subendocardially on the right side of the septum. In contrast to earlier stages, the third “middle” branch over the crest of the septum cannot be identified (Fig. 3).

Sixteen weeks of gestation: two hearts with complete AVSD. These fetal hearts with complete AVSD are comparable to postnatal hearts with AVSD. The AV node remains in its dorsal position, although its size is smaller relative to the surrounding structures. The AV node boundaries are formed by the inferior insertion of the inferior bridging leaflet, the insertion of the so-called bridging tendon that forms the free rim of the atrial septum. The long nonbranching bundle runs superficially through the fibrous tissue.

DISCUSSION

The AV conduction system in AVSD is characterized by a posterior displacement of the AV node and a long nonbranching bundle (5). Detailed anatomic and electrophysiologic studies of the conduction system in AVSD have resulted in a major reduction of surgical AV block (5,16–18). Nevertheless, a relatively high occurrence of AV conduction disturbances that are not always related to surgery is still reported in patients with AVSD (1–4). Electrophysiologic studies in preoperative children with AVSD also demonstrated a high incidence of intra-atrial and AV-nodal conduction delay (19). We hypothesized that abnormal development of the cardiac conduction system in AVSD plays a causative role in AV conduction disturbances in patients with AVSD.

Normal development of the cardiac conduction system and the origin of its different components have been studied extensively in various species, including humans, and the use of immunohistochemical markers for the conduction system has provided new ideas (6–15,20). According to the classical ring theory by Wenink (6) and Anderson and Taylor (20), the

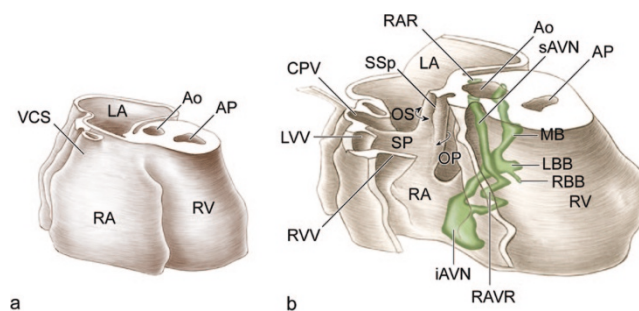


Figure 2. Three-dimensional reconstruction of the heart depicted in Fig. 1. The HNK-1 expression in the AV conduction system is indicated in green. Right lateral view with part of the RA and LA removed. The AV conduction system forms a complete figure-of-eight loop. The iAVN is connected to the nonbranching bundle. This nonbranching bundle splits into LBB and RBB but also continues anteriorly as the MB and connects to the retroaortic root branch (RAR), the sAVN, and the RAVR. Ao, aorta; AP, arteria pulmonalis; OS, ostium secundum; OP, ostium primum; LVV, left venous valve; VCS, vena cava superior.

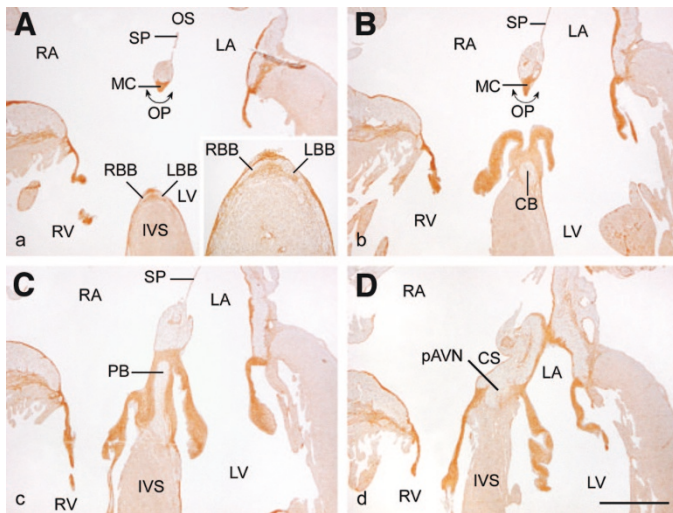


Figure 3. Ten-week-old human embryo with trisomy 21 and complete AVSD. Collagen VI and HHF-35 expression were used as tissue marker to delineate the different components of the AV conduction system. (a–d) Collagen VI expression. (a) The proximal part of the small LBB and RBB are visible. A large OP is present between LV and RV and LA and RA. The free rim of the SP is still formed by a fibrous cap (MC) or future bridging tendon. (b) The distal part of the nonbranching or common bundle (CB) just before the connection of the MC (bridging tendon) and the inferior bridging leaflet. (c) At the connection point of the MC and the inferior bridging leaflet, the long penetrating bundle (PB) runs close to the endocardium through thin fibrous tissue. (d) The iAVN in its dorsal position very close to the CS ostium. IVS, interventricular septum. Bar = 1 mm.

cardiac conduction system is derived from four separate rings of specialized myocardium between the primitive segments of the heart that partially disappear during development. The sinoatrial node originates from the sinoatrial ring, the AV node from the AV ring, and the His bundle and bundle branches from the bulboventricular ring. In mammals, the so-called truncobulbar ring completely disappears. Later, the ring concept was simplified by the “single” ring theory by Wessels *et al.* (7) with the use of the neural tissue antigen GIN2 as conduction tissue marker. They proposed that the ring of cells that surrounds the “primary” interventricular foramen form the compact AV node, His bundle and bundle branches, and the transient right AV ring bundle and retroaortic root branch. In a later study by the same group (9), it was proposed that the AV node itself was derived from the AV canal myocardium, which is more in line with the classical ring theory of Wenink (6) and Anderson and Taylor (20). With the use of another neural tissue antigen, HNK-1, we others and demonstrated in human, rat, and chicken embryos that anterior and posterior HNK-1-positive tracts of sinus venosus myocardium are present between the developing sinoatrial and two AV node primordia (8,10,11,21). These “internodal” sinus venosus connections run anteriorly through the fused venous valves (septum spurium) and posteriorly through the left and right venous valves. Studies in rat and human also report the embryologic presence of a superior and an inferior AV node primordium that appose or even fuse during cardiac development (8,10). Other studies in rats and guinea pigs have demonstrated that the superior node primordium always remains separated from the inferior node primordium by fibrous tissue (22,23).

In the present study, we investigated the embryonic and fetal cardiac conduction system in AVSD in human embryos with trisomy 21 in comparison with normal cardiac development. The results may not relate to AVSD development in other patients, because the morphology of AVSD in patients without Down syndrome can be different (24). Between 6 and 9 wk of gestation, both the developing sinoatrial and AV conduction system show transient HNK-1 antigen expression. Similar to normal development, the HNK-1 antigen expression in the sinoatrial node and other sinus venosus parts, such as the venous valves, is less intense than in the AV conduction system. The atrial HNK staining in the AVSD hearts was very faint, and we were not able to identify clear tracts or connections as observed in normal hearts. In both normal and AVSD hearts, two HNK-1-positive node-like parts of the right AV ring bundle are present. In a 7-wk-old AVSD heart, the inferior AV node lies in a dorsal position remote from the smaller superior node-like part of the right AV ring bundle, and this position does not change significantly during further development. In contrast, in a normal 7-wk-old heart, the prominent inferior AV node and the smaller superior “AV node” primordial have already apposed and connect to the HNK-1-positive sinus venosus myocardium anteriorly through the fused venous valves (septum spurium) and posteriorly through the left and right venous valve (8). During AVSD development in Down syndrome, the size and the shape of the cushions develop normally, but the amount of extracardiac mesenchyme entering the heart at the venous pole is reduced, which could play a role in the persistence of the foramen primum (25). The persistence of the foramen primum prevents normal apposition of the superior and inferior node-like parts of the right AV ring bundle and also prevents the normal input of the anterior “internodal” sinus venosus tract through the septum spurium to the definitive inferior AV node.

The inferior AV node in dorsal position is connected to a long HNK-1-positive nonbranching bundle that enters the ventricle in the dorsal part of the inlet septum. The nonbranching bundle not only divides into a positive left and right bundle branch along both sides of the inlet septum but also continues as a third HNK-1-positive “branch” over the crest of the ventricular septum. It is interesting that in AVSD hearts of 7 wk of gestation (stage 19), the superior node primordium is still connected to the third HNK-1 branch through the retroaortic root branch. In fact, the third HNK-1 branch, which is the remainder of the primary fold, and the AV conduction system temporarily form a complete figure-of-eight loop. This branch seems to vanish together with the retroaortic root branch and right AV ring bundle at later stages. The extra branch and its connection to the retroaortic ring and right AV ring bundle are identical to the connection of the middle bundle branch with the muscle arch around the aorta and AV Purkinje ring in the normal avian conduction system (26). Wessels *et al.* (7) already demonstrated that the “primary” GIN2 ring formed a complete ring at early stages of normal human development and mentioned the analogy with the mature avian conduction system. However, in normal human embryonic hearts, the anterior connection of the AV conduction system disappears later on and cannot be demonstrated using either GIN2 or

HNK-1 as conduction tissue markers after stage 17, or 6 wk of gestation (7,8). Parts of the embryonic middle bundle branch or septal branch can remain in normal postnatal human hearts and have been called dead-end tracts in the literature (27). The delayed disappearance of the anterior continuity of the AV conduction system during AVSD development is interesting, and one could speculate that it is triggered by the lack of apposition of the inferior and superior part of the right AV ring bundle. Furthermore, it suggests the potential to develop a superior AV node and bundle or even dual nodes under pathologic circumstances (28–32). The presence of dual nodes has been reported, mostly in AVSD and right isomerism, and these dual nodes can form the substrate for reentrant tachycardia (31,32).

This is the first study in the literature to describe the development of the cardiac conduction system in human embryos and fetuses with AVSD. An important difference with normal development is the observation that the superior node-like part of the right AV ring bundle never apposes to the more prominent inferior AV node. Whether fusion or apposition of this smaller superior part is necessary for normal function of the AV node remains to be determined. Nevertheless, the definitive AV node in AVSD cannot receive the anterior sino nodal input as seen in normal hearts, as a result of lack of apposition of these parts of the embryonic conduction system. These findings may be relevant with regard to the higher incidence of AV-nodal conduction delay (20) and increased vulnerability of the AV node (4) in patients with AVSD.

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