Abnormal Cardiac Conduction and Morphogenesis in Connexin40 and Connexin43 Double-Deficient Mice

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Abstract—Connexin40-deficient (Cx40−/−/Cx43+/+) and connexin43-heterozygous knockout mice (Cx40+/+ /Cx43+/−) are viable but show cardiac conduction abnormalities. The ECGs of adult double heterozygous animals (Cx40+/−/Cx43+/−) suggest additive effects of Cx40 and Cx43 haploinsufficiency on ventricular, but not on atrial, conduction. We also observed additive effects of both connexins on cardiac morphogenesis. Approximately half of the Cx40+/−/Cx43+/− embryos died during the septation period, and an additional 16% died after birth. The majority of the latter mice had cardiac hypertrophy in conjunction with common atrioventricular junction or a ventricular septal defect. All Cx40+/−/Cx43+/− progeny exhibited cardiac malformations and died neonatally. The most frequent defect was common atrioventricular junction with abnormal atrioventricular connection, which was more severe than that seen in Cx40−/−/Cx43+/− mice. Furthermore, muscular ventricular septal defects, premature closure of the ductus arteriosus, and subcutaneous edema were noticed in these embryos. Cx40+/−/Cx43−/− embryos showed the same phenotype (ie, obstructed right ventricular outflow tract) as reported for Cx40−/−/Cx43−/− mice. These findings demonstrate that Cx43 haploinsufficiency aggravates the abnormalities observed in the Cx40−/− phenotype, whereas Cx40 haploinsufficiency does not worsen the Cx43−/− phenotype. We conclude that the gap-junctional proteins Cx40 and Cx43 contribute to morphogenesis of the heart in an isotype-specific manner. (Circ Res. 2000;87:399-405.)

Key Words: mice, transgenic defects electrocardiography myocardium conduction

Gap junctions are intercellular channels that allow the exchange of ions, second-messenger molecules, and other metabolites between adjacent cells. In mammalian cardiomyocytes, gap junctions mediate electrical coupling. To date, 15 different murine connexin isoforms (ie, the prejunctional proteins of gap junctions) have been described.1–4 Cx40 and Cx43 proteins and mRNA were detected in vascular smooth muscles, endothelial cells, and a subpopulation of cardiomyocytes in the vertebrate cardiovascular system.2 In the rodent heart, the expression of both connexins is developmentally regulated.6–9 Cx40 expression is upregulated in a posterior-to-anterior fashion during cardiac development, reaching a maximum at embryonic day (ED) 14. During subsequent development, Cx40 expression becomes restricted to the atria and the ventricular conduction system. Cx43 is also gradually upregulated in both atria and ventricle during early cardiac development but reaches its highest level only at weaning. Cx43 is the main connexin isoform of the ventricular myocardium, but it is absent from the proximal ventricular conduction system in rodents.6,7

Mice heterozygous for Cx43 (Cx40+/−/Cx43+/−) are viable and show, apart from a mild dilatation of the right ventricular chamber in some cases,10 no defects in cardiac morphogenesis, whereas Cx43-deficient (Cx40+/+/Cx43−/−) mice die shortly after birth due to obstruction of the right ventricular outflow tract.11,12 There are conflicting results on the conduction velocity in the myocardium of Cx40+/−/Cx43−/− mice.13,14 Cx40-deficient (Cx40−/−/Cx43+/−) mice are viable and show reduced velocity of impulse conduction in the atria and the ventricular conductive myocardium.15–17 Spontaneous as well as inducible dysrhythmias were observed in Cx40−/−/Cx43+/− mice.18 but until now, no morphological abnormalities were associated with this genotype. In the present study, we observed, however, some Cx40+/−/Cx43+/− embryos with small septation defects.

We were interested to establish the extent to which Cx40 and Cx43 can complement one another in cardiac morphogenesis and in heart physiology. Thus, we interbred Cx40−/− and Cx43-mutant mice and characterized the genotypically different progeny through histology and ECG. We observed that haploinsufficiency of Cx40 and of Cx43, although coexpressed only in the distal part of the ventricular conductive myocardium, has additive effects on ventricular conduction in Cx40+/−/Cx43−/− mice and that haploinsufficiency of
Cx43 increased the frequency and severity of cardiac malformations in Cx40⁻/⁻ mice, whereas haploinsufficiency of Cx40 did not affect the Cx43⁻/⁻ phenotype.

Materials and Methods

Breeding and Preparation of Embryos

Cx40⁻/⁻/Cx43⁻/⁻ mice were obtained by interbreeding Cx40⁻/⁻/Cx43⁻/⁻ mice of mixed 129/Sv-C57BL/6 background. Cx40⁻/⁻/Cx43⁻/⁻ mice were mated with Cx40⁺/+/Cx43⁻/⁻ mice, which were also of mixed 129/Sv-C57BL/6 background. Double heterozygous animals (Cx40⁺/+/Cx43⁻/+ or Cx40⁻/+/Cx43⁻/+ mice) were mated with each other or with Cx40⁻/⁻/Cx43⁻/⁻ mice. The morning on which the presence of the vaginal plug was noticed was considered ED0.5. Embryos were dissected and genotyped with polymerase chain reaction or Southern blot hybridization (Figure 1) as described previously. For histological studies, the embryos were fixed in a solution of ice-cold methanol/acetic acid/water (2:2:1) for 4 hours or overnight. All experiments were carried out in accordance with the German law on animal protection.

Histological Analysis

Embryos were embedded in Paraplast plus (Sherwood Medical Co), serially sectioned at 7-μm thickness, and mounted onto polylysine-coated glass slides. The sections were stained with hematoxylin and eosin. Development of cardiac structures were evaluated with the use of a multivariate 2-factor ANOVA (factors Cx40 and Cx43, each with two levels: +/- and +/-, respectively). Probabilities of differences in the phenotypic effects of connexins on separate parts of the ECG were determined per genotype. A probability value of <0.05 was considered statistically significant.

ECG Recordings

ECG recordings were performed as previously described. All data obtained were expressed as mean±SEM. The effect of each connexin was tested with a multivariate 2-factor ANOVA (factors Cx40 and Cx43, each with two levels: +/- and +/-, respectively). Because no significant interaction was found between the connexins, the differences between the different genotypes could be tested with a Student-Newman-Keuls test. Correlations of the effects of connexins on separate parts of the ECG were determined per genotype. A probability value of <0.01 was considered statistically significant.

Results

Embryonic Lethality and Structural Heart Defects in Cx40-Deficient Mice

Cx40⁻/⁻/Cx43⁻/⁻ mice are viable and fertile, but we found fewer Cx40⁻/⁻/Cx43⁻/⁻ animals after interbreeding of Cx40⁻/⁻/Cx43⁻/⁺ mice than anticipated for mendelian inheritance (Table; compare Reference 15). We observed the expected number of Cx40⁻/⁻/Cx43⁻/⁺ embryos at ED11.5 but only half of the expected number of Cx40⁻/⁻/Cx43⁻/⁻ embryos after ED13.5 (Table). This indicates that Cx40 plays an important role in the period during which septation of the heart takes place. We examined morphologically the hearts of five Cx40⁻/⁻/Cx43⁻/⁻ mice at ED12.5 but found them to be structurally normal except for the mesenchymal cap on the free rim of the primary atrial septum, which was incompletely formed in two of the hearts (Figure 2A).

Sixteen percent of the newborn Cx40⁻/⁻/Cx43⁻/⁻ mice (17 of 106) died shortly after birth. The surviving animals developed normally, but some of the Cx40⁻/⁻/Cx43⁻/⁻ animals died as young adults at 4 to 8 weeks of age. When we analyzed the hearts of three newborn and two young adult mice, four showed myocardial hypertrophy. Three of these hearts had a small septum primum defect, considered to be the mildest form of the common atrioventricular junction (Figure 2B; see also Figure 1 online [data supplement available at http://www.circresaha.org]). In addition, one of them had a persisting interventricular foramen. Furthermore, two of the three hearts exhibited a persistent foramen ovale. In the fourth hypertrophic heart, only a ventricular septal...
Breeding and Electrophysiological Characterization of Cx40+/−/Cx43+/− Double Heterozygous Mice

To establish whether Cx40 and Cx43 have additive effects on the function of the heart, we generated Cx40+/−/Cx43+/− mice and analyzed the conduction properties of their hearts through limb lead surface ECG measurements (Figure 3). No change in the duration of the RR interval was observed in any of the genotypes, showing that the function of the sinus node is not affected by Cx40 or Cx43 heterozygosity. The duration of the P wave was increased in both Cx40+/−/Cx43+/− and Cx40+/−/Cx43+/− mice, but the measurements of Cx40+/−/Cx43+/− showed the effect was not additive. The duration of the PQ interval was not significantly different in any of the genotypes. The duration of the QRS complex was similar in Cx40+/+/Cx43+/+, Cx40+/−/Cx43+/+, and Cx44+/−/Cx43−− animals but significantly increased in double heterozygotes, showing that the small increases in the duration of the QRS complex due to Cx40 and Cx43 heterozygosity add up to a significant effect. The duration of the QTmax interval was increased in Cx40+/−/Cx43+/+ and Cx40+/−/Cx43+/− animals relative to Cx40+/+/Cx43+/+ animals. Measurement of the QTmax interval in Cx40+/−/Cx43+/− mice showed that these effects were also additive. Thus, decreased amounts of Cx40 and Cx43 protein in the heart resulted in slowed atrial depolarization and ventricular depolarization and repolarization. These effects were additive in the ventricles but not in the atria. Furthermore, the duration of the P wave always correlated with the duration of the PQ interval, and the duration of the QRS complex always correlated with the duration of the QTmax interval, except in double heterozygotes. Only in Cx40+/−/Cx43+/− animals was a correlation observed between atrial and ventricular parameters, except for the duration of the P wave and the QS interval. Cx40+/−/Cx43+/− animals did not show any correlation among the ECG parameters. There was no correlation between the cycle length (RR interval) and the other parameters in any genotypes we investigated (data not shown). This analysis shows that changes in the length of the P wave are correlated with those of the PQ interval and that changes in the duration of the QRS complex are correlated with those in the QTmax interval in all genotypes except the double heterozygotes.

Morphological Abnormalities in Embryonic Hearts of Cx40−/−/Cx43+/− or Cx40−/−/Cx43−/− Mice

We interbred Cx40−/−/Cx43+/− mice to determine whether Cx40 and Cx43 have additive functions during cardiac morphogenesis. As anticipated, we did not obtain Cx43−/− mice after birth (compare Reference 11) and found fewer than half of the expected Cx40−/−/Cx43+/− mice (Table). In addition, we did not find Cx40−/−/Cx43−/− animals at the age of 3 weeks (0 of 180 instead of ~20 of 180 expected). Similarly, interbreeding of Cx40−/−/Cx43+/− and Cx40−/−/Cx43−/− animals yielded no animals of the Cx40−/−/Cx43−− genotype in 124 mice analyzed at 3 weeks of age. Genotyping of embryos at different developmental stages showed that the Cx40−/−/Cx43−/− animals died during the first two postnatal days.

The morphology of the embryonic hearts from 21 Cx40+/−/Cx43+/− mice and 4 Cx40+/−/Cx43−/− mice from ED12.5 to ED18.5 was analyzed histologically.

Cx40−/−/Cx43+/− Mice

The most characteristic defects were seen at the atrioventricular junction. These malformations were due to a graded deficiency in normal development, indicating that the normally occurring growth of the right ventricle, the associated rightward expansion of the atrioventricular junction, and septation were inhibited to variable degrees. At ED12.5, the youngest stage investigated, the two available Cx40−/−/Cx43+/− embryos showed unaltered cardiac morphology, except that the mesenchymal cap on the free rim of the primary atrial septum was absent. At ED13.5, one of the two embryos analyzed had a persisting foramen primum of the
atrium and missed the mesenchymal cap on the primary atrial septum, whereas the other was normal. From ED14.5 on, when the interventricular foramen was closed in the wild type, all Cx40\(^{-/-}\)/Cx43\(^{-/-}\) embryonic hearts (n=17) revealed a persisting interventricular foramen (Figure 4). Thirteen of these embryos (76%) had, in addition, a persisting foramen primum, indicating the presence of a common atrioventricular junction. The common atrioventricular junction was guarded by a common valve with a single orifice in 10 of the 13 embryos (77%, Figure 5A) and by two valves in the remaining three cases (Figure 5B). If only a single orifice was present, both atrioventricular cushions had not fused, whereas the presence of two orifices showed that they did but not with the primary atrial septum. In the other four embryos, the foramen primum was closed so that right and left atrioventricular junctions had a myocardial boundary except at the location of the future membranous septum. The pattern of the atrioventricular connection ranged from absent rightward expansion of the atrioventricular connection (double inlet left ventricle, 3 of 17 cases, 18%, Figure 5A), via a small right-sided connection (6 cases, 35%, Figure 5B), to the normally balanced connection (8 cases, 47%). All embryos with double-inlet left ventricle or small right connection had a common atrioventricular junction. In all embryos with double-inlet left ventricle, the atrioventricular valve was common and the interventricular septum was malaligned with the primary atrial septum. In these hearts, the right ventricle communicated with the atria via the interventricular foramen, and its was smaller than the left ventricle. In five of the six hearts (83%) with a small right atrioventricular connection, the atrioventricular junction was guarded by a common valve, whereas the septa were malaligned in 50% of these hearts. In these cases, the right ventricle has direct access from the right atrium but was small compared with the left ventricle. Only four of the eight cases with balanced connection had a common atrioventricular junction. In these four hearts, the valve was either common (50%) or separate. Septal alignment was normal, and the sizes of both ventricles were comparable. In 10 of 13 hearts with a persisting foramen primum (77%), the primary atrial septum was not covered on its lower free rim with the mesenchymal cap (Figure 5A; see also Figure 3 online [data supplement available at http://www.circresaha.org]) that normally occupies this position. The absence of this structure was related to the presence of a common atrioventricular valve (P=0.03) (see Figure 3 online; data supplement available at http://www.circresaha.org), a lack of development of the right atrioventricular connection (P=0.03), and septal malalignment (P=0.01). At later stages, especially at ED18.5, the venous valves appeared short. This occurred because their attachment to the base of the heart was more caudal than that of control embryos. This feature was not related to the absence of a mesenchymal cap on the free rim of the primary atrial septum (P=0.63), the presence of a common atrioventricular valve (P=0.63), a lack of development of the right atrioventricular connection (P=0.62), or septal malalignment (P=0.35).

A defect in the muscular interventricular septum (Figures 5B and 5C) was observed in two of three ED18.5 embryos analyzed but not in younger embryos. However, some of these latter embryos showed a loose, random arrangement of the septal myocardium with deep canals lined by endocardium in the region where the muscular defect was found at the later stage (Figure 5E).

All of the embryos of the Cx40\(^{-/-}\)/Cx43\(^{-/-}\) genotype displayed the usual atrial arrangement (ie, the presence of a normal atrial situs). Furthermore, none of them had defects in the arterial pole of the heart. The great vessels were also inconspicuous, but surprisingly, the ductus arteriosus was fully constricted in all ED18.5 embryos (Figure 5D). Most embryos showed subcutaneous edema between ED13.5 and ED15.5, mainly in the neck and back (Figure 5F). The extent of edemas had no apparent relation to the severity of cardiac...
defects, and it was not found in any of the embryos after ED16.5.

**Cx40+/−/Cx43−/− Mice**

The four mice of this genotype that were investigated exhibited similar features as Cx40+/−/Cx43−/− mice. The ED12.5 embryo showed the typical A-loop: the right ventricle was located anteromedially relative to the left ventricle, whereas the outflow tract occupied a leftward position and turned toward the branchial arches with an acute bend (Figure 6A). Its atrioventricular canal connected both atria to the left ventricle, and the atrioventricular cushions were positioned on the left and right sides of the canal rather than on the superior and inferior side, respectively (Figure 6B). At ED15.5 and ED17.5, the Cx40+/−/Cx43−/− hearts showed the typical features of Cx40+/−/Cx43+/− hearts at the junction of the right ventricle and the outflow tract. At this junction, several interconnected or blind pouches separated by thick trabeculae were present (Figures 6C and 6D). None of the ED15.5 or ED17.5 embryos had septal defects.

**Discussion**

The Cx40 and Cx43 connexin isoforms are both expressed in the heart, showing a distinct and at some sites overlapping expression pattern. In the electrophysiological portion of the present study, we did observe additive effects of Cx40 and Cx43 heterozygosity on ventricular, but not atrial, conduction. Furthermore, cardiac morphogenesis was more severely impaired in Cx40+/−/Cx43−/− animals than in Cx40+/−/Cx43+/− animals. However, the finding that Cx43 haploinsufficiency worsened the morphological phenotype of Cx40 deficiency, whereas Cx40 haploinsufficiency did not seem to alter the morphological phenotype of Cx43 deficiency, showed that Cx40 and Cx43 functions in cardiac morphogenesis were not mutually exchangeable.

**Decreased Expression of Cx43 and Cx40 Genes in Cx40+/−/Cx43+/− Mice Results in Decreased Atrial and Ventricular Conduction**

As previously shown, ECG parameters in Cx40 heterozygous mice were only mildly affected. In fact, we observed only a 10% increase in the duration of the P wave and the QTmax interval in Cx40+/−/Cx43+/− mice compared with wild-type controls. However, substantial increases in the duration of the P wave (>50%) and PQ, QS, and QT
Intervals (>20%) are present in Cx40<sup>+/−</sup>/Cx43<sup>+/+</sup> mice. In Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> mice, the durations of the P wave and the QT<sub>int</sub> interval were significantly increased compared with those of wild-type control animals, but the PQ interval and the duration of the QRS complex were not. Our data on ventricular conduction are similar to those reported by Morley et al. However, as a result of the smaller number of animals, increases in the QT interval did not reach significance in the study by Morley et al. Ventricular depolarization was not significantly slowed in Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> mice, although different findings have been reported. Our findings support the contention that the Cx43 heterozygous mice do not experience major changes in intraventricular conduction velocity. Ventricular depolarization and repolarization are significantly slower in Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> mice compared with either Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup>, Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup>, or wild-type mice. Because Cx40 and Cx43 are coexpressed only in the distal conductive myocardium of the ventricles, these findings were probably due to the additive effects of slightly lower conduction velocities in the ventricular conduction system (where Cx40 is expressed) and in the working myocardium of the ventricles (where Cx43 is expressed). Conceptually, it could be regarded as a result of two increasing serial resistances. In mouse atria, Cx40 and Cx43 are coexpressed. Heterozygosity for either Cx40 or Cx43 has an effect on the duration of the P wave, but intriguingly, these effects were not additive. Such findings can in principle be explained by a model in which the gene dosages of Cx40 and Cx43 are responsible for different resistors in parallel.

Under anesthesia with avertin, correlation of the cycle length with any other ECG parameters was not detected in wild-type, Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup>, Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup>, or Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> animals. However, in wild-type, Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup>, or Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> mice, the duration of the P wave correlated with the PQ interval and the duration of the QRS complex correlated with the QT<sub>int</sub> interval. This suggests that adaptational changes in atrioventricular conduction velocities and in the time necessary for repolarization correlate with changes in atrial and ventricular conduction velocities, respectively. Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> and Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> mice did not differ in this respect from wild-type animals. Interestingly, these correlations are both lost in Cx40<sup>+/−</sup>/Cx43<sup>+/−</sup> mice. This finding suggests that the coordination of atrial and atrioventricular conduction, on the one hand, and that of ventricular conduction and repolarization, on the other hand, are influenced by gap-junctional communication.

Cx43 Haploinsufficiency Aggravates the Cardiac Morphological Phenotype in Cx40-Deficient Mice

Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> mice showed an increased incidence of prenatal death between ED11.5 and ED13.5. Because we did not observe overt morphological defects in the hearts of these embryos, the cause of embryonic death in Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> mice is probably functional rather than structural. The expression of the connexin genes in the embryonic heart is developmentally regulated. In the developing heart, Cx40 is upregulated in a posteroanterior manner. Cx40 mRNA is first detected in the embryonic atrium and left ventricle at ED9.5 and, after ED11.5, also in the embryonic right ventricle. Cx43 is expressed early in the ventricles but not before ED12.5 in the atria. Because Cx45 is expressed between ED8.5 and ED10.5 and is downregulated thereafter, there may be a temporary lack of gap-junctional communication in the hearts of some Cx40-deficient embryos, resulting in embryonic death.

Another “window of sensitivity” is around birth, when 16% of the Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> animals and all of the Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> animals die. The septational defects might hinder the efficient establishment of the pulmonary circulation at birth (ie, the animals cannot cope with changes in cardiac workload and finally die). The importance of Cx40 for the mechanical function of the heart is underscored by the myocardial hypertrophy that was found in the majority of Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> animals that died postnatally.

Some Cx40-deficient mice showed a mild form of common atrioventricular junction or ventricular septal defects, indicating that the septational process is sensitive to Cx40 deficiency. The cardiac malformations found in Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> mice are more severe forms of the defects observed in Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> animals. This group of cardiac malformations appears to arise as a result of a developmental arrest between ED12 (onset of cardiac septation) and ED14 (completion of cardiac septation). It is conceivable that the fusion defects developed as secondary effects due to a disturbed contractile pattern in the Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> hearts (eg, altered shear stress). However, if that were the case, it would be difficult to explain why Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> animals did not show similar septation defects but instead showed only abnormalities in the downstream portion of the heart. Alternatively, the morphological changes could be directly due to decreased intercellular gap-junctional communication.

The process of atrioventricular septation is impaired in Cx40-deficient embryos, and this impairment is worsened by haploinsufficiency for Cx43. Atrioventricular septation involves, in addition to the myocardium of the atrioventricular canal, several developmental primordia, including the endocardial cushions, the mesenchymal rim on the free edge of the primary atrial septum, the right pulmonary ridge, and the valves of the sinus venosus. The only other genetic model that is associated with defects of the atrioventricular junction is trisomy 16. Webb et al hypothesized that a failing development of the spina vestibuli underlies the maldevelopment of the atrioventricular junction in these mice. In agreement with this hypothesis, we found in Cx40<sup>−/−</sup>/Cx43<sup>+/−</sup> embryos that the absence of a mesenchymal rim on the free edge of the primary atrial septum was associated with the most pronounced abnormalities of the atrioventricular connection.

Cx40- and Cx43-mediated gap-junctional communication appears to be crucial for the complex morphogenetic process of atrioventricular septation. Nevertheless, we observed a wide spectrum in the extent to which development of the atrioventricular canal was affected. Some animals, for example, suffered only from a ventricular septal defect (compare Figure 4). The development of such a spectrum of effects would be expected, if septalation defects in the atrioventricular canal of Cx40-deficient embryos arise as a result of a disturbed heart function (see earlier). On the other hand,
small differences in the genetic background could also modulate the phenotypic appearance of individual animals.

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Supplementary figures

Fig. 1 Online Cardiac defects in the hearts of Cx40\(^{-/-}\) mice.

Panel A is a transverse section of a 2-month-old heart, panel B that of a 1-month-old heart, and panels C-E those of a single neonatal heart. Their defects of the atrioventricular junction are limited to a small hole between the superior and inferior bridging leaflets (arrows). In the neonatal heart, an interventricular foramen is still present below the aortic root (panel D). Abbreviations: AO: aorta; LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle. Bar in panels A and B, 600 \(\mu\)m; bar in panel C, D, and E, 300 \(\mu\)m.

Fig. 2 Online Cx40\(^{-/-}\) neonatal heart shown in Fig. 2B.

In this heart only a ventricular septal defect was observed. Additional sections are given to show absence of double outlet right ventricle. Abbreviations: LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle. Bar, 625 \(\mu\)m.

Fig. 3 Online Mesenchymal cap on the primary atrial septum in Cx40\(^{-/-}\)/Cx43\(^{-/-}\) embryos. Panels A, B, and C are transverse sections of the hearts at ED12.5, ED 15.5, ED 16.5, respectively, which show the absence of a mesenchymal cap on the primary atrial septum. Panel D is a transverse section of the heart at ED18.5 in which the primary atrial septum is covered with a mesenchymal cap at 18.5. Each inset shows the free rim of the primary atrial septum with high magnification. Abbreviations: LA: left atrium; LV: left ventricle; PV: pulmonary vein; RA: right atrium;
RV: right ventricle. Bar in panels A, 250 μm; bars in panel B, and C, 400 μm; bar in panel D, 500 μm.