Spectrum of Heart Malformations in Mice With Situs Solitus, Situs Inversus, and Associated Visceral Heterotaxy

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Background. We present a study of the heart malformations found in a collection of mouse fetuses of the iv/iv strain between days 16.5 and 18.5 of gestation.

Methods and Results. One hundred hearts were serially sectioned and studied by segmental analysis with a light microscope. Forty additional hearts were analyzed with a scanning microscope. Forty percent of the hearts were found to be malformed. The most frequently occurring heart malformations were persistence of the sinus venosus (9%), common atrium (17%), common atroventricular canal (24%), double-outlet right ventricle (12%), Fallot's tetralogy (8%), and transposition of the great arteries (5%). These malformations do not usually occur in isolation but rather appear in the formation of complex cardiopathies. The most severe and frequent is the combination of persistence of sinus venosus, common atrium, common atroventricular canal, and double-outlet right ventricle; this is the “bulboventricular heart.” The morphology of each lesion, as well as the degree of association, is similar to that found in human hearts with complex cardiopathies. Some of these cardiopathies appear to be directly related to formation of the cardiac loop. The iv/iv mouse appears to constitute an excellent model with which to study the etiology and pathogenesis of complex heart defects in humans. These hearts show a high phenotypic variability in the presentation of heart lesions. From a genetic viewpoint, there is a basic defect—the bulboventricular heart—which can be considered congenital. The other malformations can be considered forms frustes of the defect type.

Conclusions. The iv gene is a developmental gene that affects basic developmental mechanisms. In this regard, heart lesions may not be the primary result of the abnormal gene activity but rather are secondary to defective interactions during cardiac development. (Circulation 1991;84:2547–2558)

In 1959, Hummel and Chapman described a strain of mice in which 50% of the mice presented situs inversus. This mutation is inherited as an autosomal recessive trait.1,2 Homozygous mice carrying the iv gene (iv stands for inversus viscerum) behave as if they had lost control of visceral asymmetry, resulting in randomly determined situs. Approximately 40% of adult iv/iv mice show well-established patterns of visceral and venous heterotaxia (References 1–3 and personal observation). In addition, as occurs in humans with positional anomalies,4–8 the iv/iv mice demonstrate a wide range of heart malformations, including persistence of the sinus venosus, CA (see Abbreviations), anomalous venous drainage, CAVC, DORV, and TGA.3,9 It has been postulated that the iv/iv mouse may serve as an animal model of human syndromes characterized by positional anomalies and heart malformations, such as asplenia and polysplenia3,10–12 and Kartagener13 syndromes.

Most of the heart malformations presented by iv/iv mice do not occur as isolated entities. It has been recognized that they associate to form complex cardiopathies.3 However, the exact nature of these associations remains unclear, and a detailed morphological analysis of each malformation has not been done. This information is essential to determine the extent to which the iv/iv mouse can be considered a model, not only for human heterotaxy syndromes but also for complex heart malformations.

The study of malformed hearts has been greatly improved by the use of sequential segmental analysis.14–19 This method considers the heart to be divided into three segments: atria, ventricular mass,
Abbreviations
ASD, atrial septal defect
CA, common atrium
CAVC, common atroventricular canal
CILV, common-inlet left ventricle
DOLV, double-outlet left ventricle
DORV, double-outlet right ventricle
LA, left atrium
LV, left ventricle
PA, pulmonary artery
PS, pulmonary stenosis
RA, right atrium
RV, right ventricle
TAt, tricuspid atresia
TF, tetralogy of Fallot
TGA, transposition of the great arteries
VSD, interventricular septal defect

and arterial trunks. These segments are connected at the atroventricular and ventriculoarterial junctions. By describing the anatomy of each segment, that of the segmental connections, and the accompanying anomalies, each heart can be fully and accurately described.

We report a study of the heart malformations found in a collection of 100 iv/iv fetuses between days 16.5 and 18.5 of gestation. At these stages, the period of heart organogenesis is completed, and the different parts of the heart show the morphological relations typical of the adult. We analyze the characteristics of each heart, the frequency and distribution of malformations, their degrees of association, and the relation between the presence of malformations and the direction of the cardiac loop. We found a close similarity between the heart malformations presented by the iv/iv mouse and those found in humans. The iv/iv mouse appears to constitute an excellent model with which to study the etiology and pathogenesis of these defects in humans.

Methods
Homozygous iv/iv mice of the congenic strain C57BL/6iv were kept in open facilities and administered food and water ad libitum. The mouse colony has been maintained by brother-sister mating, irrespective of the visceral situs of each individual. For reproduction, each male was mated with two females. The morning in which the vaginal plug was observed was taken as day 0.5 of gestation. At appropriate dates, the mothers were anesthetized with ether, the abdominal cavity was opened, and the uterus was extracted and placed into warm saline. One hundred fetuses between days 16.5 and 18.5 of gestation were thus obtained and fixed for 24 hours in Bouin’s fixative. After fixation, the fetuses were examined for visceral and venous heterotaxia, and the hearts were extracted. Then, the hearts were dehydrated in graded ethanol and embedded in Paraplast. Each heart was serially sectioned at 7-μm (most of them in the frontal plane) and stained with hematoxylin and eosin. Five additional hearts of the Swiss albino strain were processed as absolute controls.

Forty additional embryos of the same gestational age were obtained as above and processed for scanning electron microscopy (SEM). The hearts were perfused first with saline to cleanse free of blood and then with 5% glutaraldehyde in phosphate-buffered saline (PBS) (pH 7.3). After perfusion, the hearts were immersed in fresh fixative for 3–5 hours. Then, the hearts were dissected in PBS under a binocular microscope, dehydrated in graded acetone, and dried by the critical point method using CO₂ as the transitional fluid. Dried specimens were mounted on aluminum stubs, coated with gold, and viewed with a Philips 501 SEM.

Results
Positional anomalies in the 100 fetuses studied included interruption of the posterior vena cava, posterior vena cava–azygos vein continuity, preduodenal portal vein, thoracoabdominal discordance, and splenic anomalies. We have not studied the spleen of these fetuses histologically, but splenic anomalies included partial bisection of the spleen by the mesogastrium, polysplenia (two to four splenic segments), and two cases of asplenia. Asplenia, however, is difficult to determine; the spleen in adults is sometimes of minimal size, and a very small spleen displaced in the abdominal cavity may have been overlooked in these two fetuses. The presence of a very small splenic fragment located on the caudal pole of the spleen was considered normal. In two fetuses with thoracoabdominal discordance, the spleen had an elongated shape and appeared to be transversely located in the epigastrium. The patterns of visceral and venous heterotaxia occurred in both situs solitus and situs inversus, were similar to those reported previously for these mice,1–3 and therefore will not be repeated here. Nevertheless, it is noteworthy that the severity of the heterotaxia correlated positively with the frequency and severity of heart malformations.3

Of the 100 fetuses examined by serial sectioning, 45 showed the apex pointing to the left side of the thorax (levocardia) and 53 showed it pointing to the right (dextrocardia). Two additional hearts were located in a midline position (mesocardia). Thirty-eight percent of the hearts showing levocardia, 39% of those showing dextrocardia, and the two hearts showing mesocardia had cardiac malformations. A total of 40 hearts were malformed. The complete segmental study of these 40 hearts is shown in Table 1.

The first step in segmental analysis is the determination of the atrial situs. However, the mouse does not have atrial appendages that could be used for the determination of atrial position. Nevertheless, when the heart is sectioned frontally (Figure 1), there are some morphological differences between the two atria. Under the light microscope, at the level of the atrioventricular junction, the right atrium shows a
### Table 1. Complete Segmental Study of 40 Hearts

<table>
<thead>
<tr>
<th>Atrial situs</th>
<th>Loop</th>
<th>AV alignment</th>
<th>Mode of AV connection</th>
<th>Ventricular dominance</th>
<th>Ventriculoarterial alignment</th>
<th>Ao-pulmonary artery relation</th>
<th>Set analysis</th>
<th>Additional malformations</th>
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<tbody>
<tr>
<td>Ind</td>
<td>L</td>
<td>Ind</td>
<td>CAVC</td>
<td>Bivent</td>
<td>DORV</td>
<td>Ao levo</td>
<td>{A,L,L}</td>
<td>Sinus ven, CA, subao VSD</td>
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<tr>
<td>Inv</td>
<td>L</td>
<td>Con</td>
<td>CAVC</td>
<td>...</td>
<td>DORV</td>
<td>Side-by-side</td>
<td>{I,L,L}</td>
<td>Sinus ven, CA, subpa VSD</td>
</tr>
<tr>
<td>Inv</td>
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<td>CAVC</td>
<td>Bivent</td>
<td>DORV</td>
<td>Ao levo</td>
<td>{I,L,L}</td>
<td>ASD II, subao VSD</td>
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<td>Con</td>
<td>CAVC</td>
<td>Bivent</td>
<td>DORV</td>
<td>Side-by-side</td>
<td>{I,L,L}</td>
<td>CA, subao VSD</td>
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<tr>
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<td>L</td>
<td>Ind</td>
<td>CAVC</td>
<td>Bivent</td>
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<td>Ao levo</td>
<td>{A,L,L}</td>
<td>ASD I, subpa VSD</td>
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<tr>
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<td>L</td>
<td>Ind</td>
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<td>Left</td>
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<td>{A,L,L}</td>
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<tr>
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<td>Bivent</td>
<td>DORV</td>
<td>Side-by-side</td>
<td>{A,D,D}</td>
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<td>Bivent</td>
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<td>Anterior Ao</td>
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<td>CA, subpa, VSD, straddling MV</td>
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<td>Side-by-side</td>
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<td>CA, subao subpa VSD</td>
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<td>Con</td>
<td>CAVC</td>
<td>Bivent</td>
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<td>{S,D,S}</td>
<td>ASD I, subao VSD</td>
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<tr>
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<td>TGA</td>
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<td>Bivent</td>
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<tr>
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<tr>
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<tr>
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<td>Con</td>
<td>Normal</td>
<td>...</td>
<td>Con</td>
<td>Ao anterior</td>
<td>{S,D,S}</td>
<td>ASD II, noncommitted VSD</td>
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<tr>
<td>Inv</td>
<td>L</td>
<td>Con</td>
<td>Normal</td>
<td>...</td>
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<tr>
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<td>Normal</td>
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<tr>
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<td>Con</td>
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<td>{I,L,I}</td>
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<td>Ind</td>
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<td>{A,D,S}</td>
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AV, atrioventricular; PA, pulmonary artery; Ind, indeterminate; Inv, inversus; Con, concordant; CILV, common-inlet left ventricle; TAT, tricuspid atresia; CAVC, common AV canal; Int, intermediate; Bivent, biventricular; DORV, double-outlet right ventricle; TF, tetralogy of Fallot; TGA, transposition of the great arteries; DOLV, double-outlet left ventricle; Ao, aortic; levo, levoposition; dextro, dextroposition; Pos, posterior; Ant, anterior; ven, venous; CA, common atrium; subao, subaortic; subpa, subpulmonary; VSD, interventricular septal defect; ASD, atrial septal defect; MV, mitral valve; TV, tricuspid valve
globular appearance with a blunt inferior border. The left atrium has a triangular, unciiform appearance with a thin inferior border (Figures 1A and 1B). The same appearance is observed with SEM (Figure 1C). These differences between the two atria are maintained even in the presence of specific heart malformations (Figures 1B and 1C). In addition, when the two atria are examined externally with SEM, the left atrium shows a thinner border than the right atrium, with several vertical indentations (see Figures 1C, 2, and 3). By using these landmarks, we were able to identify the atrial situs in the hearts (Table 1). Despite these guidelines, the atrial situs could not be identified in 15 of the 40 malformed hearts. These hearts have been classified as of indeterminate atrial situs. We have not used the term "ambiguous" because this term implies the recognition of some specific atrial anatomy. Identification of the right horn of the sinus venosus is also difficult because, on the one hand, mice show two superior venae cavae, and, on the other hand, the sinus venosus is often malformed in our hearts. Nevertheless, SEM shows (see Figures 5, 7, and 9) that hearts with similar malformations present atria that are nearly equal in size and shape. Therefore, most of these hearts show isomeric atria. The term "atrial isomerism" is not used here because, in humans, it refers to isomerism of the atrial appendages. Tables 2–4 give summaries of the anomalies found in the different segments and intersegmental connections. No cases of discordant atrioventricular connection or dextrorotation were found. As can be seen from the
Note region of mitral-aortic continuity (asterisk in panel b) in left ventricle. Magnification, ×33.

Figure 3. Scanning electron micrograph depicting a “normal” iv/iv mouse heart at 16.5 days of gestation. Situs inversus, L loop. This heart is mirror image of heart shown in Figure 2. Magnification, ×48.

Table 2. Atrioventricular Alignment

<table>
<thead>
<tr>
<th>Alignment</th>
<th>No. of mice</th>
</tr>
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<tbody>
<tr>
<td>Concordant</td>
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</tr>
<tr>
<td>Discordant</td>
<td>0</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>11</td>
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<tr>
<td>Tricuspid atresia</td>
<td>2</td>
</tr>
<tr>
<td>Common-inlet left ventricle</td>
<td>6</td>
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</table>

mediated bridging across the crest of the ventricular septum (see Figures 5, 9, and 10) and was attached to the vicinity of the right margin of the ventricular crest by a single papillary muscle. In four cases, the right end of the anterior leaflet was attached halfway down the right side of the interventricular septum; this leaflet never reached the anterior ventricular wall. The common valve was clearly unbalanced on six occasions, and the valvular annulus was displaced over the left ventricle. All six cases were classified as CILV and coursed with minimal bridging of the anterior leaflet. In four of them, the anterior leaflet was cleft. Five of the six cases of CILV showed hypoplasia of the right ventricle.

Every case of CAVC showed a VSD of variable size located in a basal position. The presence of additional, small VSDs, located in the muscular part of the interventricular septum, was observed in 20% of the hearts with CAVC. Many of these appeared to be the result of a lack of ventricular compaction (see Figures 6 and 9). A CA (Figure 6) occurred in 17 of the 24 cases of CAVC. In these cases, the only remnant of the interatrial septum was the presence of a muscular ridge along the cranial aspect of the CA (see also Figure 1C). In the other hearts with CAVC, there was an ASD of septum primum type in four cases (Figure 5) and ASDs of septum secundum type in three cases. Persistence of the sinus venosus (Figure 6) accompanied nine of the 23 cases of CAVC. The sinus venosus appeared as a
saccular third chamber that opened into the atrium through a circular orifice with no sinus valves. The opening of the sinus venosus was located in the middle or slightly displaced to the right of the CA. On two occasions, this malformation was less severe; both the location and shape of the sinus venosus were closer to that of normal hearts.

**Double-Outlet Right Ventricle**

We have found DORV on 12 occasions. The external aspect of these hearts depended on the severity of the malformation. The most severely malformed hearts had a piriform appearance in which no apex or interventricular sulcus could be recognized (Figure 7). In other cases, both the heart apex and the interventricular sulcus, although displaced, could be recognized (Figure 8). The external appearance of these hearts appears to depend on the internal development. The most severe lesions had very maldeveloped interventricular septa, with multiple VSDs and anomalies of ventricular compaction (see Figure 6).

The hearts with DORV had great anatomic variability. Table 5 shows this aspect, interrelating the position of the VSD and the arterial relation. In half of our cases of DORV, the pulmonary infundibulum was divided into two chambers by the presence of a displaced septomarginal trabecula. Although this circumstance is difficult to follow in serial sections, it can be clearly seen with SEM (Figure 9). All 12 cases of DORV showed aortomitral fibrous discontinuity, one of them presenting mitropulmonary continuity.

![Figure 5](image1.png)

*Figure 5.* Scanning electron micrographs illustrating appearance of common atrioventricular valve in iv/iv mouse heart at 18.5 days of gestation with common atrioventricular canal. Indeterminate atrial situs, D loop. Heart has been dissected from frontal, and the two halves are presented. Panel a: Ventral segment of specimen. Panel b: Dorsal segment. Common valve shows five leaflets. Anterior valve leaflet (asterisk in panel a) extends from left ventricle (LV) to right side of interventricular septum. This leaflet shows intermediate bridging supported by a single papillary muscle. An ASD of septum primum type can be observed in panel b. Septum primum and septum secundum are marked by arrowheads. Arrow indicates opening of sinus venosus. Magnification, ×42.

![Figure 6](image2.png)

*Figure 6.* Scanning electron micrograph showing iv/iv mouse heart at 16.5 days of gestation with common atrioventricular canal, double-outlet right ventricle (not shown), common atrium, and sinus venosus malformation. Indeterminate atrial situs, L loop. Heart has been dissected frontally; only dorsal side of specimen is presented. Atria are isomorphic, being similar in size and shape. Only remnant of interatrial septum is a crest that runs along cranial aspect of common atrium (arrow). Opening of sinus venosus (asterisk) is circular, being slightly displaced toward the morphological right side of the heart. No sinus valves are apparent. Common valve orifice appears in center of micrograph. Interventricular septum shows lack of trabecular compaction. Magnification, ×29.

**Table 3. Mode of Atrioventricular Connection**

<table>
<thead>
<tr>
<th>Mode</th>
<th>No. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
</tr>
<tr>
<td>CAVC</td>
<td>19</td>
</tr>
<tr>
<td>CAVC (intermediate form)</td>
<td>5</td>
</tr>
<tr>
<td>Tricuspid atresia</td>
<td>2</td>
</tr>
</tbody>
</table>

CAVC, common atrioventricular canal.
Eight cases of DORV coexisted with the complete form of CAVC (see Figures 6 and 9).

Tetralogy of Fallot

We found TF on eight occasions (Figure 10). This diagnosis was made when 50% of the aortic annulus overrode the VSD. It must be stressed, however, that our cases of TF did not have subinfundibular pulmonary stenosis (see “Discussion”). The aorta was dextroposed (levoposed in inverted hearts) with respect to the pulmonary artery in five cases, and the two arteries presented a normal relation in the other three cases.

Four cases of TF showed aortomitrail fibrous discontinuity, two showed aortotricuspid continuity, one showed aortomitrail and aortotricuspid continuity, and one showed aortovalvular fibrous continuity. Six cases of TF were accompanied by CAVC (Figure 10).

Transposition of the Great Arteries

We found five cases of TGA. All five cases coexisted with VSD, ASD (see Table 1), and aortomitrail discontinuity. The aorta was anterior (Figure 11) to the PA in four occasions and posterior in the fifth case.

Other gross heart malformations were much less frequent. We found one case of DOLV, two cases of atrial juxtaposition, and two cases of tricuspid atresia. The two hearts with tricuspid atresia showed remnants of valvular tissue (Figure 1B). For this reason, they have not been classified as of absent atrioventricular connection.

Aortic Dextroposition

“Aortic dextroposition” is used to indicate abnormal displacement of the aorta toward the right (toward the left—or levoposition—in inverted hearts) in relation to the pulmonary artery. It is used as a mere description of an abnormal relation between the two great arteries and does not imply a clinical diagnosis.

Most cases of DORV and TF had aortic dextroposition (or levoposition). In four additional hearts, corresponding to cases of arterioventricular concordance (see Table 1), the aorta was found to be dextroposed with respect to the pulmonary artery. In these cases, there were no other significant structural modifications. Three of these four cases

<table>
<thead>
<tr>
<th>Table 4. Arterioventricular Alignment</th>
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<tbody>
<tr>
<td>Alignment</td>
</tr>
<tr>
<td>Concordant</td>
</tr>
<tr>
<td>DORV</td>
</tr>
<tr>
<td>DOLV</td>
</tr>
<tr>
<td>TF</td>
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<tr>
<td>Discordant (TGA)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5. Interventricular Septal Defects and Arterial Relations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position of the interventricular septal defect</td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Subaortic</td>
</tr>
<tr>
<td>Subpulmonary</td>
</tr>
<tr>
<td>Subaorta and sub-pulmonary artery</td>
</tr>
<tr>
<td>Noncommitted</td>
</tr>
</tbody>
</table>
showed a subaortic VSD, and the fourth occurred as an isolated anomaly.

**Association of Malformations: Relation to Cardiac Loop**

Only five hearts showed a single malformation—most often, ASD or VSD. The average number of malformations per heart was found to be 3.5. The most frequent associations were those of CAVC with CA and persistence of the sinus venosus and that of CAVC with arterioventricular anomalies. Eighty-five percent of our CAVCs were accompanied by DORV, TF, or TGA.

When heart malformations are related to the direction of the cardiac loop, some specific associations are found. The association of CAVC and DORV is more frequent in the L loop, whereas the association of CAVC and TF is more frequent in the D loop. Ten of 12 cases of DORV were found in the L loop, and six of eight cases of TF were found in the D loop ($p<0.001$). Also, five of the six cases of CILV were found in the D loop ($p<0.005$). However, the small number of cases of CILV makes this significance doubtful.

**Discussion**

The main goal of the present study was to study the heart malformations presented by iv/iv mice. Heart malformations in these mice occur in a setting of visceral and venous positional anomalies. Forty percent of the fetuses showed cardiac malformations. These malformations rarely occurred in isolation but instead appeared in the formation of complex cardiopathies.

This study shows the presence of some morphological differences between the two atria that permit identification of the atrial situs. However, approxi- mately one third of the malformed hearts have been classified as of indeterminate atrial situs, although SEM shows these hearts to have isomorphic atria. The difficulty in identifying the atrial position in malformed iv/iv hearts has been recognized. This appears to be species related and might not represent real differences with respect to human hearts with similar malformations. All of our hearts with atrial situs identified as solitus or inversus showed atrioventricular concordance. Atrioventricular discordance is not frequent in humans, and no cases have been reported in iv/iv mice.

We have detected a total of 24 cases of CAVC. Of these, 19 were of the complete type, and most of them showed an anterior free-floating divided leaflet, as happens in most human series. Bridging of the right end of the anterosuperior leaflet of the common atrioventricular valve across the crest of the interventricular septum is the basis of the classification of Rastelli et al. In our hearts, most cases showed minimal bridging, and they should be classified as being of type A of CAVC. The predominance of type A of Rastelli et al is frequent in large human series. In types B and C of the classification of Rastelli et al, the right margin of the leaflet extends further, reaching as far as the anterior papillary muscle. This circumstance was never observed in our mice, but this appears to be a species difference only. The papillary muscles of the tricuspid valve of the mouse arise from the right side of the interventricular septum. Thus, it would be impossible for this leaflet to reach the anterior ventricular wall. The cases in which the right margin of the anterior leaflet arises from the middle part of the interven-
tricular septum probably correspond to types B and C as described in humans.

One third of our cases of CAVC coursed with CA and persistence of the sinus venosus. In humans, this anatomic arrangement is very frequent in hearts with atrial isomerism. In our series, most of these cases occurred in indeterminate atrial situs, that is, in hearts with isomeric atria. Taken as a whole, our results reinforce the idea of the almost infinite variability of these hearts.

The hearts with DORV also showed great morphological variability, as occurs in humans. The presence of a displaced septomarginal band separating the origins of the aorta and the pulmonary artery is another variant of this malformation. One of our cases of DORV showed an anterior aorta with a subpulmonary VSD. This heart had type II of DORV, or Taussig-Bing malformation. A high degree of anatomic variability also occurred in our cases of TGA; something similar occurs in humans.
The TF in humans includes pulmonary infundibular stenosis and right ventricular hypertrophy, which were not observed in the mouse hearts. Because ventricular hypertrophy is secondary to hemodynamic modifications, the only real difference is the pulmonary stenosis. However, this anomaly is very rare in iv/iv mice, and we have found only one of these cases. Thus, the mouse hearts with TF are really examples of TF without pulmonary stenosis. This anatomic arrangement is also called the “Eisenmenger complex.” The Eisenmenger complex, however, implies the presence of pulmonary hypertension, which has not been demonstrated in the iv/iv mice.

Our percentage of malformations is lower than that previously reported for newborns of this mouse strain. This is a logical finding since newborns were obtained by selecting dead and moribund pups before they were cannibalized by their mothers. Differences in mating strategies do not appear to influence the rate of heart malformations.

Eighty-five percent of our cases of CAVC coursed with DORV, TGA, or TF. This association is also very frequent in humans. Other associations such as CAVC–CILV–ventriculoarterial anomalies and CAVC–CA–persistence of the sinus venosus are also frequent in human hearts with complex cardiopathies. This is especially frequent in cardiopathies of genetic origin. On the whole, the heart malformations presented by the iv/iv mouse, as well as their degree of association, are similar to those found in humans. Small differences such as the inability to identify the atrial situs in some cases or the differences in the right end of the common atrioventricular valve are due to species differences. Although these particular aspects in humans are most important from the surgical and prognosis perspectives, they are not very significant from the morphological or developmental viewpoints, and they should not be taken to represent significant differences from the physiological viewpoint. The iv/iv mouse appears to be an excellent model for the study of the etiology and pathogenesis of complex heart defects in humans.

A curious, previously unreported, finding of the present study was that some of the heart malformations appear to be related to the formation of the cardiac loop. The CAVC-DORV association is more frequent in L loops, whereas the CAVC-TF association, and the presence of CILV, is more frequent in D loops. During early development, some of the cardiac loops in iv/iv mice are abnormal, and these abnormalities could lead to the development of specific malformations. However, at this point, this is only a working hypothesis. The development of these abnormal loops has not been followed, and we do not know whether they result in fetus death and resorption, give rise to specific malformations, or undergo some kind of morphological recovery. It is difficult to know whether a similar relation between the development of the cardiac loop and the presence of specific heart malformations also occurs in humans. Most human series either show no difference or the difference is not significant. In another report, however, most cases of CAVC-DORV occurred in situ inversus, and most cases of CAVC-TGA with pulmonary atresia occurred in situ solitus. The possibility that something similar to what is evidenced in the iv/iv mouse occurs in humans has yet to be explored.

Questions on Inheritance

The present study emphasizes the fact that the heart malformations presented by the iv/iv mouse are polymorphic in nature (different phenotypes), with the variability of anatomic expression ranging from mild cardiac affection to very severe cardiopathies. Many of the latter appear to be lethal as judged from the high number of fetal resorptions. A high level of anatomic variability also occurs in other animal models of congenital heart disease, as well as in human specimens selected because of chromosomal anomalies or familial recurrence of specific heart defects. In these populations and genetic studies, the most severe cardiopathy was the basic defect or the defect type inherited, whereas the less severe forms were considered to be forms frustes of the defect type. In the iv/iv mouse, the most severe and frequent malformation is the CAVC-DORV combination, coursing with CA and persistence of the sinus venosus. This is the bulboventricular heart and can be considered the defect type of these hearts. The other anomalies can be considered forms frustes of the basic defect.

From a genetic viewpoint, the presence of different phenotypes can be explained either by the presence of a single gene mutation with incomplete penetrance or on the basis of multifactorial inheritance with a threshold of expression beyond which congenital heart disease will occur. However, this is difficult to reconcile with the accepted view that positional anomalies in the iv/iv mouse result from the absence of genetic regulation that would permit situs to be determined at random. This apparent discrepancy can be explained if the iv gene is a developmental gene coding, not for specific structures but rather for developmental mechanisms. A defect in this type of gene results in pleiotropic effects not only in target organs (the heart in this case) but also in other areas of the body as well. This could explain simultaneously the high variability in the phenotypic expression of the heart and the several patterns of visceral and venous heterotaxia. Heart malformations could then be considered not as the primary effect of the abnormal gene activity but rather as secondary to defective interactive processes during cardiac development (see Reference 49).

We have demonstrated that some specific malformations appear to be directly related to the formation of the cardiac loop; that is, they may be due to positional anomalies of the cardiac segments. However, other heart anomalies appear to be unrelated to looping, or the relation is not followed as easily. The iv locus has recently been mapped to a linkage group.
in chromosome 12 of the mouse, but we have not identified the gene products or their effects at the cellular and subcellular levels. It is possible that in addition to the modification of some basic developmental mechanism(s), the iv gene could have some effects at other levels of biological organization. We are currently exploring the pathogenesis of heart defects in the iv/iv mouse and looking for possible mechanisms that could explain the high anatomic variability of these hearts.

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