PERSISTENT COMMON ATRIOVENTRICULAR CANAL OF THE COMPLETE FORM ASSOCIATED WITH ANOMALIES OF THE AORTIC ARCH SYSTEM IN WKY/NCRJ RAT FETUSES

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Atrioventricular (AV) septal defect with a common AV orifice was found in two near-term rat fetuses, which are descendants of an inbred strain, known to genetically develop tetralogy of Fallot, hypertrophic cardiomyopathy, etc. In one fetus the anterior bridging leaflet was almost entirely committed to the left ventricle but in the other it protruded slightly into the right also, coinciding with type A or type B in humans, respectively. The latter fetus had also a subaortic ventricular septal defect with overriding of the aorta and a double aortic arch. Both fetuses had a narrow pulmonary infundibulum with a muscular band, a dysplastic pulmonary valve, and a markedly hypoplastic ductus arteriosus. Complete AV septal defect and tetralogy of Fallot may be linked genetically, with some common underlying developmental processes.

(*Jpn Circ J* 1994; **58**: 720–726)

PERSISTENT common atrioventricular (AV) canal of the complete form is characterized by an AV septal defect with a common AV orifice, with the valve straddling both ventricles^{1–3}. The author came across two near term rat fetuses from the WKY/NCrj strain with this anomaly. Most rats of this inbred strain spontaneously develop tetralogy of Fallot, pulmonary valve dysplasia and/or hypertrophic cardiomyopathy in association with hypoplasia of the ductus arteriosus, occasionally together with anomalies of the aortic arch such as a right or double aortic arch and aberrant subclavian artery^{4–8}. Some knowledge has already been obtained from this rat strain of the fetal development⁵, genetic characteristics⁸, and functional aspects of these anomalies⁹.

Key words:

Hypoplastic ductus arteriosus

CASE REPORTS

Protocol. With the mother rats under urethane anesthesia, the fetuses were removed on days 19–21 of gestation. The day of identification of sperm in the vaginal smear after an overnight mating was considered day 0 of gestation. (The full term in rats is 21–22 days.) The umbilical cord and placenta was kept intact so that bleeding would not affect the size of the heart and vessels. They were immersed in 10% formalin maintained at 18°C to 20°C in order to keep the influence of temperature on the contractile state of the heart and vessels minimal and constant; a lower temperature leads to an obvious constriction of great arteries whereas a higher one causes a marked constriction of the ventricles.

The fetuses of day 21 of gestation had their thorax, abdomen, and diaphragm widely incised before immersion in formalin to fix the ductus arteriosus before its physiological

(Received October 1, 1993; accepted March 18, 1994)

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This study was supported in part by Grant No. 02807096 from the Ministry of Education, Science, and Culture of Japan

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Fig.1. Case 1. A and B, Right dorsal and dorsocaudal views, showing a common atrioventricular orifice, double aortic arch (circle=right arch), small ductus arteriosus (white arrow), and a single artery diverging from the pulmonary trunk, which then gives off right and left main pulmonary arteries. Star=anterior (ventral) bridging leaflet (ABL). Asterisk=posterior (dorsal) bridging leaflet (PBL). Black arrows=anterior (AL) and posterior (PL) tricuspid leaflets with chorda of anterior papillary muscle between them. Arrowhead=PL of mitral valve. The ABL is committed mainly to the left ventricle and slightly to the right. The bar represents 1 mm. C, Right ventricular view, with the right ventricular free wall removed. Anterior surface of the heart is on the top and midventricular transversely cut surface is on the right. There is a perimembranous ventricular septal defect (Arrowhead), its caudal wall being the free-floating ABL (star). The ABL is attached to the crista supraventricularis (small arrows). The parietal band (white arrow) extends from the crista ventrostrally (triangle). The infundibulum is slightly narrow. Arrows=tricuspid AL and PL with anterior papillary muscle between them. D, Arterial aspect with the aortic valve removed. The left ventricular free wall was partly removed. The ventricular septal defect (arrowhead) is immediately subjacent to the posterior cusp and on the right side of the ventricular septum which deviates leftward and is hence overridden by the aorta, allowing direct communication from the right ventricle toward the aorta. Arrow=left ventricular outflow. Asterisk=posterior bridging leaflet. The ABL was in part tethered to the aortic ring. E, Left ventricular aspect through the removed ventricular free wall. The midventricular transversely cut surface is on the left. The ABL (star) is tethered to the anterior papillary muscle (small arrows). Arrows= tricuspid AL and PL. Scooping-out of the septal crest (arrowhead) is shallow and the PBL (asterisk) is not free-floating. F, The anterior cusp edge is irregular due to a nodule formation protruding toward the left cusp (arrow). A=aortic ring, P=pulmonary ring.

TABLE I QUANTIFIED DATA

	Case 1	Day 19 F1xWKY [97]*	Day 19 Wistar [63]	Case 2	Day 21 WKY [90]**	Day 21 Wistar [28]
Body weight (g)	2.1	3.1±0.3	3.2±0.2	4.4	4.8±0.4	5.2±0.5
Heart weight/body weight (mg/g)	6.3	4.8±0.5	5.0±0.3	6.5	5.1±0.5	5.2±0.4
Pulmonary outflow tract (×10 ³ μm ²)	80	NM	152±25	37	93±31 (55%)	144±21
Ductus arteriosus (×10 ³ μm ²)	22	43±6 (22%)	48±5	21	33±7 (47%)	41±4
Aortic isthmus (×10 ³ μm ²)	44	38±12 (27%)	30±4	47	35±13 (52%)	23±3
Ductus/Aortic isthmus	0.5	1.3±0.3 (42%)	1.6±0.4	0.4	1.1±0.4 (75%)	1.8±0.2

F1, first generation offspring between WKY/NCrj and normal Wistar rats. Case 1 was included in the 97 fetuses from F1xWKY backcrossings. Data other than Case 1 and Case 2 are expressed as mean±SD, obtained in previous studies^{5,8}. Cross-sectional area was corrected by body weight (g) raised to 2/3 power. Individual values in WKY/NCrj descendants were considered abnormal when they were outside the mean±2SD for Wistar rats at the same stage. Percentage in parenthesis represents the incidence of abnormal values.

NM, not measured.

*, 11% had a ventricular septal defect and 28% had pulmonary valve dysplasia.

**, 18% had a ventricular septal defect and 47% had pulmonary valve dysplasia.

constriction. Our preliminary study had shown that ductal constriction occurred within 1 h after the removal of the fetus from the uterus when the fetus attained day 20 of gestation and was not treated. Through our treatments, however, the ductus was fixed without any sign of constriction in all fetuses, as was clearly shown in our previous studies^{5,10}.

The heart and the great arteries were isolated together under a dissecting microscope (Fig. 1A) and were examined quantitatively with an eye-piece micrometer, as described in detail previously⁵. In brief, the area of ventricular septal defect (VSD) and infundibular ostium was determined by their major and minor diameters from the right ventricular aspect. The cross-sectional area of the ductus arteriosus and aortic isthmus also was determined by their major and minor diameters at their junction where their lumen size was smallest. The atria were not examined.

Case 1. Of 97 fetuses (10 litters) of day 19 of gestation obtained from the crossings between WKY/NCrj and normal Wistar rats, one female fetus had an AV septal defect

with a common AV orifice (Table)⁸. Of the 14 siblings in the same litter, 4 fetuses had tetralogy of Fallot and 3 had pulmonary valve dysplasia. The fetus with the AV septal defect also had a double aortic arch and a small ductus arteriosus (Fig. 1A, Table). A single pulmonary artery originated from the pulmonary trunk and then gave off the right and left main pulmonary arteries, an abnormal divergence often seen in WKY/NCrj rat fetuses and in rat fetuses exposed to bis-diamine^{5,8,10}.

The common AV orifice was accompanied by anterior (ventral) and posterior (dorsal) bridging leaflets, anterior and posterior leaflets of the tricuspid valve, and posterior leaflet of the mitral valve (Fig. 1A, B). The anterior bridging leaflet was committed mainly to the left ventricle but also slightly to the right (Fig. 1B), corresponding to type B¹⁻³. It was tethered to the anterior papillary muscle of the left ventricle (Fig. 1E), the AV ring (Fig. 1B), and the aortic ring (Fig. 1D), with its right end attached to the portion of the crista supraventricularis (Fig. 1C) where the papillary muscle of the conus is expected to grow.

Fig. 2. Case 2. A and B, Atrial and dorsocaudal aspects. The ABL (star) is entirely committed to the left ventricle and the AL of the tricuspid valve (arrow) is relatively large. Asterisk=PBL. Arrowhead=PL of mitral valve. C, Right ventricular aspect. The pulmonary infundibulum is narrow and the parietal band (white arrow) extends from the crista supraventricularis (triangle). There is no ventricular septal defect at the membranous portion. The papillary muscle of conus is absent. D, Aortic view with valve cusps dissected. The ABL (star) is tethered to the aortic ring, in part to the portion to which the membranous ventricular septum would normally be attached (small arrows). The ABL is in contact with the infundibular septum (triangle) and cuts off direct communication from the right ventricle to the region subjacent to the aorta. Arrow=left ventricular outflow. E, Left ventricular view through the partly removed left ventricular free wall (from a more apical side than in Fig. 1E). Star=ABL. Arrow=AL of tricuspid valve. The PBL (asterisk) is floating above the septal crest (arrowhead) which is deeply scooped out. F, The pulmonary valve is obviously thicker than the aortic valve. Normally, these two valves are equally thick. A, P, ABL, AL, PBL, and PL, as in Fig. 1.

The infundibulum was narrow, with a parietal band extending ventrorostrally from the crista. (The parietal band was defined as a structure that is normally absent but develops under pathological conditions^{4,5,10} The crista designates the muscular structure separating the pulmonary and tricuspid valves,

and corresponds to the ventriculoinfundibular fold¹¹) There was a perimembranous VSD; its caudal edge was the free-floating anterior bridging leaflet (Fig. 1C). Viewed from the aorta, the VSD was subaortic and on the right side of the ventricular septum, which deviated leftward to accommodate the

VSD and was hence overridden by the aorta (Fig. 1D).

Scooping-out of the ventricular septal crest was shallow, with the posterior bridging leaflet adhering to it, which allowed only a small interventricular communication (Fig. 1E). The cusp edge of the pulmonary valve was irregular due to a nodular protrusion (Fig. 1F). The latitudinal muscle bundle in the midwall layer of the ventricular septum was continuous in a normal way with that of the left ventricular free wall^{5,10}

Case 2. One litter of fetuses was obtained from a pair of WKY/NCrj rats on day 21 of gestation. The litter comprised 12 fetuses, of which 1 female had a complete AV septal defect, another had tetralogy, 4 had a narrow pulmonary infundibulum, and 4 had pulmonary valve dysplasia. No complete AV septal defect was found in any other WKY/NCrj rats examined previously: 226 fetuses, 110 neonates, and more than 500 adults⁴⁻⁹

There was a common AV orifice with 5 valve leaflets (Fig. 2A, B). The anterior bridging leaflet was committed almost entirely to the left ventricle, and the anterior tricuspid leaflet was relatively large (Fig. 2B), corresponding to type A¹⁻³. Its right end was tethered to the aortic ring including the portion to which the membranous ventricular septum would normally be attached (Fig. 2B, D).

The pulmonary infundibulum was narrow, and there was a ventrorostrally extending parietal band (Fig. 2C). The papillary muscle of the conus was absent. There was no perimembranous VSD (Fig. 2C, D). The ventricular septal crest was deeply scooped out and the posterior bridging leaflet was floating above the crest, allowing a large interventricular communication (Fig. 2E). The pulmonary valve was obviously thickened (Fig. 2F). The septal latitudinal muscle bundle showed a moderate degree of abnormal rightward continuity^{5,10}. The ductus arteriosus was very small but the aortic isthmus was large, as in Case 1 (Table).

One hundred and two rat fetuses on days 19-21 of gestation and 98 adult rats of the WKY/NCrj strain were available for the close observation of the mitral valve. None of them displayed a cleft in the anterior leaflet, which reached the ventricular septum, and proved to be an incomplete AV septal de-

fect.

DISCUSSION

Complete AV septal defect is attributed to a faulty development of the endocardial cushions and the AV septum. It often occurs as part of the syndrome of trisomy 21 in humans or of trisomy 16 in mice, sometimes in association with tetralogy of Fallot¹²⁻¹⁴. The present two cases were obtained from an inbred strain, in which every rat is presumed to have homozygous genotype and identical genes. Therefore the occurrence of complete AV septal defect in the same strain that frequently develops tetralogy of Fallot suggests that the two diseases are genetically linked. The occurrence of these two diseases in the same families has been reported in humans as well; of 52 offspring from adults with AV septal defects 3 had AV septal defect and 2 had tetralogy¹⁵. Furthermore, the present cases were associated with a markedly hypoplastic ductus arteriosus, as it occurs in tetralogy in the same rat strain⁵. This association may be accidental or suggest the presence of a developmental mechanism commonly underlying the two diseases.

Our previous study suggested that the hemodynamic effect of a small ductus, i.e., pressure overload on the right-sided cardiovascular system in the fetus, leads to various cardiac anomalies^{5,10}. Subaortic VSD may persist as a vent for pressure release from the right ventricle, simultaneously making the ventricular septum deviate leftward and overridden by the aorta; the shunting through the VSD may lead to hypoplasia of the pulmonary infundibulum and to enlargement of the aortic isthmus due to the downstream and upstream effects, respectively. The pulmonary and tricuspid valves may become dysplastic in response to an augmented mechanical stimulus to closure of the valves due to the elevated pressure. The rightward continuity of the septal latitudinal muscle bundle, which is favorable for generation of pressure in the right ventricle, may be formed as an adaptation to the pressure load. These were also observed in similar anomalies induced by bis-diamine¹⁰.

Development of the ductus arteriosus and septation of the extracardiac part of the aortic and pulmonary outflow tracts are normal-

ly completed earlier than the AV septation,¹⁶ supporting the possibilities in the present cases that a hypoplastic ductus was placing a pressure overload on the right ventricle while the AV septation was in progress and that an AV septal defect may have persisted as a vent. The associated narrow pulmonary infundibulum, dysplastic pulmonary valve, and large aortic isthmus seem to favor this view. Infundibular and/or pulmonary valve stenosis is occasionally associated with AV septal defect in humans also.^{2,13} Furthermore, complete AV septal defect occurred in many of the rat fetuses exposed to bis-diamine, mostly in association with truncus arteriosus;^{17,18} the truncus induced by bis-diamine is likely to result from an early developmental arrest and regression of the sixth aortic arch arteries.¹⁰ In the trisomy 16 mice as well, complete AV septal defect is mostly associated with truncus arteriosus or a hypoplastic pulmonary trunk, often together with aortic arch anomalies.¹⁴

An interventricular communication at any level can play the role of a vent in the presence of a unilateral pressure load. If so, why was an AV septal defect much rarer than a perimembranous VSD in this rat strain? The AV septation normally develops somewhat earlier than does the septation of the interventricular foramen at the membranous portion.¹⁶ The foramen that normally is still present when the AV septation is in progress will alleviate the disturbing effect of the elevated right ventricular pressure on the AV septation. Thus, in order for an AV septal defect to persist, it is required that pressure overload is imposed early on and further that the time lag between the closure of the interventricular foramen and the AV septation is considerably shortened either by earlier than usual occurrence of the former or by later development of the latter. Such a parallel development of septation under pressure overload could result in concurrence of tetralogy and AV septal defect. In this context, if the septation of the interventricular foramen progressed earlier than did the AV septation, an isolated AV septal defect could persist; the presence of an AV septal defect as a vent may relieve the pressure elevation so that the interventricular foramen can close.

The above speculation is in part supported

by the following experimental result. Mother rats which were treated with bis-diamine on days 9 to 10 of gestation produced 48 fetuses near term, all with truncus arteriosus, 44 of which had an AV septal defect and only 4 had a perimembranous VSD; 90 fetuses from mothers treated on days 11 to 12 included 33 with truncus and 35 with a hypoplastic pulmonary trunk, with an AV septal defect occurring rarely (in 4 of the 33 and in 1 of the 35) but with a perimembranous VSD persisting frequently (in 29 of the 33 and in 24 of the 35).¹⁸ This result suggests that an early exposure to the teratogen causes truncus arteriosus more frequently, i.e., involution of the sixth aortic arches, and that the early involution would exert hemodynamic effects on the cardiac septation at an early stage including the AV septation.

Another possibility is that ductal hypoplasia may occur secondarily to cardiac anomalies; pulmonary infundibular and/or valvular stenosis may cause a flow decrease through the ductus, leading to ductal hypoplasia or agenesis.¹⁹⁻²¹ In WKY/NCrj rat fetuses, however, the pulmonary arteries often displayed wall thickening and luminal narrowing in association with a small ductus.^{5,8} The luminal narrowing, but not the wall thickening, could have resulted from the downstream effect through the narrow proximal pulmonary outflow. The wall thickening as well as the luminal narrowing can be explained by pressure elevation in the pulmonary trunk due to a small ductus. In addition, the cardiac valves on the right side were selectively involved in dysplasia often both in the same rat, which can be explained by the augmented mechanical stimulus common to them but not to the valves on the left. These facts seem to favor the view that hypoplasia of the ductus, like the double aortic arch, may occur primarily as an expression of anomalous development of the aortic arch system.

The present hypothesis is based on only two rat fetuses; one possibility that still remains to be excluded is that there may be primary disorders in the development of the endocardial cushions such as cell proliferation, migration, and adhesion.¹⁴ However, the above hypothesis suggests that the aortic arch system, including the sixth arch arteries, deserves to be examined in human cases of

complete AV septal defect, particularly in the syndrome of trisomy 21. In this regard, the anomalies of the aortic arch system including agenesis of the ductus are well known in cases of tetralogy of humans¹⁹⁻²¹ and dogs²² as well. In a human fetal case of trisomy 21, agenesis of the ductus was associated with complete AV septal defect and tetralogy¹⁴

Acknowledgment

The author is grateful to Dr. Gisela C. Mautner and Dr. Susanne L. Mautner, for reviewing the manuscript.

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