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Clinical Patent Ductus Arteriosus in Adult Genetically Epilepsy-Prone Rats

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Persistent patent ductus arteriosus (PDA) and clinically silent PDAs are relatively common congenital cardiac defects in humans. We report here the occurrence of symptomatic PDA in adults from a colony of genetically epilepsy-prone rats (GEPRs). Affected rats displayed severe ventral edema. Echocardiography revealed PDA in several animals. Necropsy findings included cardiomegaly, hepatic hyperemia and centrilobular necrosis indicative of passive congestion, and vascular changes consistent with pulmonary hypertension. All affected rats were descendants of one of two brother–sister breeding pairs established from a single litter in April 2000. Clinically silent PDAs were also detected in the colony. Histological examination of the ligamentum arteriosus showed normal vascular tissue in asymptomatic GEPR and Sprague-Dawley rats. PDAs are likely to have a genetic component in the GEPR colony and may provide a novel model for the study of pathogenesis and therapy of this condition.

The ductus arteriosus is a vascular conduit that connects the pulmonary artery and descending aorta. In the fetus, this conduit allows blood to bypass the high-resistance and non-functional pulmonary circulation. The shunt normally closes spontaneously in the neonate, typically within 1 week after birth (17, 31). However, persistent patent ductus arteriosus (PDA) is a relatively common congenital cardiac defect, affecting approximately 1 in 2,000 infants per year in the United States (8, 25, 39). Treatment strategies include surgical ligation and medical treatment with indomethacin or other cyclooxygenase inhibitors. However, treatment failures and adverse side effects are common, and the ideal treatment strategy remains controversial, especially in low-birth-weight infants (6, 10, 11, 21, 23, 28).

The left-to-right shunting that occurs with PDA allows increased flow of blood through the pulmonary circulation. As a result of increased pulmonary flow, a volume load is imposed on the pulmonary vasculature (arteries, capillaries, and veins), left heart, and proximal aorta. Animals with significant shunting are predisposed to develop left-side volume overload with subsequent left-side congestive heart failure. In response, increased resistance within the pulmonary vasculature may limit the amount of shunting. This increased resistance can result in severe pulmonary hypertension that can cause cessation or even reversal of ductal flow. The resulting increased pressure load on the right heart can lead to clinical signs of right heart failure.

Genetically epilepsy-prone rats (GEPRs) currently exist as two independently derived strains that display distinctive characteristic seizures in response to auditory stimulation (30). Rats of the GEPR-9 strain display clonic–tonic seizures, whereas rats of the GEPR-3 strain develop only clonic seizures in response to the same stimulus (30). These two strains were derived via selective breeding of Sprague-Dawley stock and have, in general, been maintained via brother–sister mating for over 40 generations (15, 30). Seizure susceptibility is 90% to 99% penetrant in GEPR-9 and 92% to 97% penetrant in GEPR-3, with some sexual dimorphism (15). In addition to susceptibility to audiogenic seizures, GEPRs also demonstrate impaired hearing (5, 29), thyroid hypofunction (24), and immunologic impairments (4, 32).

We report here the occurrence of a new phenotype, PDA, in a GEPR-3 colony. These PDAs resulted in heart failure in a subset of rats. Further study of these rats is warranted to assess their utility as novel animal models for PDA and pulmonary hypertension.

Case Report

Animals and husbandry. A GEPR-3 colony was established at the Southern Illinois University School of Medicine (SIUSM) in the spring of 2000. The founder animals were obtained from the Genetic Resource section of the National Institutes of Health and have been maintained at SIUSM as a closed colony with brother–sister mating since that time. Offspring are routinely weaned at 21 to 23 days of age into single-sex groups. Typically, rats are tested for seizure susceptibility at approximately 6 weeks of age by using standard criteria (5, 26) and are then maintained in the colony for eventual use as experimental or replacement breeder animals. The colony currently comprises approximately 175 male and female animals of various ages.

The GEPR-3 colony at SIUSM is housed under conventional conditions in a dedicated room as a closed colony. Rats are maintained under a 12:12-h light:dark cycle on hardwood bedding at a room temperature of 21 to 22°C and a relative humidity of 40 to 60%. Food (LabDiet 5001, PMI Nutrition International, Brentwood, Mo.) and water are available ad libitum. The colony is free of known infection with *Mycoplasma pulmonis* and common rat viruses (rat coronavirus, Sendai virus, pneumonia virus of mice, parvovirus, and Theiler's murine encephalomyelitis virus), as indicated by periodic serological testing of both sentinel and colony animals.

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Table 1. Signalment of and findings from symptomatic and asymptomatic GEPR-3 rats

GEPR-3 (SIUSM ID)	Sex	Age at death	Echo	Clinical pathology	Clinical signs	Post-mortem findings: heart and liver	Post-mortem findings:lungs
Symptomatic							
6/9/02-A*	Male	13 m	PDA	WNL	Ventral edema, thoracic effusion	Cardiomegaly, PDA, hepatic hyperemia	Mild pulmonary arterial medial hypertrophy and moderate numbers of heart failure cells
6/9/02-B*	Male	16 m	ND	ND	Ventral edema	Cardiomegaly, PDA, atrial thrombi, mild hepatic hyperemia and centrilobular necrosis	ND
6/19/02	Male	12 m	PDA	WNL	Ventral edema, ascites, thoracic effusion	Cardiomegaly, PDA, hepatic hyperemia and venous hypertrophy	Mild pulmonary arterial medial hypertrophy and moderate numbers of heart failure cells
9/2/02-A*	Female	8 m	ND	ND	Ventral edema	Cardiomegaly, PDA, hepatic centrilobular necrosis	Very mild pulmonary arterial medial hypertrophy and few heart failure cells
9/2/02-B*	Male	9 m	ND	ND	Ventral edema, ascites, thoracic effusion	Cardiomegaly, PDA, hepatic centrilobular necrosis and venous hypertrophy	Mild pulmonary arterial medial hypertrophy and few heart failure cells
9/23/02	Female	12 m	ND	ND	Ventral edema	Cardiomegaly	ND
10/19/02	Female	12 m	ND	ND	Ventral edema, cyanosis	Cardiomegaly, pericardial and thoracic effusion	ND
5/8/03	Male	9 m	ND	ND	Ventral edema	Cardiomegaly, PDA	Cardiac hypertrophy, focal mineralization of pulmonary artery
5/31/03-A*	Female	14 m	ND	ND	Dyspnea, edema	Cardiomegaly, pleural effusion	ND
5/31/03-B*	Female	14 m	ND	ND	Ventral edema, chromodaccyrhea	Cardiomegaly, severe pleural effusion, ascites	ND
6/3/03-A*	Female	12 m	ND	ND	Ventral edema	Pulmonary congestion, cardiomegaly, pleural effusion	ND
6/3/03-B*	Male	13 m	ND	ND	Edema	ND	ND
Asymptomatic							
9/2/02-C*	Male	9 m	NSL	WNL	NSL	Residual vascular tissue in LA	NSL
NA	Male	11 m	NSL	WNL	NSL	Residual vascular tissue in LA	Very mild pulmonary arterial medial hypertrophy and few heart failure cells
NA	Male	NA	NSL	WNL	NSL	Residual vascular tissue in LA	Very mild pulmonary arterial medial hypertrophy and few heart failure cells

Animals whose IDs include the same numerical portion and that are flagged by * are litter mates.
Echo, findings on echocardiography; LA, ligamentum arteriosum; NA, not available; ND, not done; NSL, no significant lesions; PDA, patent ductus arteriosus; WNL, within normal limits.

Case history and diagnostic approach. All diagnostic testing was performed under an approved institutional animal care and use protocol. During the period between May 2003 and June 2004, six adult male and six adult female rats displayed sudden onset of severe ventral edema and, in some cases, dyspnea (Table 1). Rats were euthanized by an inhaled overdose of carbon dioxide. A gross necropsy examination was performed on all rats, and selected tissues were collected for histopathologic analysis. For histopathologic examination, tissues were fixed in 10% neutral-buffered formalin, and sections (thickness, 5 μm) were stained with hematoxylin and eosin. Lung sections were also stained with Verhoeff–van Giesen technique to assess pulmonary vasculature.

Two affected rats were examined by echocardiography, and thoracic radiographs were obtained from one. Serum was collected and analyzed for glucose, urea nitrogen, creatinine, sodium, potassium, chloride, total protein, albumin, globulin, calcium, cholesterol, total bilirubin, alanine transaminase, and alkaline phosphatase by the Clinical Pathology Laboratory of the University of Missouri College of Veterinary Medicine. Three age-matched asymptomatic rats from the same colony were assessed using echocardiography, gross and histologic examination of selected tissues, and clinical chemistries. Thoracic radiographs were obtained from two additional age-matched asymptomatic rats.

Echocardiography. Two-dimensional (2D) and M-mode echocardiography were performed, and mean values for multiple parameters were calculated (Table 2). The two rats that presented with signs of respiratory distress and ventral edema were found to have PDAs. Rats with PDAs had marked increases in left atrial diameter in 2D and M-mode views, left ventricular internal dimension in diastole and systole, and left atrial-to-aorta ratio, compared with normal rats. In rats with PDA, color flow and spectral Doppler evaluation of the ductus were also evaluated. Color flow analysis of the right ventricular outflow tract (RVOT) in affected rats identified a predominant left-to-right shunt, with turbulent flow in the RVOT (Fig. 1). A low-velocity (2 m/sec), left-to-right shunt between the descending aorta and pulmonary artery (PA) was confirmed by spectral analysis. This velocity of blood flow between the systemic and pulmonary circulations is consistent with pulmonary hypertension (Fig. 2). In the three asymptomatic rats, interrogation of RVOT and PA revealed no abnormal pulmonary flow, with all flow occurring from the RVOT into the PA (Fig. 1).

Thoracic radiographs from one affected rat revealed pleural

Table 2. Mean parameters from two-dimensional and M-mode echocardiography of rats with patent ductus arteriosus (PDA)

Parameter	PDA (n = 2)	Asymptomatic (n = 3)	*P*
LAD	1.075	0.6	0.002*
IVSd	0.230	0.207	0.357
LVIDd	1.300	0.860	0.016*
LVPWd	0.260	0.223	0.504
IVSs	0.245	0.233	0.518
LVIDs	1.030	0.570	0.016*
LVPWs	0.290	0.303	0.780
%FS	20.950	33.567	0.104
LA (M)	1.100	0.563	0.008*
Ao (M)	0.405	0.460	0.593
LA/Ao	2.875	1.240	0.032*

*, Statistically significant ($P \leq 0.05$, Student *t* test) differences between asymptomatic rats versus rats with symptomatic PDA.
LAD, Left atrial dimension (2-Dimension); IVSd and IVSs, interventricular septum (diastole and systole, respectively); LVIDd and LVIDs, left ventricular internal dimension (diastole and systole, respectively); %FS, percent fractional shortening; LA (M), left atrial dimension (M-mode); Ao (M), aortic dimension (M-mode); LA/Ao, ratio between LA and Ao M-mode measures.

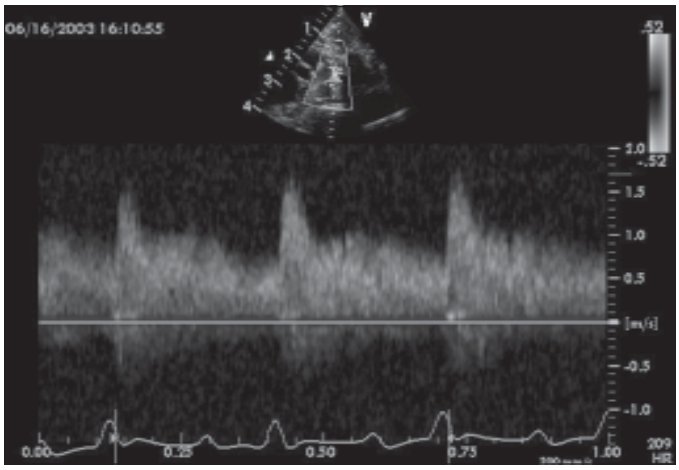

Figure 2. Spectral Doppler tracing of ductal flow in a rat with a PDA. The x-axis denotes time, and the y-axis reflects flow velocity in m/s. Peak flow velocity of 2 m/sec (normal, 5 m/sec) from left to right suggests a reduced systemic to pulmonary pressure gradient, consistent with increased pulmonary pressure (pulmonary hypertension). The degree of pulmonary hypertension is not severe enough to reverse the shunt from right-to-left.

effusion that precluded evaluation of the heart. No cardiac abnormalities were detected by radiography in two asymptomatic rats examined.

Post-mortem findings. Post-mortem examinations were performed in all rats. On gross examination, all 10 rats had various combinations of subcutaneous edema, ascites, pleural effusion, hepatomegaly, cardiomegaly, dilated pulmonary trunks with thickened arterial walls, and PDAs (Fig. 3). Atrial thrombi were present in one rat. Asymptomatic rats had no gross cardiovascular abnormalities, although the structure presumed to be the ligamentum arteriosum (LA) was larger than expected. An attempt to flush dye through one such structure was successful, suggesting that these rats also had retention of a ductus arteriosus.

Histologic examination of the heart of one affected rat revealed myocardial hypertrophy and mild fibrosis. Lungs of four affected rats were examined and revealed medial hypertrophy and occasional mild intimal thickening of small pulmonary arteries and arterioles (Fig. 4). These lesions are consistent with lesions seen in people with pulmonary hypertension and, according to the Heath–Edwards grading scale, could be classified as grade one (7). Varying numbers of pigment-laden macrophages were present in alveoli of affected rats. These findings are consistent with the so-called "heart failure cells" that result from either passive hyperemia of the lungs (left-side heart failure) or pulmonary hypertension with subsequent capillary rupture and intraalveolar microhemorrhages (40). The PDA of one rat was examined histologically and was found to have a typical vascular wall structure with a patent lumen. Examination of livers revealed lesions ranging from mild patchy centrilobular hyperemia to centrilobular necrosis with moderate associated hyperemia. In addition, the walls of central veins were often thickened. These findings are consistent with passive congestion and associated anoxia secondary to right-heart failure.

In all three asymptomatic GEPR-3s, the structures identified grossly as LAs had typical vascular wall structure on histological exam, confirming that these rats also had retention of ductus arteriosus. In two of three unaffected rats, mild medial smooth muscle hyperplasia was evident in a few pulmonary arteries and arterioles.

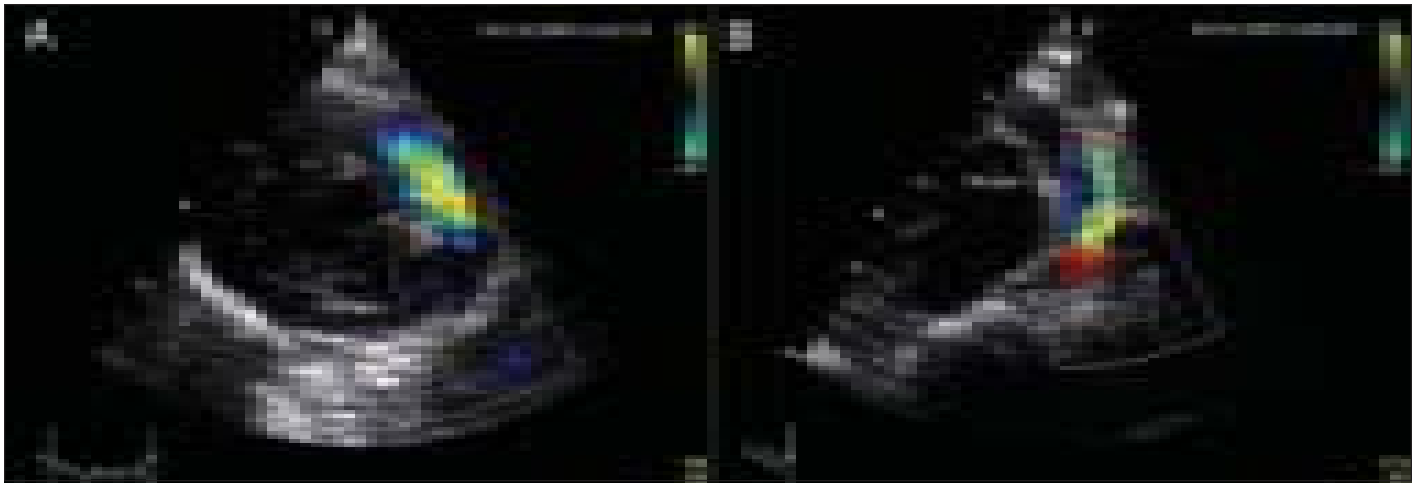

Figure 1. Doppler color flow mapping of the right ventricular outflow tract (RVOT) in a normal rat (A) and a rat with a PDA (B). Note the predominantly blue color suggesting normal RVOT flow (away from the probe) in (A) compared to the primarily red color representing ductal flow (toward the probe) becoming turbulent (mosaic) as blood enters the pulmonary artery in (B).

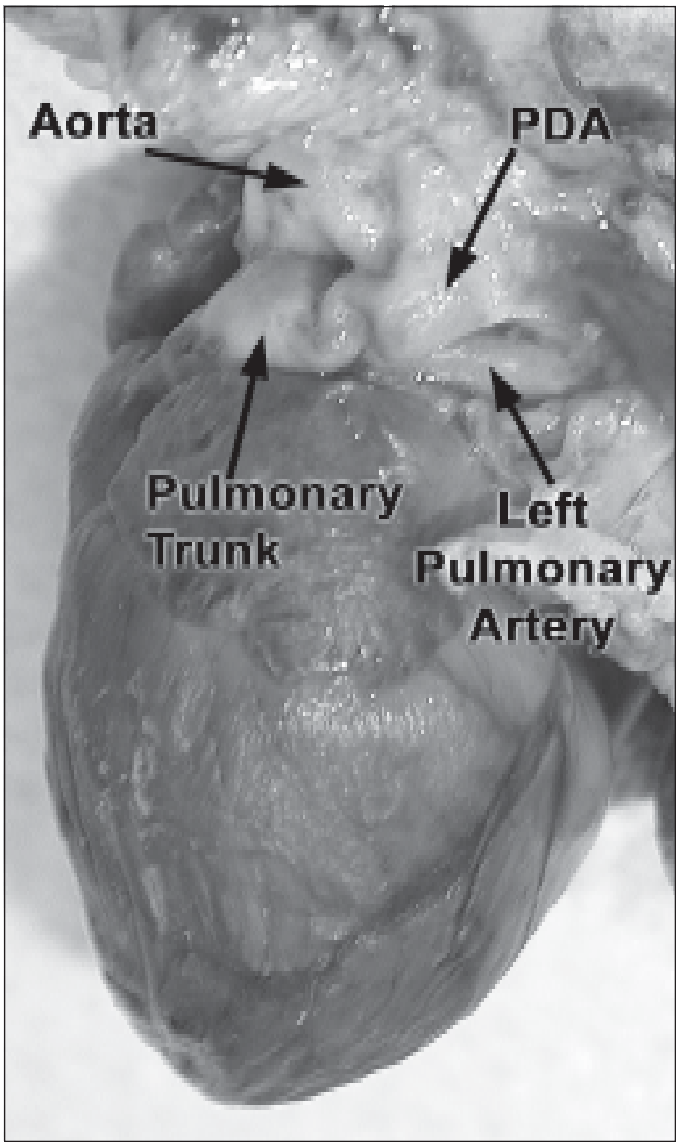

Figure 3. Heart and great vessels from a rat with a PDA and clinical signs of heart failure. Note enlarged pulmonary trunk and tortuous dilated vascular channel (PDA) connecting the pulmonary artery and aorta.

Genealogy of affected rats. All clinically affected rats were related through two related brother–sister breeding pairs, one designated as 4/7/00(L)(LN) and the other as 4/7/00(U)(U) (Table 3).

Assessment of other rat strains for PDA. Because the ductus arteriosus was retained in several adult asymptomatic GEPR-3s, we sought to assess whether common strains or stocks of rats also retained this structure. To this end, seven adult Sprague-Dawley (SD) (five male; two female) and six adult F344 (three male; three female) rats were examined for PDA. Histological examination of the LA/PDA region of two male and two female adult SD rats revealed fatty and thin ligamentous tissue. However, another three SD males showed LA/PDA with typical vascular wall structure, two with obvious lumina. Three adult female and two adult male F344 rats showed fatty and thin ligamentous tissue in the LA/PDA region. However, one male had typical vascular wall structure.

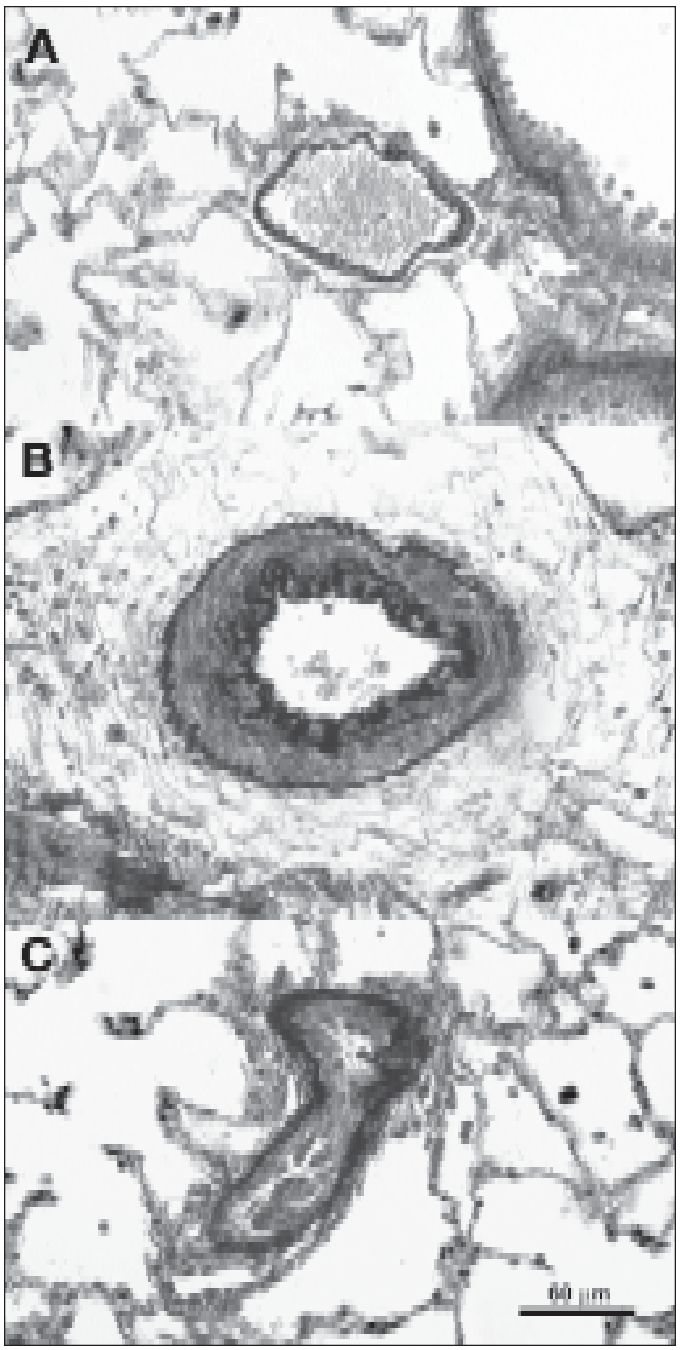

Figure 4. Photomicrographs of sections of lungs from (A) an asymptomatic GEPR-3 and (B and C) a GEPR-3 with clinical signs of heart failure. (A) normal peribronchiolar artery with thin tunica media; (B) medial hypertrophy of a peribronchiolar artery with resulting separation of internal and external elastic laminae; (C) cellular intimal hyperplasia. Verhoeff-van Giesen stain; Bar, 60 µm.

Discussion

We present here a novel observation of the familial persistence of the fetal PDA in a colony of GEPR-3s. Affected rats presented with signs of congestive heart failure, including respiratory distress and ventral edema. The presence of biventricular enlargement, dilated pulmonary trunk and artery, and medial hypertrophy of pulmonary arteries and arterioles are consistent with a left-to-right shunt and subsequent pulmonary hyperten-

Table 3. Maternal lineage of GEPR-3 rats with symptomatic patent ductus arteriosus (PDA)

Generation from maternal founder (column 1)					
1	2	3	4	5	6
0/7/00(L)(LN)[1]	10/4/00B	5/11/01A	8/31/01E	*6/19/02	
		6/29/01C	*6/9/02A *6/9/02B		
			2/16/02	*9/23/02 *10/19/02	
			5/1/02	10/17/02A	*6/3/03A *6/3/03B
4/7/00(U)(U)	12/10/00	6/29/01A	5/5/02	*9/2/02A *9/2/02B	*5/8/03 *5/31/03A *5/31/03B

Animal designations are investigator-assigned animal identification numbers, which are based on the date of birth, the litter number, and the results of testing for susceptibility to audiogenic seizures. Designations of "A" or "B" with the same birth date indicate littermates. All female breeders and the male parent developed clonic seizures in response to a loud tone. Brother–sister mating was used throughout.

Identification numbers in each column denote offspring from dam in preceding column. Only litters relevant to the genealogy of animals with clinical PDAs are shown.

*, Animals with PDA that developed clinical disease.

sion. The identification of a systemic–pulmonary connection between the aorta and pulmonary artery, as demonstrated by echocardiography, is diagnostic for the presence of PDA.

PDA is a common abnormality that has been well described in humans, dogs, cats, and numerous other species. PDA is the persistence of a fetal connection between the descending aorta and pulmonary artery. In the fetus, this connection allows shunting of blood from the pulmonary circulation to the systemic circulation (right to left) to bypass the nonfunctional lungs. Local and circulating prostaglandins have a critical role in maintaining distension of the ductus arteriosus in the fetus (16, 37).

In the neonate, multiple factors, particularly rising partial pressure of oxygen within the circulation, contribute to closure of the ductus arteriosus. Changes in maternal and fetal prostaglandins also likely contribute to the closure of the ductus (3, 6, 37). Neonatal mice that are deficient in cyclooxygenase-1 and cyclooxygenase-2 show failure of ductus arteriosus closure and increased mortality (19), as do mice that are deficient in the prostaglandin E_2 EP_4 receptor (27, 35). In newborn Wistar rats, closure is delayed if neonates are maintained at low ambient temperatures (38).

In pre-term infants, developmental and physiological factors are likely to contribute to the pathogenesis of PDA (6). However, genetic factors also influence the development of PDA in humans (22, 39). The Char syndrome is an autosomal dominant disorder that is characterized by PDA and other features (2). This condition has been studied in two large families by using linkage analysis and positional cloning. These analyses have revealed mutations in the *TFAP2B* gene (33, 34, 41), a neural crest-related transcription factor (20), that appear to cause the syndrome. In our colony, all clinically affected rats were descendants of related founder breeder pairs and represent sub-lines that originated from those animals. These relationships suggest an underlying genetic etiology for PDA in our colony.

Patency of the ductus arteriosus after birth allows continued communication between the aorta and pulmonary artery. During postnatal development, increasing aortic pressures coupled with falling pulmonary pressures allow a pressure gradient to develop between the systemic and pulmonary circulations. This gradient represents the driving force that allows systemic-to-pulmonary (left-to-right) shunting of blood. The existence of left-to-right shunting at the level of the great vessels allows an increased flow of blood through the pulmonary circulation. The magnitude of the shunt depends on resistance across the ductus relative to systemic resistance. The major factors influencing ductal resistance are the size of the duct itself and the pulmonary vascular resistance. A small ductus will restrict flow and allow maintenance of a normal pressure gradient between the two circulations. A large ductus will provide little resistance, resulting in massive left-to-right shunting. As a result of increased pulmonary flow, a volume load is imposed on the pulmonary vasculature (arteries, capillaries, and veins), left heart, and proximal aorta. Animals with pronounced shunting are predisposed to left-side volume overload with subsequent left-side congestive heart failure.

In response to marked pulmonary over-circulation, the pulmonary vasculature may increase its resistance, thus limiting the amount of shunting. This increase in resistance can create pulmonary hypertension that, when severe, can result in cessation or even reversal of ductal flow. Pulmonary hypertension causes the right heart to experience an increased pressure load. Under these circumstances, the right heart may fail, and shunt reversal may induce Eisenmenger-type physiology and cyanotic heart disease.

In the GEPR-3s presented here, the PDAs were likely of sufficient size to result in marked left-to-right shunting with subsequent chronic cardiovascular disease that ultimately resulted in pulmonary hypertension, cardiac decompensation, and acute onset of right heart failure. Medial hypertrophy of pulmonary vessels, the presence of heart failure cells, and echocardiographic evidence of attenuated left-to-right flow and a shunt velocity that was less than expected with normal pulmonary pressures are consistent with pulmonary hypertension caused by chronic left-to-right shunting. In addition, the presence of acute hepatic hyperemia and necrosis, edema, ascites, and pleural effusion is suggestive of right heart failure.

The degree of pulmonary hypertension was probably not severe enough to reverse the direction of shunting, but reversal might have occurred in time, had decompensation not ensued. Species differences are reported in the development of Eisenmenger-type physiology (1), and little information is available on the development of right-to-left shunting in rats with PDAs. Further study of related rats may facilitate identification of a subset of individuals that manifest a right-to-left shunt and clinical signs of cyanotic heart disease.

Given that these founder breeder pairs were established in 2000, the absence of clinical PDA prior to 2003 seems surprising if one hypothesizes a genetic link to the founder stock. However, development of this phenotype may reflect patterns of research use. During the past 2 years, colony animals were euthanized for experimental purposes very soon after they reached sexual maturity, such that relatively few animals in the colony attained the ages associated with clinical signs in the cases reported here. A decline in animal use has resulted in more aged animals being maintained in the colony, thus facilitating detection of age-related clinical cardiac failure. Unfortunately, the numbers of af-

fected animals/litters at this point are too low to assess genetic factors of this disease such as mode of inheritance. Future studies, including a more extensive pedigree analysis, are warranted to further characterize this novel phenotype.

The finding of milder pulmonary vascular lesions in unaffected GEPR-3 cohorts with histologic evidence of retention of the ductus arteriosus but no echocardiographic evidence of shunting suggests that pulmonary resistance may have compensated for smaller PDAs in these rats. Whether these PDAs would ultimately become functional shunts is unknown and warrants further study.

The detection of PDAs in presumed normal SD rats was a particularly surprising finding. However, clinically silent PDAs, which cannot be detected by auscultation but are found incidentally when echocardiography is performed for some other reason, also are reported in humans (1 per 500 to 1,000 persons) (9, 18). Moreover, as explained earlier, pulmonary resistance may compensate for small PDAs and result in relatively normal cardiovascular physiology. This observation that clinically asymptomatic PDAs may occur in some SD rats requires that caution be used in interpreting hemodynamic data from this commonly used outbred stock. Similar cautions have been advanced with regard to the use of WKY rats (36). A high incidence of ventricular septal defects and biventricular hypertrophy, as well as PDA, has been reported in WKY rats (12-14, 26, 36), which are often used as the normotensive control strain in studies of spontaneously hypertensive rats.

In summary, we identified a subset of rats of the GEPR-3 strain that developed late onset heart failure as the result of patent ductus arteriosus. The finding of this phenotype in rats descended from related founder pairs suggests a possible genetic etiology that, if documented, may result in a novel model for the study of various cardiovascular diseases including PDA and pulmonary hypertension.

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