Spontaneous mitral valve prolapse in a breeding colony of rhesus monkeys

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ABSTRACT Mitral valve prolapse was observed in 26 of 92 animals in a harem breeding colony of rhesus monkeys (Macaca mulatta). The affected animals had a systolic murmur best auscultated over the mitral region with the animal in a sitting position. Mid-to-late systolic clicks were also heard. Phonocardiographic examination also demonstrated systolic murmurs and clicks in six of 16 animals. Twenty-three of the animals were studied by M mode and/or two-dimensional echocardiography. The diagnosis was confirmed in 12 animals that had a murmur during the examination. Electrocardiograms revealed T wave abnormalities in five animals and left or right ventricular hypertrophy in five. Four adult animals that died during the course of the study were confirmed at necropsy as having prolapse of the posterior and/or anterior mitral valve leaflets into the atrium. Analysis of the breeding records suggested that mitral valve prolapse was a dominant genetic trait with an approximate birth incidence of 16% to 20% in the colony. The existence of mitral valve prolapse in a nonhuman primate species provides a unique opportunity to study the disease in an experimental animal.


MITRAL VALVE PROLAPSE is a well-described entity in humans. The presence of the typical murmur and systolic clicks, along with confirmation by echocardiography or angiocardiography, may be used to diagnose the condition.1,2,3 The disease has been reported to affect 6% or more of the population, more commonly occurring in women; it is believed to be transmitted as an autosomal dominant trait, but the penetrance of the gene and the reasons for the sexual predominance are not completely understood.1,2,4,5 Generally, mitral valve prolapse is a benign condition but may be associated with a variety of complications.1,2,6

The pathogenesis of the disease is a source of controversy and it is not known in all cases whether the myxomatous changes in the valves are primary or secondary.1,2,7,8 Investigations into the pathogenesis and associated complications of the condition have been hampered by the lack of a suitable animal preparation that can be manipulated experimentally.

This study describes the occurrence of spontaneous mitral valve prolapse in a unique colony of rhesus monkeys (Macaca mulatta), which may represent a suitable experimental animal for study of the human disease.

Materials and methods

Animals. The rhesus monkeys were housed in harem breeding groups of eight to 14 adult females per adult male. Offspring were raised with the family unit until 3 years of age. Individual breeding records were kept on all members of the colony. The colony has been maintained as a closed breeding colony and purposely outbred since its inception in 1975. Family groups were housed in chain-link outdoor runs with free access to indoor concrete block and chain-link runs. Water was provided by a central watering system ad libitum, and food (Purina Monkey Chow, Ralston Purina Co.) was provided once a day.

Anesthesia. All auscultatory, echocardiographic, phonocardiographic, and electrocardiographic examinations were performed while the animals were sedated with 10 mg/kg im ketamine hydrochloride (Ketaset, Bristol Laboratories). Previously, a pilot study was performed to determine that auscultation of the murmur and echocardiographic examinations would not be altered by ketamine anesthesia. Two animals diagnosed as having mitral valve prolapse were restrained manually while echocardiographic and auscultatory studies were performed. Ketamine was then administered intramuscularly and no changes were noted in the echocardiographic recordings after induction of anesthesia.

Auscultation. The colony was auscultated on five occasions from April 1982 to April 1984. All auscultations were carried...
out independently by at least two people. The presence of systolic clicks and mid-to-late systolic murmurs was not recorded unless both observers agreed that they were present. A consensus was reached on the grading of the murmur after its presence was determined. Animals were auscultated in dorsal, right lateral, and left lateral recumbency, and in a sitting position. Monkeys over 2 years of age were considered to be unaffected if they remained free of detectable murmurs or clicks on auscultation after the second year. In addition to the colony described in this study, a separate unrelated group of 60 rhesus monkeys maintained by the Johns Hopkins University School of Medicine and a breeding colony of 81 rhesus monkeys at the National Institutes of Health were screened by auscultation for the presence of heart murmurs. Both groups of control monkeys were housed in individual cages.

**Echocardiography and phonocardiography.** A commercially available two-dimensional and M mode echocardiographic recorder (ATL Mark 300 IC) with a 5 MHz transducer was used. Multiple long- and short-axis recordings of the heart were made with the monkey in dorsal, left lateral, and right lateral recumbency, and in a sitting position. Twenty-nine animals (23 affected, six control) were examined by two-dimensional echocardiography and 27 (21 affected, six control) by M mode echocardiography.

The diagnosis of mitral valve prolapse of two-dimensional echocardiography was made by demonstration of a leaflet bulging into the left atrium, as previously reported. A posterior or displacement of the mitral valve past the annular plane during ventricular systole was considered to be diagnostic. The diagnosis of mitral valve prolapse on M mode echocardiography was made by demonstration of a deflection of 2 mm or more of a mitral valve leaflet posterior to the "C" point during systole.

Phonocardiograms (Irex System II) were obtained in 21 animals (15 affected, six control). Systolic murmurs and/or systolic clicks characteristic of mitral valve prolapse were sought.

**Electrocardiograms.** Standard six-lead electrocardiograms (leads I, II, III, aVR, aVL, and aVF) were obtained with a commercially available electrocardiograph (Hewlett-Packard 1511A) to screen for T wave abnormalities and atrial and/or ventricular arrhythmias that have been associated with mitral valve prolapse in humans. Electrocardiographic tracings were compared with standards for the species, as previously reported.

**Necropsy.** Complete necropsies were performed on 26 rhesus monkeys during the course of this study by the pathology service of the Division of Comparative Medicine, Johns Hopkins Medical School. Fourteen of the monkeys were from the same colony as the individuals being studied. The others were from the second, unrelated colony maintained at The Johns Hopkins University School of Medicine.

Two of the monkeys studied were aborted fetuses. Three were less than 1 year of age, and two were juveniles between 1 and 4 years of age. The remainder were adults ranging in age from 4 to 25 years.

None of the animals died of primary cardiac disease. Three of the animals died of cerebral venous thrombosis, five of shigellosis, one of Entamoeba histolytica infection, one of dystocia, and two of trauma; two were aborted macerated fetuses, and the remainder were adults from a separate colony than the one under study, killed as a part of other experimental protocols. Four of these animals (table 1, cases 1 to 4) had been diagnosed as having mitral valve prolapse by auscultation antemortem. The other 22 animals did not have detectable heart murmurs antemortem and were used as controls for the necropsy studies.

Samples were taken of all tissues, including sections of anterior and posterior mitral valves, tricuspid valves, and their attached papillary muscles and tendinous chordae. All tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 6 μm. Sections were stained with hematoxylin and eosin, and all cardiac sections were also stained with Verhoeff-van Giesen, Masson's trichrome, and alcan blue/periodic acid-Schiff stains.

**Results**

**Auscultation.** Twenty-six of 92 monkeys (21 males, 71 females) in the colony were identified as having heart murmurs consistent with mitral valve prolapse (table 1) after screening the entire colony five times. Ten were males and 16 were females. The animals ranged in age from 2 months to greater than 10 years of age when the murmurs were first detected in the colony. The murmurs were characterized as being mid-to-late systolic, with an occasional mid-to-late systolic click best auscultated over the mitral region of the chest. The murmurs were loudest when the animals were placed in a sitting position with the knees and waist flexed. The murmur would characteristically disappear when the position of the animal was changed. Murmurs varied in intensity from grades I to V to as high as VI. The intensity of the murmur varied between auscultations of the same animal and frequently varied in intensity during a single auscultation. The murmurs could not be demonstrated in all animals every time they were auscultated. Only one of 60 animals in the second Johns Hopkins colony and one of 81 in the NIH colony used as control populations had the typical mid-to-late systolic murmur. Heart murmurs were never heard in animals used as controls for the studies.

**Echocardiography and phonocardiography.** Twenty-three of the 26 animals with demonstrated heart murmurs were examined by two-dimensional echocardiography, 21 of 26 by M mode echocardiography, and 15 of 26 by phonocardiography (table 1). Five of the animals (cases 7, 8, 17, 21, and 24) had a positive M mode echocardiogram (figure 1). Eight animals (cases 6, 8, 9, 16, 18, 19, 20, and 25) had a positive two-dimensional echocardiogram (figures 2 and 3). One animal (case 8) was positive on both M mode and two-dimensional echocardiography. An intermittent systolic click was demonstrated on phonocardiograms of three animals (cases 7, 16, and 17), early systolic murmurs were demonstrated on phonocardiograms of four animals (cases 8, 16, 19, and 25), and one animal (case 16) was demonstrated to have both a murmur and a click. Only animals in which murmurs could be auscultated at the time of the echocardiographic and phonocardiographic examinations had positive findings. Six control animals without heart murmurs had

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negative findings on two-dimensional and M mode echocardiography and phonocardiography.

Electrocardiograms. Standard six-lead electrocardiograms were obtained from the 23 animals that had echocardiographic examinations. T wave abnormalities were demonstrated in five animals (cases 4, 7, 8, 9, and 12). These included large, notched T waves and T waves that varied in shape and size during the recording. Three animals (cases 5, 10, and 11) were demonstrated to have left ventricular hypertrophy based on wide QRS complexes and a mean electrical axis of less than 30 degrees. One of these animals (case 11) also had left atrial hypertrophy based on a p wave measurement of 60 msec duration. Two animals (cases 8 and 24) were demonstrated to have right atrial and ventricular hypertrophy based on mean electrical QRS axis determinations of greater than 100 degrees and the presence of s waves in leads I, II, and III. A Ta wave was present in lead II on one of these animals (case 8). Arrhythmias were not detected in any of the tracings.

Necropsy findings. Four adult animals with mitral valve prolapse died during the period of time in which the colony was being studied (table 2).

Case 1. A female that had been caught in the wild as an adult 10 years before her death had ballooning of the central scallop of the posterior mitral valve into the atrium. There was also rolling of the edges of the leaflets and nodular thickenings of the attachments of both the anterior and posterior mitral valves. Microscopically, the posterior leaflet of the mitral valve had a fibrous thickening of the dorsal surface. There was an accumulation of loose elastic and fractured collagen tissue in the leaflet. The animal also had cerebral venous thrombosis.

Case 2. A male that had been caught in the wild as an adult 6 years before his death had similar findings.
Under gross examination there was prolapse and ballooning of the central scallop of the posterior leaflet of the mitral valve into the atrium (figure 4). There was a small area of myocardial hemorrhage in the left ventricular septum and jet lesions were present on the wall of the left atrium. Microscopically, there were slight myxomatous changes and accumulation of fractured elastic and collagen fibers in the posterior leaflet of the mitral valve (figures 5 and 6). Subendocardial and myocardial hemorrhages in the interventricular septum were confirmed. This animal also had cerebral venous thrombosis.

**Case 3.** A female that had been caught in the wild as an adult 11 years before her death died after abdominal surgery for pregnancy complications. She had ballooning and prolapse of all scallops of the posterior mitral valve into the atrium. Microscopically there was myxomatous degeneration of the affected leaflets with fibrosis of the dorsal surface of the leaflet and disarray of the collagen fibers in the valve.

**Case 4.** A 6-year-old colony-born female died unexpectedly without clinical signs. She had slight ballooning and prolapse into the atrium of the lateral and medial margins of the anterior mitral valve and of the lateral scallop of the posterior mitral valve. The affected anterior and posterior leaflets had myxomatous degeneration and fibrosis of the dorsal surface of the valves microscopically. There was also slight disarray and fragmentation of the collagen fibers in the posterior leaflet.

Twenty-two other rhesus monkeys varying in age from aborted fetuses to 21 years of age underwent necropsy during the course of this study. None of these animals had the lesions of mitral valve prolapse. One other animal was demonstrated to have cerebral venous thrombosis without pathologic changes of the heart valves.

**Genetic analysis.** Only one family line affected with the disorder has progressed into the third generation (figure 7). The progenitor female in the line is case 1 in table 1, and was demonstrated to have the disease at necropsy. She had three daughters (cases 5, 8, and 16),
TABLE 2
Necropsy findings of mitral valves of animals with mitral valve prolapse

<table>
<thead>
<tr>
<th>Prolapsing leaflet</th>
<th>Microscopic changes of valve a</th>
<th>Cause of death</th>
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<tbody>
<tr>
<td></td>
<td>Anterior cusp</td>
<td>Lateral</td>
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<td>Case 1</td>
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<td>Case 2</td>
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<tr>
<td>Case 3</td>
<td></td>
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<tr>
<td>Case 4</td>
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aMyxomatous degeneration, fibrosis of dorsal surface of leaflet, and collagen fiber abnormalities.

The unaffected third generation offspring are under 2 years of age and are not yet diagnosed as having the disease. The original father in this line died of Entamoeba histolytica infection during the course of this study, and no pathologic changes were found in his heart valves. This information indicates that mitral valve prolapse is probably a dominant genetic trait in these monkeys. Since both males and females in the colony are affected, it is not likely to be sex-linked.

Analysis of birth records for 2 years during the course of this study shows that five of 30 animals (four females, one male) born in 1981 and five of 25 animals (one female, four males) born in 1982 developed mitral valve prolapse. This gives an approximate incidence of 16% to 20% of live births in the colony becoming affected before 2 years of age, without an apparent sexual predisposition.
Auscultation alone has been used to diagnose mitral valve prolapse in humans. The presence of a position-dependent, intermittent systolic murmur and a mid-to-late systolic click have been reported to be specific for mitral valve prolapse.1,2 This is consistent with the murmurs auscultated in our colony. The typical murmur of mitral valve prolapse was heard in only one of 60 monkeys in a second Johns Hopkins colony and in one of 81 monkeys in an NIH colony. It appears that our study colony either represents a unique group of animals or that the development of the condition is in some way related to their environment.

Unlike humans,1,2 the monkeys in our colony could not be demonstrated to have mitral valve prolapse by echocardiography unless the typical murmur was present at the time of the examination. None of the animals represented in table 1 with negative echocardiographic findings had a murmur at the time of the examination.

Discussion

This study describes the first reported occurrence of spontaneous mitral valve prolapse in a colony of rhesus monkeys with a known genetic background. The occurrence of mitral valve prolapse in one dog is the only previously published report of this disease in a nonhuman species.12

Others have compared the myxomatous changes associated with valvular endocardiosis in aged dogs with human mitral valve prolapse.13,14 However, important differences exist between this preparation and the human disorder. In valvular endocardiosis the lesions are not confined to the mitral valve; the clinical murmur is that of valvular regurgitation and does not vary in intensity or occurrence like that of mitral valve prolapse; prolapse of the mitral valve into the left atrium does not occur unless there is rupture of a tendinous chorda.

Mitral valve prolapse has been produced experimentally in dogs by creating papillary muscle dysfunction.15 However, because this condition was produced experimentally under ischemic conditions it may not represent the spontaneous human situation as accurately as the currently documented condition in nonhuman primates.

FIGURE 5. Photomicrograph of posterior leaflet of the mitral valve of a monkey with mitral valve prolapse demonstrating myxomatous degeneration of the valve (arrow). PM = papillary muscle; M = myocardium. (Alcian blue/periodic acid–Schiff; original magnification ×7.)

FIGURE 6. Photomicrograph of posterior leaflet of the mitral valve of a normal monkey. PM = papillary muscle; M = myocardium. (Alcian blue/periodic acid–Schiff; original magnification ×7.)
Six animals with heart murmurs (cases 5, 10, 11, 12, 15, and 23) at the time of echocardiographic examination could not be diagnosed by either two-dimensional or M mode echocardiography. Echocardiographic examinations are further complicated by the small size of the animals' hearts and the normal heart rate of greater than 150 beats/min. Further screening of the colony and improvements in echocardiographic equipment may alter these findings.

Phonocardiography was performed in a portion of the animals to document the presence of a systolic click and murmur. It was not performed simultaneously with the echocardiographic examinations so the findings were not always consonant with the results of auscultatory examinations done at the time of echocardiography. Nevertheless, none of the animals with a negative phonocardiographic finding had a murmur at the time of the examination, and all of the animals with a murmur at the time of the examination had positive phonocardiographic tracings.

Electrocardiographic tracings from anesthetized animals showed the presence of T wave abnormalities, which is consistent with findings in humans. It is not known whether the atrial and ventricular hypertrophy detected by the electrocardiographic tracings was directly related to the diagnosis of mitral valve prolapse. Arrhythmias were not demonstrated in anesthetized animals. Holter monitor studies in awake animals may yield different results.

The necropsy findings of mitral valve prolapse in four animals from the colony seem to confirm our clinical diagnoses. The gross pathologic changes were analogous to those reported in humans. Microscopically, the myxomatous changes, collagen abnormalities, and fibrosis of the dorsal surface seen in these valves was not as well developed as would be expected in humans. Similar myxomatous changes of the ventricular aspect of mitral valves without valvular deformities or prolapse have been reported in aged rhesus monkeys. This may indicate that the myxomatous changes of mitral valve prolapse in rhesus monkeys may be secondary rather than primary. This is a matter of controversy in humans and, indeed, mitral valve prolapse may be a common end point of a variety of conditions. None of the affected animals died of primary cardiac disease. It is not thought that the cerebral venous thrombosis in two animals was related to mitral valve prolapse, since this event has been reported in monkeys from the same colony without valvular lesions. More detailed studies with greater numbers of affected animals are needed to determine the pathogenesis of mitral valve prolapse in this colony.

The colony has not been followed through enough generations of breeding to determine whether mitral valve prolapse is clearly the result of a dominant genetic trait. It is tempting to speculate that this is the case, since necropsy of the progenitor female in the family line discussed in this study (table 3) confirmed the presence of the condition and her mate did not have any pathologic changes of the mitral valve at necropsy. Since the condition has been diagnosed in males in the colony, it probably not a sex-linked trait, although male-to-male transmission has not been shown. After analysis of birth records for 2 years, mitral valve prolapse in these monkeys may not be associated with a higher incidence in females, as is the case in humans. Definitive breeding studies will have to be performed with the third generation offspring when
they become sexually mature. Our colony also includes four matings in which both parents were affected. Of the four progeny, two are known to have mitral valve prolapse and the other two are too young (under 1 year) for a diagnosis to be established. Mitral valve prolapse in the affected progeny was of similar severity to that in their parents. In our colony, a production colony, males are more likely to be sold after weaning and females may be kept as future breeders. This biases our colony population toward the retention of affected females.

The presence of mitral valve prolapse in a breeding colony of rhesus monkeys offers a unique opportunity to study the pathogenesis of this disorder in a nonhuman primate preparation.

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