New Embryological Evidence for the Formation of Quadricuspid Aortic Valves in the Syrian Hamster (*Mesocricetus auratus*)

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Summary

A Syrian hamster embryo, aged 11 days and 4 h post-coitus, had a developing quadricuspid aortic valve. The septation of the cardiac outflow tract was confined to the distal part of the conotruncus. There was a conspicuous recess in the anlage that normally gives rise to the left aortic valve cushion. Globular endothelial cells arranged in several layers were present at the luminal side of the recess. The present findings support the hypothesis that in the Syrian hamster, quadricuspid aortic valves result from the division of one of the three mesenchymal anlagen that give rise to normal aortic valves. In addition, they indicate that the division of the anlage is due to the invagination of the endothelial layer that covers its luminal side. The invagination of the endothelium starts at a very early stage of the valvulogenesis, namely, when the conotruncal ridges begin to fuse at the distal portion of the embryonic cardiac outflow tract.

Introduction

In man, congenital quadricuspid aortic valve is a rare abnormality (James et al., 1991); thus, Simonds (1923) found a prevalence of 0.008% in 25,666 necropsies, and Feldman et al. (1990) a prevalence of 0.043% in 13,805 echocardiographies. In animals, it has been reported in an adult greater white-toothed shrew (*Crocidura russula*) (Durán, 1996) and in a Syrian hamster embryo (Fernández et al., 1994).

The morphogenesis of quadricuspid aortic valves remains unknown in man. In the case described by Fernández et al. (1994), the developing aortic valve had four cushions instead of three. It was concluded that the supernumerary cushion probably resulted from the division of one of the three mesenchymal anlagen that give rise to normal aortic valves. However, it remained unclear whether the division of the anlage was due to its defective growth or to invagination of the endothelial layer that covered its luminal side. We report here the finding of a further quadricuspid aortic valve in a Syrian hamster...
embryo from an earlier developmental stage, which provides new information on this question.

**Materials and Methods**

The present embryo with a developing quadricuspid aortic valve was found unexpectedly in the course of a current study on the formation of the cardiac semilunar valves in Syrian hamster embryos. The characteristics of the Syrian hamster colony and the breeding procedure have been described elsewhere (Fernández et al., 1998), and as far as is known the animals have never been exposed to teratogenic agents.

In the study that gave rise to the observation reported here, pregnant females are killed with ether and embryos are obtained by laparotomy and uterotomy. Thereafter, they are freed from fetal membranes and their total length is measured. The age of the embryos is calculated from the time of coitus (“day 0, hour 0”).

The heart of the affected embryo was fixed in 10% neutral formalin buffered with magnesium carbonate, and processed by the paraffin wax method. Serial sections, cut transversely at 5 μm, were stained with Delafield’s haematoxylin-yellowish eosin.

In this report, the terms “proximal” and “distal” are used to describe the location of the conotruncal components with regard to the ventricle.

**Results**

The affected embryo was aged 11 days and 4 h postcoitus (total length = 8.5 mm). In this specimen, the septation of the cardiac outflow tract was confined to the distal part of the conotruncus. The conotruncal ridges (swellings) were still separated from each other at the proximal portion of the conotruncus (Fig. 1A). At this level, each ridge displayed a circular profile and consisted of a mixed population of (1) neural crest-derived ectomesenchymal cells that formed the so-called ectomesenchymal prong of the ridge, and (2) mesodermal-derived mesenchymal cells arising by delamination from the endothelial layer covering the ridge. At the middle portion of the conotruncus (Fig. 1B), the ridges had an oval contour, and the prongs occupied the central zone of the ridges. More distally, the ridges were fused to each other along their central regions, whereas their cephalic and caudal margins remained separated (Fig. 1C), constituting the anlagen of the right and left aortic valve cushions and of the pulmonary valve cushion, respectively. The left conotruncal ridge had a conspicuous recess at the distal level of the conotruncus (Fig. 1D). The recess was located at the caudal margin of the ridge, i.e., at the anlage of the
left aortic valve cushion. Globular endothelial cells arranged in several layers were present at the luminal side of the recess (Fig. 1E). The cells of the layer next to the underlying mesenchyme possessed several cytoplasmic processes that extended towards the extracellular matrix (Fig. 1E).

The anlagen of the other aortic and pulmonary valve cushions were normal.

**Discussion**

Embryological findings concerning quadricuspid aortic valves are uncommon, and the present observation is only the second such report. The preceding case was that of the Syrian hamster embryo mentioned above (Fernández et al., 1994). In that embryo, aged 12 days and 2 h postcoitus, the septation of the conotruncus had concluded, and the developing aortic valve consisted of four valve cushions, a dorsal, a left, and two rights, each showing a mild degree of excavation. The two right cushions were joined to each other at the level of their basal portions.

This finding contradicted the classical hypotheses on the formation of quadricuspid aortic valves, which implicated mechanisms such as anomalous septation of the conotruncus (Simonds, 1923; Koletsky, 1941; Wyatt and Goldenberg, 1948; Peretz et al., 1969; Hurwitz and Roberts, 1973; Coeurderoy et al., 1986), anomalous excavation of one of the valve cushions (Simonds, 1923), and septation of a normal valve cushion as the result of an inflammatory episode (Salvatore et al., 1976). The arrangement of the two right cushions strongly suggested that the supernumerary cushion resulted from the division of the anlage that normally gives rise to the right-ventral valve cushion. However, the possibility that quadricuspid aortic valves result from the interpolation of an extra swelling in the common trunk (Simonds, 1923; Hurwitz and Roberts, 1973; Coeurderoy et al., 1986) could not be ruled out.

The specimen described herein provided information concerning an earlier stage of the morphogenesis of quadricuspid aortic valves, namely, the stage at which the septation of the cardiac outflow tract begins at the distal portion of the conotruncus. In the affected embryo there was no evidence of abnormal mesenchymal proliferations in the common trunk; interpolation of an extra pad as a possible cause of aortic valve malformation can therefore be excluded.

The arrangement and morphology of the cells located at the recess of the anlage of the left aortic valve cushion indicated that the division of the anlage was due to invagination of the endothelial layer, and not to the defective growth of the anlage from the outset. This mechanism is similar to that proposed recently for the formation of quadricuspid pulmonary valves in Syrian hamsters (Fernández et al., 1998). Nonetheless, the fact that quadricuspid aortic and pulmonary valves occur independently (Fernández et al., 1998) indicates that the primary factors inducing the development of these defective valves operate independently from each other.

Our findings indicate that, at least in the Syrian hamster, quadricuspid aortic valves result from the division of one of the three anlagen that normally give rise to the right, left, and dorsal aortic valve cushions. In addition, they prove that the division of the valve cushion anlage starts at a very early stage.
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of valvulogenesis, namely, when the conotruncal ridges begin to fuse at the distal portion of the embryonic cardiac outflow tract. Such a division has now been observed in both the right and left aortic anlagen. Whether it may also occur in the dorsal aortic anlage remains an open question.

The endothelial cells located in the deepest part of the recess seemed to display a morphology intermediate between that of endothelial and mesenchymal cells. This suggests that transformation of endothelium into cushion mesenchyme is implicated in the division of the valve cushion anlage. It is well known that epithelio-mesenchymal transformation plays a fundamental role in the formation of the mesenchymal conotruncal ridges (Markwald et al., 1977; Fitzharris, 1981). In the chick, transformation of endothelium into mesenchymal mesenchyme may be responsible for the fusion of the conotruncal ridges (Hurle et al., 1990), which constitutes the first step in the septation of the cardiac outflow tract. However, further investigation with specific immunohistochemical markers for the detection of epithelio-mesenchymal transformation is needed.

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