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On the Study of Developmntal Genetics of Isolated Pulmonary Hypoplasia*)

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ABSTRACT

We have carried out the study on the heart anomalies of the DHS mice and Pdn mice fetuses and newborns which are known to produce genetically induced polydactyly. The results have revealed that the heart anomalies are mainly isolated hypoplasia of the pulmonary artery and that these anomalies are not always associated with polydactyly. The isolated hypoplasia of the pulmonary artery show a tendency to develop among uterine sibilings. These might suggest that the genetic traits of heart anomalies are controlled by the polygenic or multifactorial inheritances.

INTRODUCTION

Some kinds of teratogens have been given to pregnant rats in our laboratory to produce particularly a high incidence of heart anomalies for the purpose of making morphological observations of the development of this anomaly and studies of the mechanisms involved[12,13,18,19]. In recent years, some species of mice with genetic anomalies have been developed and used in the area of developmental genetics to make clear the developmental mechanisms of various anomalies[2,4,6,9,10,21]. We have learned that fetuses with polydactyly show a tendency to have a complication of heart anomalies by practical experience of performing numerous autopsies of human fetuses. Therefore, with the purpose of discovering and breeding species of mice with heart anomalies, we have begun inbreeding DHS and Pdn mice which are known to be strains with a high incidence of polydactyly. We are going to make clear some causes of heart anomalies by seeking and breeding the strains with genetic cardiovascular anomalies and comparing those findings of abnormal morphogenesis of the heart with those of experimentally induced heart anomalies.

MATERIALS AND METHODS

1) DHS mice

The DHS mice are a strain known as a dd group which is characteristic of its high incidence of polydactyly[3]. Its genetic polydactyl traits haven't been known yet. The experimental DHS mice are those inbred and raised at the Laboratory of Experimental Animals, Faculty of Science, Hokkaido University. Their mating and breeding have been continued in our laboratory. The total 46 pairs have been mated in our laboratory and 236 fetuses have been bred.

2) Pdn mice

A new type of mouse called Polydactyly Nagoya (gene symbol: Pdn) was found by Hayasaka of Nagoya University in the course of breeding JCL and ICR mice[5]. The genetic analysis indicates that the polydactyly is an autosomal dominant trait. According to Naruse and Kameyama[11], the homozygous fetuses or newborns have 1-3 extra digits both on the fore- and hindlimbs on the preaxial side. Some of them show one or some of such characteristics as exencephaly, cleft palate, open eyelid, short tibia and fibula, deformed sternum and hypoplastic olfactory bulb. Many of the heterozygotes have one extra first digit bifurcated at the distal phalanx of the forelimbs. These Pdn mice were given to our Institute by the

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Research Institute of Environmental Medicine, Nagoya University. They have been breeding for four generations in our laboratory. The feed for them has been Oriental solid food. Those fetuses that died after birth were subjected to autopsy, and from time to time normally delivered newborns were also sacrificed. An observation of heart is performed under a stereomicroscope and the diagnosis has been made.

RESULTS AND DISCUSSION

1) DHS mice

The results obtained from observations made during the course of inbreeding will be confine primarily to the findings of polydactyly, fatal developmental retardation and heart anomalies.

Polydactyly: As it is difficulf to present all the pedigree charts of mice which have been inbred, a few will be shown in Figures 1, A-D. The polydactyl anomaly has been observed in the living mice (Fig. 1-A) as well as in those that died after birth (Fig. 1, B and C). This implies that the polydactyly is not always associated with the fatal developmental retardation. A polydactyl anomaly has seldom oc-

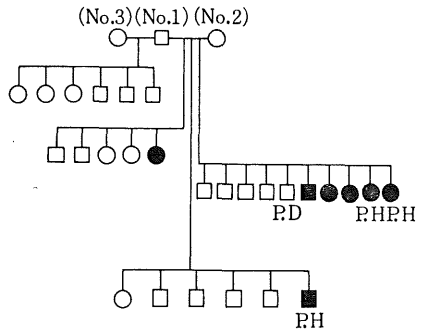

Fig. 1-A

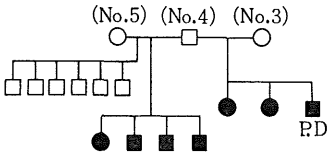

Fig. 1-B

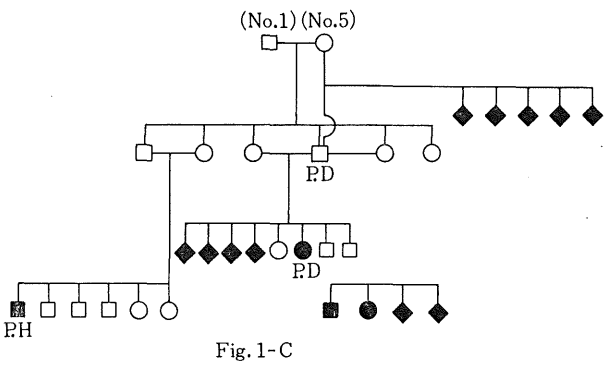

Fig. 1-C

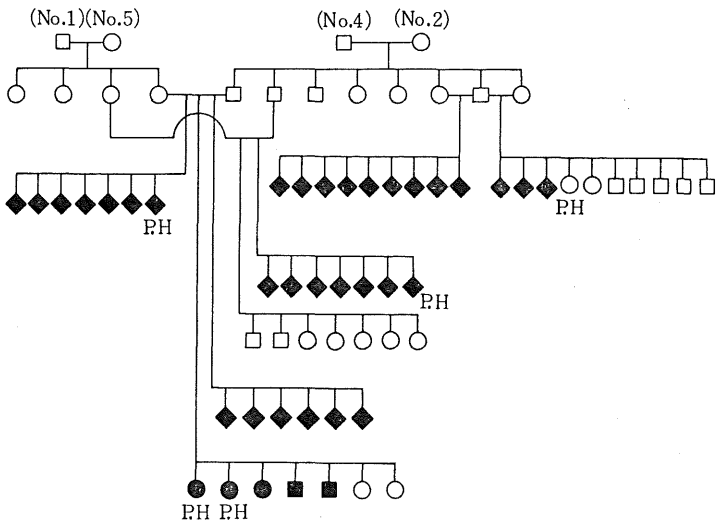

Fig. 1-D

Fig. 1, A-D. Some examples of pedigree chart of DHS mice. This implies that polydactyly and heart anomaly are at times associated with the fatal developmental retardation, but at times not associated. (No. 1)-(No. 5) show the mark numbers of mice.

□, ○ : male, or famale mouse, normal.

■, ● : male, or female mouse of still birth or neonatal death with developmental retardation.

♦ : sex unknown mouse, as they were sacrificed by parents.

P . H : isolated pulmonary hypoplasia

P . D : polydactyly

curred in the pedigree charts. Consequently, it is impossible to establish the genetic background from these charts. In case of man, an isolated or familial occurrence of polydactyly is considered to be a dominant genetic trait. When such occurrence is considered a part of pleiotropic syndrome, the polydactyly is regarded as a recessive trait.

Fatal developmental retardation: Some of the mice that died soon after birth have a body weight of much less than that of the normal ones (Fig. 2). Such mice mount to 30 (7.8%) out of 236, but no major anomalies associated with heart are found by autopsy. This might suggest that they should have some fatal developmental retardation factors. The number of newborns is as few as 3 or 4 in some mice, which suggests that abortion should have taken place during the early fetal stages and that fetuses should have been delivered prematurely.

Heart anomalies: We have found a few fetuses with heart anomalies in the strain of one of the 4 adult female mice (Fig. 1, No. 2). Therefore, it was decided to carry out various mating combinations with that strain and examine the hearts of the fetuses. The heart anomaly found in these cases is an isolated hypoplasia of the pulmonary artery (Fig. 3). A characteristic finding is that heart anomalies show a tendency to develop in uterine sibilings (Fig. 1, A and D). Generally, the causes of heart anomalies are considerd to be the interrelation of genetic and environmental factors as in the case of other anomalies, and the cause for a high incidence in uterine sibilings implicates the possibility of involvement of polygenic and/or multifactorial inheritances. Heart anomalies are different from external anomalies in that they can be confirmed only by autopsy, but as severe anomalies are fatal, it is difficult to preserve the strain by mating.

Sex ratio: The total 236 mice consist of 167 survivors (86 males and 81 females) and 69 sex unknown neonates sacrificed by their parents. Polydactyly is found in 3 males and 4 females, indicating no significant difference in sex, while developmental retardation is found in many cases.

2) Pdn mice

In our laboratory, the four pairs of parents of Pdn mice in the fourteenth generation have been breeding for four generations (From F 14

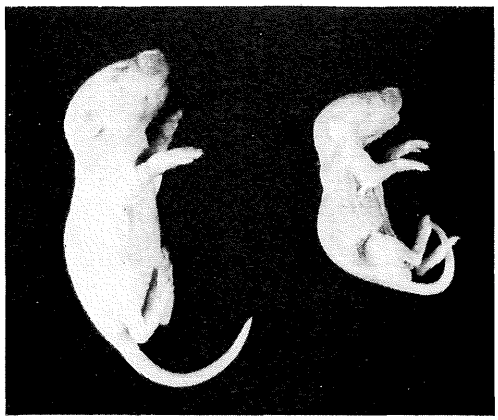

Fig. 2. DHS mice newborns on day 20 of gestation, showing fatal developmental retardation (right), compared to normal dvelopment (left).

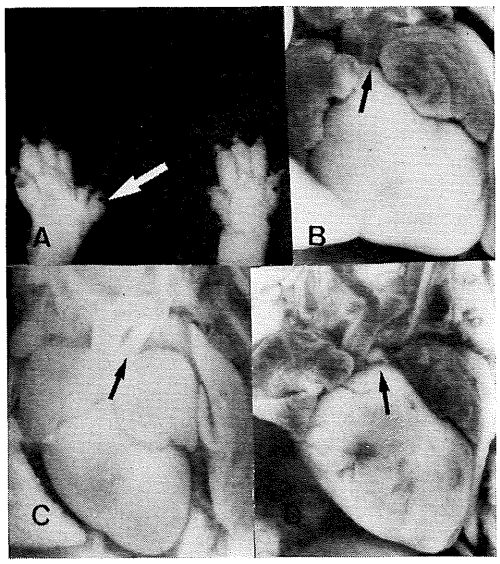

Fig. 3, A–D. Findings of heart anomalies and polydactyly of DHS mice.
A: Ventral view of the hindlimbs on day 20 of gestation. One extra-digit on the preaxial side (↑) of the right hindlimb is observed. The left hindlimb is normal.
B: Pulmonary hypoplasia (↑) of femal on day 18 of gestation. This case correspond to the alive female case in Figure 1-D.
C and D: Pulmonary hypoplasia (↑) of the male seen in Figure 1-A and 1-D respectively. The pulmonary arteries of these still birth fetuses are small in diameter than aorta. In these hearts no interatrial of ventricular septal defects were observed.

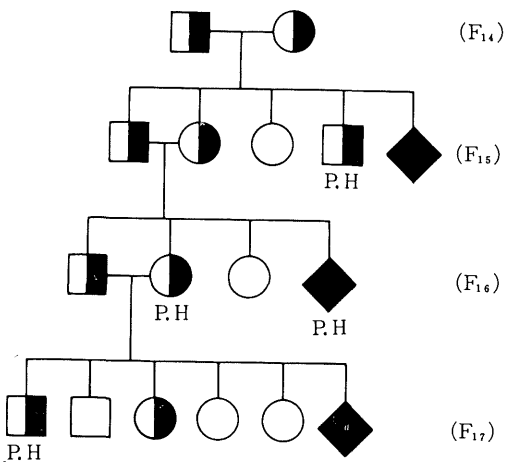

Fig. 4. An example of pedigree chart of Pdn mice. Isolated pulmonary hyperplasia (P. H) are seen in homo, and heterozygous newborns both in male and female.
◆ : homozygous mewborn with polydactyly, sex unknown, genotype: Pdn/Pdn, neonatal death and /or sacrificed by parents.
□, ○ : male, or female homozygous newborn, genotype: +/+, alive.
◨, ◑ : male, or female heterozygous newborn with polydactyly, genotype: Pdn/+, alive.
F14-17 show from 14th to 17th generations of Pdn mice respectively.

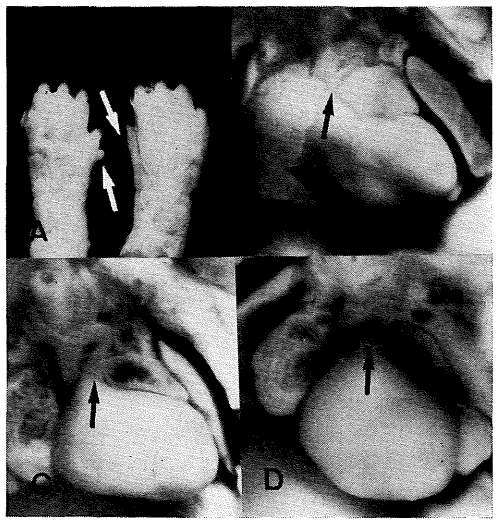

Fig. 5, A-D. Findings of heart anomalies and polydactyly of Pdn mice.
A: Preaxial digites of bilateral hindlimbs (↑) of homozygous newborn.
B, C and D: Frontal view of isolated pulmonary hypoplasia (↑) of sex unknown homozygous neonatal death (B), male (C) and female (D) heterozygous fetuses on day 18 of gestation.

to F 17 in Fig. 4). Figure 4 shows an example of their pedigree chart. In this Figure, we can see no relation between heart anomalies and polydactyly in both cases of homo- and heterozygous newborns. All the heart anomalies are isolated pulmonary hypoplasia unrelated to atrial or ventricular septal defects (Fig. 5). It is considered normal in fetuses that the aorta and the pulmonary artery are almost equal in diameter, because the circulating blood runs more through the pulmonary artery than through the aorta. The aorta gets larger in diameter after birth. In this study we have examined hearts in the fetal period of mice and stillborn or newborn mice died right after birth and made a diagnosis of pulmonary hypoplasia when the pulmonary artery is smaller than the aorta in diameter. This heart anomaly is seen both in homo- and heterozygous fetuses and is not always associated with polydactyly. From this pedigree chart, the genetic trait of isolated hypoplasia of pulmonary artery in this strain might be multifactorial inheritance which consists of polygenic effects associated with some environmental factors. Up to date, there have been a few report about the study on the genetically induced cardiovascular anomalies. Hummel and Chapman[7] describe a mutation in the mouse inherited as a single autosomal recessive trait in which 50% of homozygotes have situs inversus. William and Layton[20] find many kinds of cardiovascular anomalies in this mutant. They suggest that situs inversus and heart anomalies are not a single gene. Heterotaxia, on the other hand, may be under more conventional genetic control. Jantine et al.[8] find left hypoplastic heart syndrome in minipig and indicate that a high frequency of these anomalies in this inbred strain is a hereditary malformation. Rychter et al.[17] show that ventricular septal defect is a heterogenous inborn malformation, either a sequel or primary disturbance of ventricular septal development in Siller's strain of brown leghorn chicken. Patterson[14-16] points out five common cardiovascular anomalies, that is, Patent ductus arteriosus, Subvalvular aortic stenosis, Conotruncal septum defects, Persistent right aortic arch and Pulmonary stenosis are determined by lesionspecific genetic factors that have a higher frequency in some breeds of dogs. Buchanan[1] also shows the genetically induced

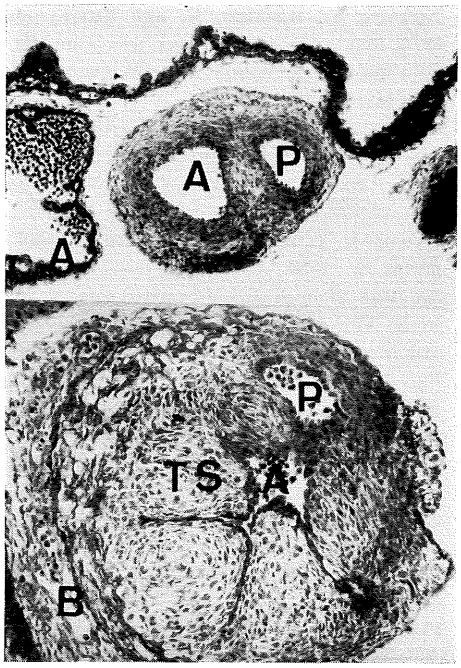

Fig. 6, A, B. Transvers sections of pulmonary hypoplasia experimentary induced in rat embryonic heart on day 14 of gestation.
6-A : hypoplastic pulmonary artery (P) is seen in the truncal region.
A: Aorta
6-B : At the horizontal level of primordium of the aortic valve, hypoplastic pulmonary artery (P), abnormaly formed truncal swellings (TS) and aorta (A) are observed.

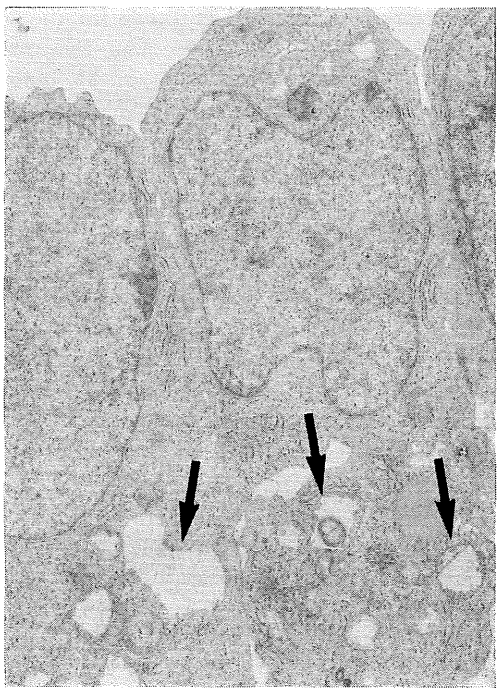

Fig. 7. Electronmicrograph of endothelial cells of truncal swelling at the level of primordium of the aortic valve on day 14 of gestation in the rat embryonic heart. Four days after administration of Bisdiamine to the pregnant rats, some vacuolations (←) are seen in the abnormal cubic epithelial cells of the truncus.

Patent ductus arteriosus in dogs. The apparently high risk of congenital cordiac malformations in purebred suggests that the causative factors are single genes or polygenic sets that behave as recessives.

Addendum:

We have been producing experimentally a high incidence of pulmonary hypoplasia associated with other heart anomalies in rat embryonic hearts, giving 0.2 mg of Bis (dichloroacetyl) diamine to pregnant rats on the 10th day of gestation. We have been also examining abnormally developing hearts morphologically, and it is suggested that pulmonary hypoplasia should be produced by abnormal or unbalanced fusion of the dextro superior and sinistro inferior truncal swellings with aorticopulmonary septum (Fig. 6) (OKAMOTO, N. et al. going to publish.). Electron microscopic examination has revealed that some vacuolations mainly due to swellings of mitochondria are seen in the endothelial cells of abnormally developing truncal swellings (Fig. 7). In order to clear up the causes of pulmonary hypoplasia, a further comparative study of morphogenesis of the genetical pulmonary hypoplasia and the experimentally induced one must be made. In this study we have found that there is no distinctive relation between the traits of external anomaly and heart anomaly, that is, the genetic trait of pulmonary hypoplasia is not the same as that of polydactyly. We would like to find out a type of external anomaly which can be used as an index to produce a complication with heart anomalies by successive repetition of mating and breeding. It is hoped that the results of this observation will serve as a milepost in this direction.

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