Hereditary Transmission of Tetralogy of Fallot, Cardiac Hypertrophy, and Anomalies of Great Vessels in WKY/NCrj Rats

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ABSTRACT. We examined 78 fetuses on d 21 of gestation (G21) and 83 neonates on d 2 after birth (A2), which were first generation offspring of WKY rats mated with normal Wistar rats (F1). In addition, we examined six groups of fetuses on d 19 of gestation (G19): 65 Wistar rats, 111 WKY rats, 85 F1, 100 F1 × F1, 92 F1 × Wistar, and 97 F1 × WKY progeny. In the F1 at G19, G21, and A2, there were abnormalities of the pulmonary valve, pulmonary outflow tract, architecture of muscle bundle, and pulmonary arterial branch, as well as hypoplastic ductus arteriosus and postnatal cardiac hypertrophy, similarly in males and females but at a lower incidence and to a lesser extent than in the WKY rats. Severe pulmonary valve dysplasia and ventricular septal defect with overriding of the aorta (tetralogy of Fallot), usually associated with a markedly small ductus, were not present or were very rare in the F1 and the F1 × Wistar but were present in the F1 × WKY and in the F1 × F1 less prevalently than in the WKY. The size of the ductus showed a continuous distribution in all of the six groups; there was a large skewing toward lower values in the WKY, the F1 × WKY, and the F1 × F1. These results suggest that cardiovascular anomalies of WKY rats are transmitted as autosomal recessive or incomplete autosomal dominant traits with an incomplete penetrance and variable expressivity or as polygenic traits. Chromosomal analysis of 31 WKY fetuses revealed no abberations specifically related to the development of cardiovascular malformations. (*Pediatr Res* 30: 227–231, 1991)

Abbreviations

PV, pulmonary valve
PA, pulmonary artery
VSD, ventricular septal defect
F1, first generation hybrids
VFW, ventricular free wall
G, day of gestation
A, day after birth

In epidemiologic studies of congenital heart disease, genetic factors have been strongly suspected on the basis of familial

occurrence in humans (1–9) and animals (10–19). The aspects of genetic participation are chromosomal aberrations (1, 2, 19–22), single gene Mendelian inheritance (1, 2, 6–9), or polygenic inheritance in which multiple genes with equal and small effect act additively (10, 11). Environmental influences, such as specific teratogenic chemicals and viruses or as yet unidentified and unassessable factors, are also being considered in the etiology of congenital anomalies (1, 23). A prevailing hypothesis explains that multifactorial etiology with genetic-environmental interactions, be it linked with polygene or several major genes, is the ultimate cause in a vast majority of cases (1, 2, 23–25).

We have already described morphologic features of tetralogy of Fallot, PV dysplasia, VSD, abnormal muscle bundle architecture, cardiac hypertrophy, pulmonary hypertension, and anomalies of the great vessels, including hypoplasia of the ductus arteriosus spontaneously occurring in WKY/NCrj rats (15–18). In this study, we carried out mating experiments and chromosomal analysis with the rats. The use of these rats in genetic studies should be particularly beneficial, because the rat strain is an established inbred one maintained under conditions with no specific teratogenic exposure and with minimal variations of other environmental factors.

MATERIALS AND METHODS

Animals and matings. The WKY rats used for matings were produced by successive selective breeding based on electrocardiographic findings (16) in an air-conditioned room free of specific pathogens. The unrelated Wistar rats with normal cardiovascular systems (15–18) were purchased from Shimizu Jikken Zairyo Inc. (Kyoto, Japan); they were maintained by a random breeding in a closed colony under conventional conditions. Several weeks before matings and throughout pregnant intervals, all of the parents were exposed to the same laboratory environment by being housed, side by side, in suspended wire-bottom cages, with the same laboratory food and sterilized water available *ad libitum.* Pregnancy was confirmed by identifying sperm in the vaginal smear, and the next day was considered to be the 1st d of gestation.

First, we mated reciprocally four male WKY rats with four female Wistar rats and two female WKY rats with two male Wistar rats. All of the first litters (83 neonates) were sampled at A2, and the second litters (78 fetuses) were sampled at G21. The results in these F1 were compared with those from WKY and from Wistar rats at the same stage obtained in the previous study (18). We next examined six groups of fetuses at G19: 63 Wistar rats, 111 WKY rats, 85 F1, 100 F1 × F1, 92 F1 × Wistar, and 97 F1 × WKY progeny. The F1 × F1 progeny were produced

by sibling mating. Some of the Wistar and WKY rats that were mated with the F1 were not the parents of the F1.

For proper interpretation of the present experimental project and its results, it should be mentioned here that the WKY/NCrj strain of rats is an established inbred one and, therefore all of the rats are presumed to have a homozygous genotype and identical genes including, if any, abnormal ones. In every F1, therefore, all of the alleles including the allele of interest, derived from the WKY gametes of either sex, are considered to be present in heterozygous form and identical in every individual. Similarly, one fourth of the F1 × F1 progeny are considered homozygous and half of them are heterozygous for the allele of interest. Furthermore, half of the F1 × WKY progeny should be homozygous and half of them heterozygous for the allele, and half of the F1 × Wistar progeny should be heterozygous for the allele.

Methods of identifying phenotypes. The hearts, great vessels, and lungs were isolated in the same way as in the previous study (18). We then examined in the same way the cross-sectional area of the ductus arteriosus and the aortic isthmus, the appearance of cardiac valves, VSD, pulmonary outflow tract, architecture of the middle latitudinal muscle bundle, wall thickness/lumen radius ratio of the PA branch, and the width of the ventricular septum, left VFW, and right VFW.

Chromosomal analysis. Thirty-six WKY rat fetuses at G18–19 (three litters) and 13 normal Wistar rat fetuses were examined. With the mother rats under urethane anesthesia, the fetuses were rapidly removed from the uterus. The caudal ⅓ of the body was available for chromosomal analysis. The specimen was minced and cultured in RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with a 15% FCS for 3–5 d. Cells were treated with colcemid (Hyclone Laboratory Inc., Logan, UT) at a concentration of 0.08 μg/mL, then trypsinized, and further treated with 0.075 M KCl for 15 min and fixed in methanol-acetic acid solutions with a mixture ratio of 3:1. The cells at metaphase were spread by air-drying method and were G-banded with trypsin. They were then arranged according to the scheme and nomenclature for G-bands of rat chromosomes (26). The rostral ⅔ of the body was soon immersed in 10% formalin for anatomic examination of the cardiovascular system. Hypoplasia of the ductus was estimated qualitatively.

Statistical analysis. The data are expressed as mean ± SD. Each parameter was judged to be abnormal with reference to the mean ± 2 SD for the Wistar rats. Because data in males and females were similar in every group, and because various abnormalities occurred in the hybrid groups irrespective of WKY paternity or maternity, the data were analyzed together in every group. The incidence of abnormalities between the groups was compared by the χ^2 test.

RESULTS

F1 at G21 and A2. Severe PV dysplasia occurred only in one of the 78 fetuses and one of the 83 neonates of WKY rats; mild thickening of the PV was present in 27–28% of the F1 at both stages, and the incidence was significantly lower than in the WKY (Table 1). VSD was present in one of the F1 fetuses. The pulmonary outflow tract was narrow in 35 and 24% of the F1 at G21 and A2, respectively, about half the incidence in the WKY.

The wall thickness/lumen radius ratio of the PA branch in the F1 was abnormally low in 17% at G21 and in 15% at A2, the incidence being similar to that in the WKY; however, the range of the ratio was narrower in the F1 than in the WKY at both stages. The PA branch lesion in the F1 always occurred in the absence of VSD or severe PV dysplasia.

The abnormality of the septal middle latitudinal muscle bundle was present in the F1 fetuses and neonates (37 and 34%, respectively) less frequently than in the WKY (81 and 85%, respectively).

The heart wt/body wt of the F1 at A2 was abnormally high in 24%, about half the incidence in the WKY. A very high weight ratio greater than 7.0 was seen in only one of the F1 neonates, whereas it was noted in 16% of the WKY neonates. The mean ventricular septum/left VFW and right VFW/left VFW in the F1 were intermediate between the Wistar and the WKY at both G21 and A2, as was the incidence of abnormally high individual ratios.

The ductus arteriosus was abnormally small in 21% of the F1 fetuses, about half as prevalent as in the WKY (47%). However, the smallest size of the ductus was 28 μm in the F1 whereas it was smaller than this in 18% of the WKY, with the smallest one being 9 μm, The size of the ductus corrected by the size of the aortic isthmus, the ductus/aorta ratio, was abnormally low in more fetuses than was the uncorrected size in both the WKY (75 *versus* 47%) and the F1 fetuses (40 *versus* 21%). A double or right aortic arch was present in seven of the 133 WKY but in none of the 161 F1.

G19 fetuses. Severe PV dysplasia was seen only in the WKY (14%), the F1 × WKY (6%), and the F1 × F1 (3%), less prevalently in the latter two than in the WKY (Table 2). There was no VSD in the F1 and only one small VSD in the F1 × Wistar, but in 3% of the F1 × F1 and in 11% of the F1 × WKY, the incidence in the latter was about one third that in the WKY (35%).

The abnormality in size of the ductus and the aortic isthmus occurred at G19 to a somewhat lesser extent than at G21 in both the WKY and the F1. The mean and incidence of abnormal values of the ductus and the ductus/aorta ratio decreased in descending order from the WKY rats to the WKY × F1, F1 × F1 or F1, F1 × Wistar, and Wistar rats. The distribution of the ductus/aorta ratio was continuous in all six groups (Fig. 1); it was widely ranged because of a large skewing toward lower values in the WKY, the F1 × WKY, and the F1 × F1. This pattern was much the same when the ductus size was not corrected by the aorta. Extremely low ductus/aorta ratios (<1.0) were present almost exclusively in the WKY, the F1 × WKY, and the F1 × F1 fetuses, the incidence in the latter two (8 and 5%, respectively) being significantly lower than in the WKY (21%); VSD and/or severe PV dysplasia almost always occurred in association with these very small ductus in all of the groups.

Abnormal branching of the PA (18) occurred often in the F1 × WKY (29%) and the WKY fetuses (52%), but rarely in the F1 (8%), the F1 × F1 (10%), and the F1 × Wistar (2%). A double or right aortic arch was present in one of the F1 and one of the F1 × F1 and in two of the F1 × WKY. There was a retroesophageal right subclavian artery in one of the F1 × Wistar. One F1 × WKY fetus had a common atrioventricular canal in association with a double aortic arch and a small ductus.

Chromosomes. Of the 31 WKY fetuses in which the cell culture was successful, there was a VSD of various sizes in 12 and a mild or severe PV-dysplasia in 14, both occurring together in nine. There were small ductus in 17, double aortic arch in one, and abnormal PA branching in 13. A clonal chromosomal translocation, t(1;5)(p11;q13), was present in one WKY fetus, in which, however, there was no definite cardiovascular lesion. The remaining 30 WKY and 13 Wistar fetuses had normal karyotypes.

DISCUSSION

Environmental influences are not likely a major factor for the development of anomalies in the rats described here because all of the parents were housed under the same controlled conditions with very small individual variations. The F1 showed various abnormalities irrespective of maternity, suggesting that either intrauterine environment or cytoplasmic inheritance does not participate in the anomalous development. An independent paternal contribution to the development of tetralogy of Fallot and membranous VSD has been demonstrated in Keeshond dogs and Long-Evans rats (10, 13).

In humans, various congenital cardiac malformations occur frequently under the presence of chromosomal anomalies (65–

Table 1. *Data from Wistar and WKY rats and F1 at G21 and A2**

	G21 fetuses			A2 neonates		
	Wistar [28]	WKY [90]	F1 [78]	Wistar [23]	WKY [49]	F1 [83]
Body wt (g)	5.16 ± 0.52	4.81 ± 0.46	5.35 ± 0.59	8.36 ± 0.59	7.08 ± 0.97	8.44 ± 0.87
PV dysplasia		17% (30%)	1% (27%)†		13% (43%)	1% (28%)‡
VSD		18%	1%‡		10%	0%‡
POT§	144 ± 21	93 ± 31 / 55%	113 ± 24 / 35%†	225 ± 39	132 ± 45 / 53%	178 ± 37 / 24%†
WT/LR of PA branch	0.21 ± 0.02	0.23 ± 0.06 / 19%	0.23 ± 0.04 / 17%	0.13 ± 0.02	0.15 ± 0.04 / 26%	0.14 ± 0.03 / 15%
Abnormal MLB	1.5 ± 0.7	−0.8 ± 1.4 / <0, 81%	0.1 ± 1.0 / <0, 37%‡	5.1 ± 0.6	1.7 ± 1.3 / <3, 85%	3.0 ± 1.2 / <3, 34%‡
Heart wt/body wt (mg/g)	5.24 ± 0.39	5.09 ± 0.53 / 8%	5.23 ± 0.48 / 1%	5.78 ± 0.22	6.38 ± 0.89 / 51%	5.86 ± 0.48 / 24%‡
IVS/LVFW	1.37 ± 0.12	1.61 ± 0.17 / 45%	1.47 ± 0.15 / 25%†	1.07 ± 0.09	1.27 ± 0.18 / 53%	1.14 ± 0.12 / 20%†
RVFW/LVFW	0.90 ± 0.10	1.00 ± 0.10 / 20%	0.95 ± 0.09 / 10%	0.43 ± 0.05	0.69 ± 0.12 / 87%	0.53 ± 0.11 / 48%‡
Ductus§	41 ± 4	33 ± 7 / 47%	38 ± 5 / 21%‡			
Aorta§	23 ± 3	35 ± 13 / 51%	27 ± 5 / 29%‡	28 ± 3	30 ± 5 / 15%	29 ± 3 / 5%†
Ductus/Aorta	1.83 ± 0.21	1.11 ± 0.38 / 75%	1.47 ± 0.28 / 40%‡			

* Data are mean ± SD. Brackets, number of rats; %, percentage of rats with abnormalities; POT, pulmonary outflow tract; WT/LR, wall thickness/lumen radius ratio; MLB, middle latitudinal muscle bundle; IVS, ventricular septum; LVFW, left ventricular free wall; RVFW, right ventricular free wall.
† $p < 0.05$; significantly smaller than the percentage in WKY rats.
‡ $p < 0.01$; significantly smaller than the percentage in WKY rats.
§ ($\times 10^3 \mu m^2$) corrected by ⅔ power of body wt (g).

Table 2. *Data in G19 fetuses from various matings**

	Wistar [63]	WistarxF1 [92]	F1 [85]	F1xF1 [100]	F1xWKY [97]	WKY [111]
PV dysplasia	0%	0% (5%)	0% (15%)	3% (16%)	6% (22%)	14% (37%)†
VSD	0%	1%	0%	3%	11%†	35%†
Ductus‡	48.6 ± 4.6	45.9 ± 3.7 / 5%	44.4 ± 4.0 / 15%	44.7 ± 5.4 / 16%	43.3 ± 5.8 / 22%	41.2 ± 7.8 / 36%§
Aorta‡	28.7 ± 3.4	29.8 ± 3.7 / 5%	31.5 ± 4.4 / 19%§	31.6 ± 5.6 / 18%	33.1 ± 5.9 / 27%	37.4 ± 10.6 / 40%
Ductus/Aorta	1.69 ± 0.18	1.55 ± 0.18 / 12%§	1.42 ± 0.17 / 26%§	1.44 ± 0.26 / 30%	1.34 ± 0.25 / 42%	1.20 ± 0.37 / 59%§
Abnormal PA branching	0%	2%	8%	10%	29%†	52%†
Double or right aortic arch			1	1	2	
Others		1‖			1¶	

* Data, mean ± SD. Brackets, number of rats; %, percentage of rats with abnormalities; Parentheses, thickening of the cusps with no marked distortion.
† $p < 0.01$; for comparison with just the left column.
‡ ($\times 10^3 \mu m^2$) corrected by ⅔ power of body wt (g).
§ $p < 0.05$; for comparison with just the left column.
‖ Retroesophageal right subclavian artery.
¶ Common atrioventricular canal.

99%), but the association of chromosomal anomalies with cardiac malformations is relatively low (2–5%) (1, 2, 20–22). Our present results suggest that chromosomal abnormality is not related to the development of cardiovascular malformations in WKY rats.

The cardiovascular anomalies in WKY rats did or did not occur in various forms and degrees of severity in the present and previous studies (16–18). This may suggest that there are different alleles of interest, each of which is responsible for each diverse lesion. However, they occurred, isolated or in association, irrespective of the type and severity of abnormalities of parental rats. Therefore, it seems reasonable to explain this phenomenon by referring to the concept "penetrance and expressivity"; once a gene is penetrant, it will frequently exhibit a variable expressivity (27). The concept is thought to be due to two factors: genetic background and environmental influence (27). If so, however, the question still remains as to what determines the unstable expression of various anomalies in WKY rats because the two factors should be most minimal owing to their genetic homogeneity and the identical environment. Also, in an inbred strain of the mouse, various skeletal anomalies occurred independent of the traits of the parental mice (28, 29).

Severe PV dysplasia and VSD, being not expressed or rarely expressed in the F1 but being expressed in the WKY, the F1 ×
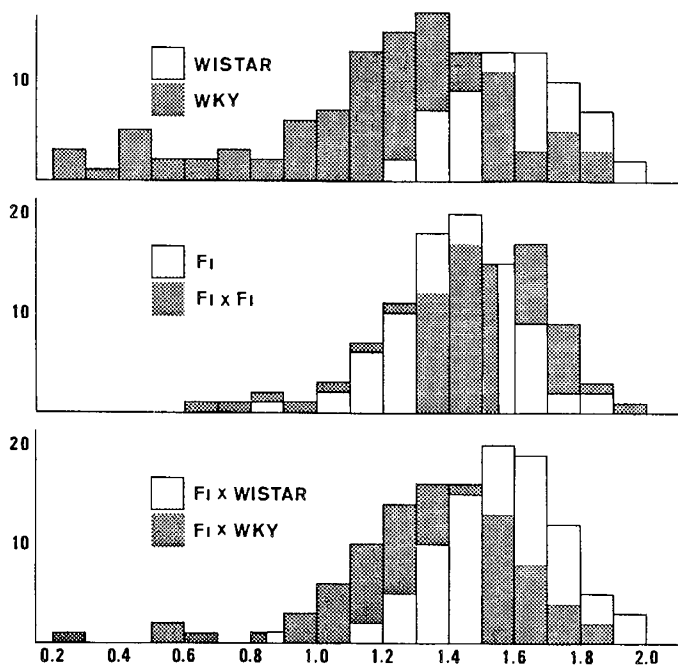
Fig. 1. Histograms showing the distribution of the ductus/aorta ratio of cross-sectional area in various mating groups. *Horizontal scale,* ductus/aorta ratio. *Vertical scale,* number of rats. The distribution in WKY, F1 × F1, and F1 × WKY (*dotted columns*) is wide because of a marked skewing toward lower values, not depicting a normal curve. The other three groups show almost symmetric normal curves.

WKY, and the F1 × F1, are considered to be autosomal recessive traits. The phenotypic expression of other lesions in the F1, including hypoplasia of the ductus, is consistent with autosomal dominant inheritance; in this case also, incomplete penetrance and variable expressivity seem to explain the much lower incidence of the lesions than expected (100%) from the genotype of the F1. Furthermore, the incidence and severity of most traits were significantly lower in the F1 than in the WKY rats. This difference may be explained by "incomplete dominance"; the presence of an allele in a heterozygote does not necessarily define the phenotype, depending on the nature of the allele at the paired locus (27). This phenomenon is also seen in other congenital anomalies (28-30).

In our previous study (18), we suggested that various cardiovascular lesions could occur secondarily to the hemodynamic effects exerted by an increase in flow impedance through a hypoplastic ductus. A heart lesion that apparently bears no developmental relationship to another cardiac defect may be a manifestation of a common mechanism of maldevelopment due to a core lesion (2). This idea leads us to the assumption that a threshold for each abnormality is present along a continuous scale of the ductus size and the threshold, each varying according to the corresponding lesion. Thus, the threshold for a lesion with low incidence such as VSD or severe PV dysplasia should be high.

The expression of a trait that is characterized by a quantifiability and a continuous distribution of values with a normal curve, such as length or color, is explained by polygenic inheritance; the greater the accumulation of the genes, the greater becomes the expression of the trait (10, 11, 31). This theory may also explain the continuous distribution of the ductus size in the WKY rats. There are, however, some difficulties. The distribution of the ductus size ranged most widely in the WKY although the genes of interest are considered to have the greatest accumulation and homogeneity in the WKY rats. When highly inbred lines continue to exhibit variation in a polygenic trait, the variation is attributed to environmental rather than genetic causes (31). Thus, the widest distribution of the ductus size in the WKY

should imply that hypoplasia of the ductus cannot be explained simply as a polygenic trait. A similar distribution of various skeletal anomalies in an inbred mouse strain has been explained by multifactorial inheritance with environmental influences (28, 29).

The present results are not fully concordant with either single gene Mendelian inheritance or polygenic inheritance, as far as the known traits are concerned, without the invoking of a strong participation of highly variable environmental influences. It has been stated in the theory of multifactorial inheritance that a major maldevelopment can be produced by a minor environmental stimulus in the early stage of development (1, 2, 23, 24), although we have no clue as to its identification at present. Furthermore, we know little yet about the cardinal defect that, as the primary expression of the genetic defect, underlies the various traits. Even hypoplasia of the ductus must be an expression of remote gene effects rather than the primary one (29, 32). The genetic and environmental problems in the WKY rats thus should be left open until the underlying biochemical process or the presence of causal molecular defects is ultimately ascertained (32).

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Announcement

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The Society for Adolescent Medicine will hold its annual meeting from March 19 to March 22, 1992 in Washington, DC at the Omni Shoreham Hotel. The theme for this meeting will be "Adolescent Health Policy and Advocacy." In addition to addressing this topic, the Society will present new material on a broad range of issues important to adolescent physical and emotional health, including AIDS, teenage sexuality and pregnancy, eating disorders, depression, and risk-taking behaviors such as drug and alcohol use. Meeting presentations include all-day workshops, 3-hour clinically oriented workshops, research networking breakfasts and luncheon seminars, scientific research paper presentations, and poster sessions, as well as the prestigious Gallagher Lecture Series. CME/CEUs are available. *For additional information, contact*: the Society for Adolescent Medicine, Suite 120, 19401 E. U.S. Highway 40, Independence, MO 64055 or call (816) 795-8336.