

VISCERAL INVERSION AND ASSOCIATED ANOMALIES IN THE MOUSE

KATHARINE P. HUMMEL AND DOROTHY B. CHAPMAN*

SITUS inversus viscerum has been described rarely in mice, and reports in the literature are limited almost exclusively to cases noted at autopsy⁶. Among 1,817 mice autopsied for visceral anomalies, Bagg¹ found only one with reversed viscera. Strong¹¹ found one among descendants of methylcholanthrene-treated mice and remarked that over a period of 25 years, he had observed only one other in a colony of 210,000 mice. Hummel⁷ reported finding seven among 350 adults of the BALB/c strain autopsied, and none among mice of eight other inbred strains. Tihen, Charles and Sipple¹² found none in examining more than 11,500 hybrids of a cross between mice of strains C57BL and DBA, but in a stock constituted by crossing these two strains with the BALB/c and Swiss, they found animals in which the condition was inherited as an autosomal recessive with incomplete penetrance. In addition to left-right transposition of viscera and blood vessels, manifestations included abnormalities in spleen shape, lung lobation, and position of postcaval and hepatic portal veins. Mortality was high in affected animals; they were small, sickly-looking, and in many cases appeared to be hydrocephalic.

Since 1948, one more case of situs inversus viscerum has been found in a mouse of the BALB/c strain in our colony. The animal, a male identified as transposed at birth, was raised and bred, and many descendants, including backcrosses, were examined. Neither situs inversus nor other abnormalities were found and it was concluded that in this case, the condition was not genetic. At about the same time, two newborn mice

with stomachs on the right, rather than the left, were noticed in a litter of 12, born to a pair (brother and sister) of mice in a non-inbred stock homozygous for myelencephalic blebs (*mymy*). This pair of mice had several more litters; in a total of 43 offspring examined, five with transposed viscera were found. At autopsy, the parents were found to be normal with respect to situs inversus. Three of the five survived, were bred and produced healthy offspring with transposed viscera and abnormalities of blood vessels, spleen, liver and lungs. Studies have shown that this syndrome of visceral inversion and accompanying abnormalities is inherited as a single autosomal recessive gene (symbol *iv*) with incomplete penetrance. Thus in its inheritance and expression, this mutation is similar to that described by Tihen and associates, but as their mutation is believed to be extinct, its identity with *iv* can not be tested.

Mice with reversed viscera can be identified as soon after birth as milk has been ingested and until they are four to five days old, after which time, the stomachs can not be seen readily through the thickened skin. Using stomach and also spleen position, mice were classified neonatally for visceral inversions; for the observation of other abnormalities not involving stomach position, autopsies were necessary.

Several types of matings were made. Mice with transposed viscera were mated together and with normal animals of strains DBA, BALB/c and C3H. The F₁ hybrids were mated together and backcrossed to "reversed" animals. In this way, a stock of mice homozygous for situs inversus was established. At

* Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This investigation was supported in part by a research grant RG 4636 from the National Institutes of Health, Public Health Service.

TABLE I. Classification by phenotype of mice homozygous for *iv*

	1		2		3		4		5		6		13		Total unreversed	
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
♀	82	31	8	0.7	2	2	26	10	5	2	10	4	6	2	139	53
♂	63	26	4	1	3	8	20	8	2	0.8	19	8	2	0.8	113	46
Total	145	29	12	0.9	5	9	46	9	7	1	29	6	8	1.5	252	50
♀	66	25	10	4	3	9	21	8	5	2	18	7	1	0.3	124	47
♂	84	34	5	2	3	3	10	4	3	1	22	9	4	2	131	54
Total	150	30	15	3	6	6	31	6	8	1	40	8	5	0.9	255	50
♀	148	56	18	7	5	3+9	47	18	10	4	28	11	7	3	263	52
♂	147	60	9	4	6	6	30	12	5	2	41	17	6	2	244	48
Total	295	58	27	5	11	11	77	15	15	3	69	14	13	3	507	—

the same time, there was selection against mice homozygous for myelencephalic blebs. Offspring of all matings were examined at birth, and were autopsied either neonatally, at weaning, or after their periods of reproductive usefulness. They were then classified according to the pattern of anomalies found.

Morphology

Asymmetrical structures observed to be anomalous in position or shape in the *situs inversus* mice are listed, and the normal as well as abnormal conditions described briefly.

Abdominal viscera

Liver: divided into median, caudate, and right and left lateral lobes. The median lobe is deeply notched and contains the gall bladder; the caudate lobe surrounds the oesophagus in the lesser curvature of the stomach; and the right lateral lobe is subdivided into anterior and posterior portions. The more caudal or posterior portion of the right lateral lobe lies medial to the anterior pole of the right kidney and receives the renal segment of the inferior vena cava.

Stomach: lies in the anterior left quadrant of the abdominal cavity with the forestomach on the left and the pylorus on the right.

Spleen: a flat, elongated oval body lying along the greater curvature of the stomach.

Kidneys: the left is posterior (caudal) to the right.

Thoracic viscera

Lungs: divided into five lobes, one from the left bronchus and four from the right, one of the latter being centrally located.

Heart: apex is on left, formed by the more muscular systemic ventricle; larger right atrium receives both of the superior venae cavae, as well as the hepatic segment of the inferior vena cava.

Veins

Superior venae cavae: right and left; the left is longer and passes dorsal to the base of the heart to reach the right atrium.

Azygos: located to the left of the aorta and vertebrae against the dorsal wall of the thoracic cavity; receives branches from both right and left intercostal spaces and joins the left superior vena cava. A hemiazygos vein, if present, collects from the right posterior intercostal spaces and joins the azygos in the 9th or 10th intercostal space.

Inferior vena cava: a single vessel lying to the right and slightly ventral to the aorta in the lumbar region. After receiving the left and right renal veins, it enters the posterior division of the right lateral lobe of the liver. It receives hepatic veins in the liver, emerges from the median lobe, pierces the diaphragm and as the hepatic segment traverses the thoracic cavity to enter the right atrium together

with the left superior vena cava.

Hepatic portal: formed by union of veins from the intestine, pancreas and spleen, it passes dorsal to the duodenum to enter the median lobe of the liver close to the gall bladder.

In mice with the genotype *iziv*, all of these structures were found transposed right to left in a mirror image. Both complete and partial reversals were found, and in addition, other anomalies were observed. In the liver, all or part of the caudate lobe might be missing, and the posterior subdivision of the right lateral lobe reduced or missing. In the latter case, the renal segment of the inferior vena cava did not enter the liver but continued by way of the azygos vein and superior vena cava to the right atrium, and the hepatic segment was attenuated.

Since the azygos vein and the inferior vena cava normally develop on opposite sides of the body, one or other must be in abnormal position for them to be continuous. Two situations were observed. The inferior caval and azygos veins were continuous on the right side as is normal for the inferior vena cava, or continuous on the left, the normal position of the azygos vein.

In the hepatic portal anomaly, the vein passed ventral to the duodenum on its way to the median lobe of the liver. Lung lobation varied from 1 lobe each side to 4 each side in various combinations which will not be listed in detail. The spleen varied in shape, and position, being thickened, shortened, constricted in the middle, dorsal to the stomach, or extending across the body.

As a rule, all of the viscera were transposed together, although in a few cases, abdominal and thoracic viscera were not concordant. Most of the animals fell into 12 categories with regard to transpositions of viscera and blood vessel patterns. In the first 6 of these, the abdominal viscera were not reversed, and the animals would have been marked as normal soon after birth. Animals of groups 7 through 12 had reversed stomachs which would have served to identify them at birth. Groups 13 and 14 included the few cases where the transpositions and blood vessels did not fall into specific patterns. The 14 categories used to classify mice of the *iziv* genotype are listed.

Viscera not reversed

1. Normal relationship, position and shape of all asymmetrical structures.

2. Like 1, but with the hepatic portal anomaly.
3. Inferior vena cava on left (abnormal), joining left azygos vein (normal).
4. Like 3, but with the hepatic portal anomaly.
5. Inferior vena cava on right (normal), joining right azygos vein (abnormal).
6. Like 5, but with hepatic portal anomaly.
13. Blood vessels in miscellaneous abnormal patterns.

Viscera reversed

7. Mirror image of 1. (complete "normal reversal").
8. Mirror image of 2; like 7, but with hepatic portal anomaly.
9. Mirror image of 3.
10. Mirror image of 4; like 9, but with hepatic portal anomaly.
11. Mirror image of 5.
12. Mirror image of 6; like 11, but with hepatic portal anomaly.
14. Blood vessels in miscellaneous abnormal patterns.

Abnormal lung and liver lobations, and abnormal spleen shapes and positions occurred among mice of all groups except 1 and 7.

In all, 507 mice born to parents with transposed viscera were autopsied and classified. Table I summarizes the observations for both males and females. From these data, it is apparent that only 50 percent of the mice would have been classified as *situs inversus* by observation of stomach position; autopsy showed that actually 71.4 percent of the population was affected. Only 145 of the 252 mice classified as "unreversed" proved on autopsy to be entirely normal in respect to *situs inversus* and associated anomalies. These 145 mice (group 1) were the normal overlaps and represented about one half of the "unreversed" animals and 28.6 percent of the *iziv* mice classified. A like proportion of the 255 mice with reversed viscera were mirror images of normal. These 150 mice (group 7), designated as complete "normal reversals," made up 29.6 percent of the observed population and 58.8 percent of the "reversed" animals.

The largest group (41.8 percent) was composed of the 212 mice of groups 2 through 6 and 8 through 14, with partial transpositions and blood vessel anomalies. In 172 or 81 percent of these 212 animals, the inferior vena cava by-passed the liver and joined the azygos; in 173 or 82 percent the hepatic portal

TABLE II. Incidences of abnormalities among mice homozygous for *iv*

Abnormality	No. Observed			Abnormal			Total		
	♀	♂	Total	♀	♂	Total			
Transpositions	263	244	507	181	68.8%	181	72.2%	362	71.4%
Hepatic portal anomaly	263	244	507	93	35.4%	80	32.7%	173	34.1%
Postcava-azygos anomaly	263	244	507	90	34.2%	82	33.6%	172	33.9%
Lung lobation	270	247	526	56	20.7%	50	19.5%	106	20.1%
Spleen shape or position	272	247	519	78	28.6%	73	29.5%	151	29.0%

anomaly was present; and 146 mice or 69 percent had both of these anomalous patterns. Very few animals, on the other hand, had the inferior vena cava anomaly without the hepatic portal anomaly (26 mice or 12.6 percent) or the hepatic portal anomaly without the inferior vena cava anomaly (27 mice or 12.7 percent).

It is also noteworthy that among the 172 mice in which the azygos vein and superior vena cava served to return blood from the posterior part of the body to the heart, the azygos vein had developed in normal relationship to the aorta in approximately one half of the cases (88), and the inferior vena cava had developed normally in approximately one half (84).

Although it might appear that there were more "normal reversal" males than females, the difference is not significant ($P = 0.2-0.1$); in fact there were no differences in any of the groups in regard to sex.

Different numbers of mice were observed for abnormal spleens and abnormal lung lobations, as it was sometimes impossible to determine the course of blood vessels or shapes of spleens in animals which were found dead. In 526 offspring of animals with visceral inversion, 106 or 20 percent had abnormal patterns of lung lobation; in 519, 151 or 29 percent had spleens, abnormal in shape or in position relative to the greater curvature of the stomach. In neither group were there differences in incidence between males and females. These observations are summarized in Table II, and compared with incidences of visceral transposition and anomalous blood vessel pattern.

Inheritance

Observations on the penetrance of situs inversus in the different types of crosses are summarized in Table III. No mice with abnormalities were found in hybrids produced by crossing "reversed" animals (*iviv*) with normal animals (*++*) of the three inbred strains, BALB/c, DBA and C3H_o. When the F_1 hybrids were mated together ($iv^+ \times iv^+$), less than half of the expected number of abnormal forms were found in the F_2 generation, even after careful examination at autopsy. Although penetrance was higher in backcrosses ($iv^+ \times iviv$) and in intercrosses ($iviv \times iviv$), it was still reduced. Normal phenotypes, derived from intercrosses between affected animals, when mated to "reversed"

animals gave the same proportion of abnormal offspring as did matings between "reversed" animals, indicating that the phenotypically normal animals were indeed normal overlaps with the *iviv* genotype.

From these data, it was concluded that the visceral inversions and associated abnormalities are inherited as a single autosomal recessive character with incomplete penetrance.

Discussion

Whereas the situs inversus mice described by Tihen *et al.*¹² were small and sickly with swollen craniums and soft skulls, ours were healthy and were usually fair breeders. Some selection for fertility was necessary; among 177 males and 321 females placed in breeding pens, 39 and 66 respectively (21 percent) were discarded for infertility after suitable trial periods. No reason for this was apparent at autopsy.

Malformations of the heart, aorta and pulmonary arteries, such as those associated with situs inversus in man^{5, 13} and those produced experimentally in rats by subjecting pregnant females to teratogenic agents^{4, 14} have not been observed in our situs inversus mice. The chambers of the heart, associated vessels and lungs were always transposed together so that normal relationships were maintained. Whether the azygos vein entered the left or right superior vena cava appeared to be a matter of which embryonic vessel persisted and not of malformation of the heart.

Visceral inversion has been more extensively studied in man than in any other animal. It is rather generally agreed that the condition is not closely related to twinning although of all monozygous pairs, conjoined twins exhibit the most reversed asymmetry^{5, 10, 13}. There is considerable evidence from studies of inheritance that situs inversus in man is due to a single recessive gene. However, it is so often associated with other abnormalities such as bronchiectasis, nasal polyps, and malformations of the heart, skeletal parts and other entities that it has been suggested that the inversion in some cases is a secondary rather than a primary gene effect. Torgersen¹³ in a study of 168 cases concluded that, "the ratio of affected to normal persons does not accord with the supposition that a single recessive gene is always the cause of situs inversus."

Union between inferior vena cava and azygos vein has been observed in other mammals. Cooper³ in describing this anomaly in

TABLE III. The inheritance of situs inversus viscerum

Cross	No.	Abnormal			
		Observed	Expected	Penetrance %	
$iv^+ \times iv^+$	509	53 10.4%	127 25%	41.7	
$iv^+ \times iviv$	29	9 31.0%	14.5 50%	62.1	
$iviv \times iviv$	606	429 70.8%	606 100%	70.8	
normal \times "reversed"	99	67 67.7%	99 100%	67.7	
"reversed" \times "reversed"	507	362 71.4%	507 100%	71.4	

the dog, cited 30 reported cases in man as well as occurrences in the guinea pig, cat, and deer. He believed that the postcaval-azygos continuation probably represented persistence of the right embryonic supracardinal vein throughout its length. In man, cat and many other mammals, the embryonic supracardinal and subcardinal veins are important in the development of the adult inferior vena cava and azygos vein⁸. In the adult rat, on the other hand, the supra- and subcardinals play a lesser role and it is the right embryonic postcardinal vein that persists as the inferior vena cava caudal to the diaphragm (Butler²). The azygos vein of the rat and mouse differs from that of the cat, dog, rabbit and man in position, being on the left side and entering the left superior vena cava; description of its derivation is not included in Butler's study. Assuming that mouse and rat development are similar, the inferior vena cava-azygos junction could represent either persistence of the postcardinal-supracardinal anastomoses in the region of the diaphragm or the persistence of the embryonic postcardinal vein over its entire length⁹.

The frequent association between abnormality in liver lobation, inferior vena cava bypass of the liver, and ventral position of the hepatic portal vein can not be accidental, and is indicative of a common cause. This has an important bearing on a fundamental problem in vertebrate development, the cause of normal asymmetry of unpaired organs and blood vessels. It seems hopeful that the embryonic processes important in establishing asymmetries can be defined through studies of development of these mice in which reversed asymmetry occurs as the result of gene action.

Summary

Situs inversus viscerum, inherited as a single recessive character with incomplete penetrance has been described for the second time in mice. Expression includes left-right transposition of thoracic and abdominal viscera and associated blood vessels, anomalous relationship of postcaval and azygos veins, anomalous position of the hepatic portal vein,

abnormalities in spleen position and shape, and in liver and lung lobation.

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