

Random determination of a developmental process

Reversal of normal visceral asymmetry in the mouse

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MIRROR IMAGE reversal of normal visceral asymmetry is unusual in vertebrates. In man it is quite rare, occurring in about 1:10,000 adults, and although it appears to be genetically determined, the exact mode of inheritance is not clear. The only experimental genetic studies have been with mutant

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mice. The first was reported by Tihen, et al.¹⁴ and the results suggested an autosomal recessive gene (symbol *v*) with 50 percent penetrance. Homozygous animals were sickly, most died at a few weeks of age, and the mutation died out. In 1959 a similar mutation was recovered from a noninbred stock of mice by Hummel and Chapman⁵, who assigned the gene symbol *iv*. Homozygous (*iv/iv*) animals were viable but only 50 percent expressed *situs inversus*. In matings between homozygotes, the situs of the offspring was independent of parental situs. In 42 percent of homozygotes there was discordance in the situs of some of the large veins of the thorax or abdomen with that of the rest of the body. This heterotaxia occurred independently of *situs inversus* in seven pairs of patterns (Table I). Heterozygotes (*+/iv*) were anatomically normal.

Table I. Patterns of malformations in mice homozygous for *iv*; data of Hummel and Chapman⁵ compared with present study

	Hummel and Chapman* 507 Mice		Present study* 441 Mice	
	<i>situs solitus</i> %	<i>situs inversus</i> %	<i>situs solitus</i> %	<i>situs inversus</i> %
Normal relationship, position, and shape of all asymmetrical structures	28.6	29.6	37.9	36.3
Anomaly of hepatic portal vein	2.4	3.0	1.8	2.0
Discordant inferior vena cava joining concordant azygous vein	1.0	1.2	2.0	3.4
Discordant inferior vena cava joining concordant azygous vein and anomaly of hepatic portal vein	9.1	6.1	3.6	4.5
Discordant azygous vein joining concordant inferior vena cava	1.4	1.6	0.9	2.0
Discordant azygous vein joining concordant inferior vena cava and anomaly of hepatic portal vein	5.7	7.9	2.0	2.7
Blood vessels in miscellaneous abnormal patterns	1.6	1.0	0.2	0.9
Total	49.7	50.3	48.5	51.5

* The data of Hummel and Chapman⁵ are derived from autopsies of offspring of matings in which both parents had *situs inversus*. The pattern of matings in the present study is given in Table II. Here, all mice that were products of these matings were autopsied, either neonatally, at weaning, or when no longer needed for reproduction. In four autopsied homozygotes the situs of the major thoracic organs was discordant with those of the abdomen and data from these mice are not included above. In all other cases there was concordance in situs of major organs so that situs could be unequivocally assigned. All other data are included, whether from autopsies of mice derived from matings between the 10 original *iv/iv* mice and their descendants without outcrossing, or from homozygotes obtained after outcrossing with CD-1 mice

Materials and Methods

A colony of *iv/iv* mice was established at Dartmouth in 1971 from 10 homozygotes supplied by Dr. Hummel. The mice were then in the 32nd generation of breeding in a noninbred stock. The mutation has been propagated both through an additional nine generations with little conscious inbreeding, and from homozygotes obtained after outcrossing to a vigorous stock of noninbred mice (Charles River, CD-1). All *iv/iv* pups were examined within 24 hours of birth and classified as having either *situs solitus* (normal asymmetry) or *situs inversus* (reversed asymmetry) based on the location of the milk-filled stomach seen through the translucent abdominal wall. Since there was some maternal cannibalism not all pups born were examined. When no longer needed for breeding studies all *iv/iv* mice were autopsied and the situation of the abdominal and thoracic viscera was classified according to the scheme used by Hummel and Chapman⁵. Because half of the *iv/iv* mice show *situs inversus*, living animals could be identified as homozygotes only if both parents were homozygous, or if the parents were heterozygous, only if the offspring showed *situs inversus*. Some mice with *situs solitus* from heterozygous parents were found at autopsy to have heterotaxia and thus be homozygous; however, such animals were not used for the breeding studies described here. Also, 10-day *iv/iv* embryos were examined. At this stage the mouse embryo shows an external asymmetry since it is helical in shape¹³. These *iv/iv* embryos were compared to *+/+* (CD-1) 10-day embryos.

Results

The earlier findings of Hummel and Chapman were corroborated (Table I). After more than 15 years the incidence of *situs inversus* in *iv/iv* homozygotes was still 50 percent, although heterotaxia decreased in incidence from 42 percent to 26 percent. This decrease in heterotaxia is statistically significant ($P < 0.001$, $\chi^2 = 26.6$). The heterotaxia still occurred independently of *situs inversus* and in the same patterns. Introduction of the *iv* gene into the CD-1 stock did not change the incidence of phenotypic *situs inversus*. The visceral situs of offspring was independent of that of their parents (Table II).

Examination of 10-day *iv/iv* embryos showed that 91 of 173 (53 percent) were in the shape of left handed helices (Figure 1). Examination of 127 *+/+* embryos showed only four to be in the shape of a left handed helix. None of the *iv/iv* embryos showed evidence of monozygotic (and thus monozygotic) twinning.

Discussion

These data are unusual in two respects: 1) the incidence of *situs inversus* in homozygotes (*iv/iv*) is 50 percent and resistant to selection and a change in genetic background sufficient to significantly reduce the incidence of heterotaxia; and 2) homozygotes show a variable incidence of heterotaxia that occurs in equal frequency in animals with *situs solitus* and *situs inversus*. No satisfactory model or hypothesis has been suggested that

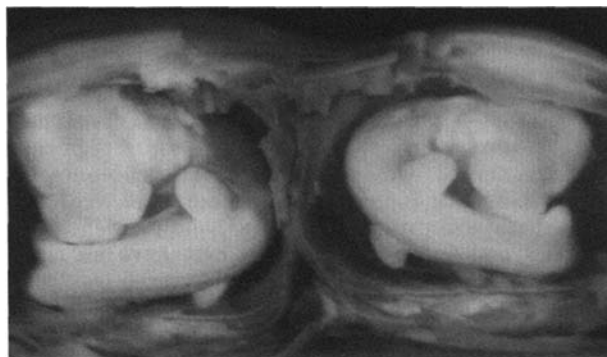


FIGURE 1—Embryos of 10-day *iv/iv* mice in utero. Note that the embryo on the left is in the shape of a right-handed helix and the one on the right in the shape of a left-handed helix.

will account for these peculiarities. The possibility that the equal number of normal and reversed mice is the consequence of mirror imaging of identical twins or of some sort of selective intrauterine death is ruled out by studies of 10-day embryos. It is proposed that the normal allele at the *iv* locus specifies *situs solitus*, i.e., normal laterality of visceral situs. Absence of this control allows situs to be determined in a totally random fashion. The heterotaxia is a consequence of this lack of developmental control. Thus, phenotypically, *situs inversus* is to be expected in only 50 percent of individuals homozygous for the *iv* gene.

This hypothesis may apply to other vertebrates. In man *situs inversus* has been reported to be inherited as an autosomal recessive trait^{3,4}, but this has been disputed because of a deficiency of *situs inversus* among sibs of index cases in family studies and a high rate of discordance of visceral situs in some monozygotic twins^{6,15}. The present hypothesis reconciles these apparent departures from autosomal recessive inheritance. Only half the individuals homozygous for the *situs inversus* gene should actually exhibit *situs inversus*.

Table II. Lack of correlation of visceral situs between parents and offspring of *iv/iv* mice

Visceral situs of parents	Litters N	Visceral situs of offspring*	
		Solitus N	Inversus N %
♀ ♂			
Solitus × solitus	15	29	35 55
Solitus × inversus	34	77	68 47
Inversus × solitus	14	26	35 57
Inversus × inversus	42	93	96 51
Total	105	225	234 51

* These data include 441 mice listed in Table I plus 18 of their littermates still alive in which visceral situs was determined by the location of the milk-filled stomach in the intact newborn pup.

This is consistent with the segregation ratios reported in some family studies^{3,6}. Only one-third concordance in monozygotic twins is to be expected since of all monozygotic twins homozygous for *situs inversus* one-quarter will be concordant for *situs inversus*, one-half will be discordant, and one-quarter will be phenotypically normal with *situs solitus* and hence not be detected. In one reported series there were five concordant and eight discordant pairs¹, and the deficiency of cases among sibs of propositi may have an additional explanation: since there is an increased incidence of congenital malformations of the heart^{2,7} in individuals with *situs inversus*, the probability of survival and thus ascertainment is decreased, particularly in surveys of adult populations. These heart malformations are probably the consequence of heterotaxia; however, it should be kept in mind that some individuals homozygous for the *situs inversus* gene will have heterotaxia but *situs solitus*. While there may be other modes of inheritance of *situs inversus* in man¹⁰, it is postulated that in all cases the production of this malformation involves the loss of control of the determination of situs.

This hypothesis may also apply to experimental or nongenetic production of *situs inversus*. In amphibia, particularly urodeles, *situs inversus* can be induced by subjecting the early embryo to a wide variety of procedures ranging from surgical manipulation to injection of tissue homogenates^{8,11,17,18}. A search of the literature has shown that regardless of the method used, it has not been possible to produce an incidence of *situs inversus* significantly greater than 50 percent. Wehrmaker¹⁷ concurs in this. In addition, the procedures that can result in *situs inversus* frequently produce stages intermediate between *situs inversus* and *situs solitus*, i.e., heterotaxia. These results can be interpreted to show that these various forms of experimental manipulation produce *situs inversus* indirectly by interfering to a variable extent with the normal developmental genetic mechanism that determines *situs solitus*.

Also, when conjoined twins are produced by constriction of the early embryo with a hair-loop, the right twin often (but never more than 50 percent of the time) shows *situs inversus* but the left one rarely does^{12,18}. This observation led Wilhelmi¹⁸ in 1921 to suggest that the left twin had something that the right one lacked, and that in the absence of this substance, visceral situs could be either *solitus* or *inversus*. That proposition is similar to the hypothesis presented here.

The procedures that have been reported to produce *situs inversus* in laboratory mammals (x-irradiation¹⁹ or trypan blue treatment¹⁶ of pregnant rats and retinoic acid treatment of pregnant hamsters⁹) result in a very low incidence of this anomaly (much less than 50 percent) and, as in amphibia, it appears that treatment interferes with the developmental determination of normal situs.

The hypothesis presented here can be tested since it predicts that it should be impossible to establish by selection a line of *iv/iv* mice having an incidence of *situs inversus* greater than 50 percent.

Summary

Situs inversus viscerum in the mouse has been shown to be inherited as an autosomal recessive trait (gene symbol *iv*) with reduced penetrance. It is hypothesized that the normal allele at the *iv* locus exhibits complete dominance and controls normal visceral asymmetry. Absence of this control allows the situs of visceral asymmetry to be determined in a random fashion. This hypothesis also appears to apply to the inheritance of *situs inversus* in man and to the experimental production of *situs inversus*.

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