Expression of the IV (Reversed and/or Heterotaxic) Phenotype in SWV Mice

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ABSTRACT 
Approximately 50% of iv/iv mice have situs inversus (mirror image reversal of viscera) and 40% have heterotaxia (anomalous arrangement of viscera). The occurrence of heterotaxia is independent of situs. Using the cross-intercross breeding system to put the iv gene on the SWV background, an occasional presumed ivl mouse was found that had an IV (situs inversus and/or heterotaxic) phenotype. Testcrosses of these reversed animals indicated an ivl genotype. Since iv is linked tightly to IgH-C on chromosome 12, we inferred the genotype with a polymorphism of IgH-C demonstrated using the polymerase chain reaction (PCR). This confirmed them to be ivl+. The expression of the IV phenotype in animals heterozygous for the iv gene may be due to an interaction of iv with an autosomal recessive gene found in SWV. We have not found the IV phenotype in heterozygous ivl+ mice following placement of the iv gene on six other inbred strains. Rarely, we also found that presumed SWV +/+ mice had the IV phenotype. Test matings of these phenodeviants, corroborated by PCR, have confirmed them to be +/+ . Although the phenotypes of the affected SWV +/+ and ivl+ mice resembled those found in iv/iv mice, the occurrence of situs inversus and heterotaxia were not independent of each other, and most of the SWV mice with the IV phenotype had heterotaxia with situs solitus.

This infrequent dominant expression of the iv gene has so far only been seen when iv is on the SWV background. These findings are consistent with the idea that this phenomenon is due to the interaction of the iv gene with another gene found so far only in the SWV strain. © 1993 Wiley-Liss, Inc.

The autosomal recessive gene iv (Hummel and Chapman, '59), which maps to chromosome 12 (Brueckner et al., '89), causes situs inversus (mirror image reversal of placement of organs) in mice. One half of iv/iv mice have situs inversus and approximately 40% have some sort of heterotaxia, an abnormal arrangement of organs in relation to each other. Heterotaxia occurs independently of visceral situs in iv/iv mice, so that the frequency of heterotaxia in mice with situs inversus is equal to its frequency in those with situs solitus (normal placement of organs). In mouse strains examined previously, ivl+ heterozygotes have demonstrated normal laterality indicating that the iv gene is a loss-of-function allele: in the absence of iv gene activity in the iv/iv mouse, laterality determination does not occur resulting in the random determination of visceral situs (Layton, '76; Kurnit et al., '87).

Heterotaxia in the context used here can be considered as residual situs inversus in a mouse with situs solitus, or residual situs solitus in one with situs inversus. Occasionally, the heterotaxia is so marked that the predominant situs cannot be determined;

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these cases are categorized as *situs ambiguous*. Most cases of *situs ambiguous* are in mice with the *situs* of the thorax discordant with that of the abdomen. Heterotaxia occurs in a number of reproducible patterns, the majority of which have been described by Hummel and Chapman ('59) and Layton ('78).

During an attempt to put the *iv* gene on various inbred backgrounds using a cross-intercross system of mating (Green, '81; Fig. 1), we encountered apparent partial dominant expression of the *iv* gene on the SWV background. In the cross-intercross mating system, a G1 (F1) generation is produced from the cross *iv*/*iv* X +/+ to yield *iv*/+ heterozygotes. Members of G1 are crossed among themselves to produce a G2 generation. Reversed G2 mice, presumably reflecting an *iv*/*iv* genotype, are then crossed with +/+ mice to produce G3 *iv*/+ mice for a second cycle. This process is repeated, usually for 10 cycles (20 generations). The even numbered generations contain one-quarter *iv*/*iv* mice, approximately one half of which should have *situs inversus*. The odd numbered generations are all *iv*/+ and should have the wild-type phenotype. This expected result was the case with six of the seven inbred strains; however, for the SWV strain, a reversed pup (based on location of the stomach) was found in G7. Since the albino SIV (*iv*/*iv*) mice were housed in the same room as the albino SWV, this unexpected finding was attributed to a mating error and the study was begun again. However, in the repeat study another reversed pup was found in G3 and still others in succeeding odd-numbered generations, which we shall call the G-odd effect.

There are two possible explanations for these findings: 1) One of the parents was a SIV (*iv*/*iv*) mouse instead of SWV (+/+), so that the reversed G-odd mice were actually *iv*/*iv*. 2) The *iv* gene is dominant on the SWV background, but with reduced penetrance.
Breeding tests to infer genotype are difficult to interpret in these animals because of reduced penetrance of the IV phenotype and the high rate of lethal congenital heart malformations associated with the IV phenotype (Layton, '78). Recently, we have demonstrated that SWV and SIV are polymorphic at the Ig-h-C locus on chromosome 12 (Hanzlik et al., '90). Since the Ig-h-C locus is linked tightly to iv, this has made it possible to distinguish if the reversed G-odd mice are iv/+ or iv/iv. Below are summarized genetic analyses that support the hypothesis that SWV has an unlinked autosomal recessive gene that interacts with iv to cause expression of the IV phenotype in iv/+ heterozygotes with reduced penetrance.

MATERIALS AND METHODS

Non-inbred iv_iv mice were obtained originally from Dr. Katherine Hummel of The Jackson Laboratory. These were kept in a small closed colony and eventually an inbred strain was produced by brother-sister mating for 20 generations. This strain is called SIV/Lay provisionally (SIV in this paper). Inbred strains A/J, AKR/J, BALB/cJ, CBA/J, C57BL/6J, and DBA/2J were obtained from The Jackson Laboratory and SWV from F.G.B. At weaning, all mice were classified and tagged by ear punch. Newborn pups were examined for the location of the milk spot, which is the white milk-filled stomach viewed through the translucent abdominal wall. Those mice with a right-sided milk spot (and thus situs inversus) were marked by clipping the tip of the tail.

The attempt to inbreed the iv gene on the SWV background using a cross-intercross system (Fig. 1) was started three times (experiments 1, 2, and 3). Although none of the experiments went the 20 generations required for inbreeding, all experiments were consistent with the unanticipated results reported herein. During experiment 1, the identification of IV phenotype was based on the position of the milk spot. During experiment 2, examinations became more thorough. In addition to using the milk spot location to identify the IV phenotype in living pups, most of the animals were autopsied excepting those that were too autolyzed or lost due to maternal cannibalism. These two methods of identifying affected mice are not equivalent. The use of the milk spot only identifies animals with a right-sided stomach and thus misses cases that have a left-sided stomach with heterotaxia. However, because of the selective loss of pups with the IV phenotype prior to weaning, autopsies done after the perinatal period miss mice with the IV phenotype that have died (usually as a result of heart malformations). Since G5 of experiment 2, all wild-type SWV (+/+ ) mice have been autopsied.

In order to test the hypothesis that the IV phenotype in G-odd iv/+ mice is due to interaction of the iv gene with another autosomal gene, two sets of experiments were done:

1. If the reversed G-odd mice were genetically different from the non-reversed G-odd mice, the incidence of reversal and heterotaxia and their association with each other in the offspring of reversed G-odd parents would be different from that in the offspring of non-reversed G-odd parents. This experiment was set up prospectively and G-odd parents of generations G-5 to G-7 were used. Results of 102 of 805 autopsies of offspring of non-reversed G-odd parents were not used in the tabulated results of this experiment because the parents of these mice, although not reversed, were found at autopsy to have the iv phenotype.

2. Two backcrosses were set up (see Fig. 3). BC1 should show the G-odd effect since approximately 1/4 of its members are heterozygous for iv and homozygous for the putative "hi" gene that is hypothesized to interact with iv/+ to result in the ic phenotype. None of these offspring can be iv/iv. RBC1 should not show the G-odd effect since no animals would be iv/+ , hi/hi. However, approximately half of them will be iv/iv so that about 1/4 should be reversed. To infer the genotype of these mice at the iv locus, we took advantage of the tight linkage of Ig-h-C with iv (Brueckner et al., '89; Hanzlik et al., '90) and of a polymorphism of Ig-h-C that enabled us to differentiate SWV from SIV. This was based on the difference in the length of a segment of Ig-h-C, which in turn was due to a variable number of tandem dinucleotide repeats of (A,C) and/or (C,T) in this segment (Hanzlik et al., '90). This difference was demonstrated using PCR. The segment from SWV was longer than that from SIV (Hanzlik et al., '90). This "PCR test" first became available during G9 of the third experiment and was used subsequently. Details about the primers and
the conditions used for the PCR, as well as the mapping of \( iv \) close to \( Igh-C \) (1 recombinant out of 201 animals examined) are given in Hanzlik et al. ('90).

RESULTS

In all three experiments attempting to place the \( iv \) gene onto the SWV background, we continued to find mice with the IV phenotype in odd numbered generations. Hereafter, we shall call this the “G-odd effect.” This unexpected finding does not appear to be the result of a breeding error; testcrosses of two reversed G5 mice with C57BL/6-\( iv/iu \) mice resulted in 4/24 and 4/24 reversed pups, respectively. This is consistent with the 25% incidence of reversal expected in an \( iv/+ \times iv/iv \) mating and not with the 50% expected if both G5 animals were \( iv/iv \). The PCR test established the \( id^+ \) genotype of 16 G9 animals with the IV phenotype from experiment 3; one animal was homozygous for the upper (SWV) allele, presumably representing a recombinant between the \( iv \) and \( Igh-C \) loci (Fig. 2; Hanzlik et al., '90).

The incidence of the IV phenotype as shown by reversal at birth and the phenotype at autopsy is given in Table 1. The incidence of this G-odd effect was independent of the sex of the reversed parent and was found with approximately equal frequency in males and females. The proportion of mice with the IV phenotype that had heterotaxia alone was much higher in the G-odd mice than in \( iv/iv \) mice as most of the affected G-odd mice had \( situs solitus \) (Table 2). This is in contrast to SWV \( iv/iv \) mice, in which \( situs inversus \) and \( situs solitus \) are equally frequent in heterotaxic mice. In 424 G1 mice, there were no cases of reversal at birth and only a single instance of IV phenotype. Note that this finding rules out an anomaly at the \( iv \) locus in the SWV mouse, as the compound heterozygote created in G1 essentially shows no IV phenotype. Assuming that the single case of the IV phenotype in G1 was a phenodeviant (which is seen in SWV mice; vide infra), we hypothesized that the increasing frequency of the G-odd effect with G-number was the result of interaction of \( iv \) with another recessive gene carried by SWV which we shall call “\( hi \).”

In an effort to evaluate the \( hi \) gene hypothesis further, we performed three breeding experiments that were consistent with this hypothesis: 1) We found that there was a significantly higher frequency of IV phenotype in the offspring of reversed G-odd mice than in the offspring of non-reversed G-odd mice (Table 2, cf. rows labeled “G-Odd Inverted \( \times \) G-Odd Inverted” and “G-Odd Solitus \( \times \) G-Odd Solitus”). This difference was consistent with the hypothesis that such reversed animals were homozygous for a second (“\( hi \)” gene). 2) Offspring of the backcross BC1 [(SWV \( \times \) SIV) \( \times \) SWV] (Fig. 3), which should contain no \( iv/iv \) mice but...
1/4 of which should be iv/+ hi/hi, showed a low incidence of IV phenotype (Table 2). It is noteworthy that all of the affected mice had heterotaxia and virtually all had situs solitus. In this respect, they resembled the affected G-odd mice rather than the "conventional" iv/iv mice (non-inbred iv/iv, C57BL/6 Lay iv/iv, and SIV/Lay iv/iv). These two backcrosses were originally done before the PCR test was available. Because of the importance of BC1, this was repeated and all seven of the mice with the IV phenotype that resulted from this backcross were iv/+ as indicated by PCR. 3) Affected offspring of the reciprocal backcross [(SWV × SIV) × SIV]; (BC1 in Table 2 and Fig. 3) resembled affected iv/iv mice: when compared with affected G-odd mice they had a relatively high incidence of situs inversus and low incidence of heterotaxia (Table 2).

We also encountered a few instances of the IV phenotype (most frequently heterotaxia with situs solitus) in putative SWV +/+ mice. We started our SWV colony in 1976; we examined 1,351 newborn pups curvierly and autopsied a relatively small number of weanlings and adults without finding the IV phenotype. However, since 1983, when we realized that there might be an association between the SWV background and reversal, we have examined all newborn SWV pups carefully and have done autopsies on virtually all SWV mice. During this period, we have encountered three in-
stances of situs inversus in 1,587 newborn SWV pups and six cases of heterotaxia with situs solitus in 1,250 autopsies. This gives an 0.3% incidence of the IV phenotype in SWV +/+ mice. Recently, using PCR to detect the Igh-C allele, we tested two of these SWV +/+ animals with the IV phenotype and have confirmed that these cases are not due to accidental crosses to SIV (Fig. 2). Unfortunately, those +/+ mice that have heterotaxia with situs solitus cannot be ascertained prior to autopsy, and thus cannot be used for breeding tests. Recently, however, a male SWV +/+ mouse (identification number 10VU41) was born with a reversed milk spot. His parents had another pup with heterotaxia and situs solitus and a pair of littermates of his parents also had a pup with situs solitus and heterotaxia. Breeding tests of this male and his close relatives have been uniformly negative. Matings with his mother and sisters produced only normal offspring. Matings between his siblings have also failed to produce any pups with the IV phenotype. At autopsy, this mouse had situs ambiguous and the PCR test was consistent with a +/+ genotype at the iv locus.

Putting the iv gene on six other inbred backgrounds (A/J, AKR/J, BALB/cJ, CBA/J, C57BL/6J, and DBA/2J), we examined 209 G-odd litters containing 1,179 pups at birth. None of these pups had the IV phenotype as demonstrated by a right-sided milk spot.

**DISCUSSION**

Tests for polymorphism at the Igh-C locus demonstrated that the instances of IV phenotype found in SWV iv/+ and +/+ mice were real and not the result of mistaking the iviv albino parent for one that is +/+ . In contrast to iv/iv mice, those showing the G-odd (n greater than or equal to 3) effect and phenodeviant SWV +/+ mice most often have heterotaxia with situs solitus (Table 2). In G1, the genotype of all animals is heterozygous at both the IV and HI loci, so little reversal is expected. This appears to represent a graded response; the mildest expression of the phenotype is heterotaxia alone. Next most frequent is heterotaxia with situs inversus. Situs inversus without heterotaxia is rare in SWV mice.

The G-odd effect is due to an interaction of the iv gene with the SWV genome, possibly with a hypothetical mutant gene that we shall call "hi." We propose that both iv and hi are loss of function mutations. The iv gene product enforces normal coordination of the sense of asymmetry among asymmetrical structures and sets a switch that determines global situs. Without the iv gene product the coordination of asymmetry is imperfect, resulting in heterotaxia, and the situs switch is set randomly. Although the hi gene product acts in a manner similar to that of iv it is weaker and acts mostly to enforce coordination of asymmetry. In its absence (in the SWV mouse) there are rare instances of heterotaxia, a few of which have situs inversus. However, in the absence of the hi gene product, iv acts as a dominant trait due to haploinsufficiency. In such a case the resulting phenotype is most likely to consist of heterotaxia, less often of heterotaxia with situs inversus, and rarely of situs inversus alone.

A number of phenodeviant traits, such as the cases of IV phenotype described here, have been found in various inbred mouse strains. For example, lateral cleft lip, open eyelids at birth, and atrial septal defects are found in the A strain (Kalter, '68; Nora et al., '68). In the C57BL/6 strain, microphthalmia or anophthalmia is relatively common (Kalter, '79). This strain also has a
low incidence of ventricular septal defects (Nora et al., '68). As we have documented, stochastic effects may play an important role in these cases (Kurnit et al., '87).

The penetrance and expression of single gene-determined phenotypes can be changed by genetic background (Juriloff et al., '87). For example, the first arch (far) mutation is lethal when homozygous due to abnormal development of structures derived from the maxillary process of the first branchial arch (Juriloff and Harris, '83). The far mutation occurred in the BALB/cGaBc strain; heterozygotes (far/+ ) in this strain are normal, but in the ICR/Bc strain most heterozygotes have some expression of far such as disrupted patterns of mystacial vibrissae (80%), hemifacial deficiencies (40%), and cleft palate (20%) (Juriloff et al., '87). Breeding studies suggest the change from recessive to partial dominance of the far mutation is an effect of genetic background due to a small number of loci rather than due to isolalleles or a difference between BALB/cGaBc and ICR/Bc in the normal wild-type (+ ) alleles at the far locus (Harris and Juriloff, '89). Another mutation, Dactylaplasia (Dac) is a dominant mutation that causes absence of the median digits of all four limbs and is a recessive lethal due to unknown causes (Chai, '81). The phenotype of dactylaplasia in Dac/+ heterozygotes depends on homozygosity for an unlinked recessive modifier (mdac/ mdac); a dominant suppressor (+/+ or mdac/+ ) causes Dac/+ heterozygotes to develop phenotypically normal digits.

The incidence of some phenodeviants traits can be changed by altering the intrauterine environment. The incidence of situs inversus, heterotaxia, and heart malformations in the Non Obese Diabetic (NOD) strain of mice is affected by the presence and severity of diabetes in the dam during early pregnancy (Morishima et al., '91). The incidence of the IV phenotype varied from .05% in the offspring of non-diabetic dams to 31% in those from dams that were diabetic during the first 3 days of pregnancy. Similar to the findings reported here for SWV, in the NOD mouse situs inversus without heterotaxia was rare. Most cases of the IV phenotype consisted of heterotaxia with situs solitus. There is some indication of clustering of the IV phenotype in the SWV iv/iv mouse, which suggests an environmental effect. Thus stochastic, genetic, and/or environmental factors may play a role in the expression of the IV phenotype.

Because the discovery of heterotaxia requires a detailed post mortem examination, it may be more prevalent in laboratory mice than is generally recognized. For example, the WB/ReJ strain of mice have a variety of azygous drainage patterns of the thorax, some of which are similar to those found in the IV phenotype (Biddle et al., '91).

Determination of laterality therefore involves at least two loci, viz., IV and HI. The finding that putative +/hi/hi SWV mice only rarely show the IV phenotype but that iv/+ hi/hi SWV mice more often show the IV phenotype indicates that the HI locus interacts with the IV locus to effect laterality.

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