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The original shaker-with-syndactylism mutation (*sy*) is a contiguous gene deletion syndrome

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Abstract

Tests for allelism among mice with four different mutant alleles at the shaker-with-syndactylism locus on mouse Chromosome (Chr) 18 provide evidence that the original radiation-induced mutation, *sy*, is a deletion including at least two genes associated with distinct phenotypes. Mice homozygous for *sy* have syndactylous feet and other skeletal malformations, are deaf, and exhibit abnormal behavior characteristic of vestibular dysfunction. Two less severe spontaneous mutations, shown to be allelic with *sy*, cause syndactylism when homozygous (hence named fused phalanges, *sy^{fp}* and *sy^{fp-2J}*), but do not affect hearing and behavior. Here we describe a third spontaneous mutation allelic with *sy* that does not affect foot morphology (hence named no syndactylism, *sy^{ns}*), but that does cause deafness and balance defects when homozygous. Complementation test results indicate that *sy^{fp}* and *sy^{fp-2J}* are alleles of the same gene, but that *sy^{ns}* is an allele of a different gene. The original *sy* mutation, therefore, includes both of the genes defined by these three spontaneous mutations. Typing of DNA markers in *sy/sy* mice revealed a deletion of approximately 1 cM in the *sy* region of Chr 18, including *D18Mit52*, *D18Mit124*, *D18Mit181*, and *D18Mit205*. The genetic relationships described here will aid in positional cloning efforts to identify the genes responsible for the disparate phenotypes associated with the *sy* locus.

Introduction

The original shaker-with-syndactylism mutation, *sy*, was induced by irradiation; many *sy/sy* homozygotes die within the first month after birth (Hertwig 1942). Homozygotes have variable fore- and hindlimb syndactylism (Gruneberg 1962), are deaf, and exhibit head-tossing and mild circling behavior caused by vestibular dysfunction (Deol 1963). A spontaneous mutation named fused phalanges, allele symbol *fp*, first reported by Hummel and Chapman (1971), was subsequently shown to be allelic with *sy* (Lane and Hummel, 1973). *sy^{fp}/sy^{fp}* mice exhibit digit fusions like *sy/sy* mice, but have normal behavior and both sexes breed; *sy/sy^{fp}* heterozygotes resemble *sy^{fp}/sy^{fp}* homozygotes (Lane and Hummel 1973). The *sy^{fp}* mutation was mapped to the middle of mouse Chromosome (Chr) 18 by Lane et al. (1981). More recently, another spontaneous mutation with the same homozygous phenotype as *sy^{fp}/sy^{fp}* was shown to be allelic with *fp* and consequently given the allele symbol *fp^{2J}* (Sweet 1996). The syndactylism associated with mutant *sy*, *sy^{fp}*, and *sy^{fp-2J}* alleles is more frequently expressed in hindlimbs than forelimbs and involves digits 2 and 3, digits 3 and 4, or digits 2, 3, and 4.

Here we report yet another spontaneous mutation at the *sy* locus. Mice homozygous for this new mutation exhibit the vestibular and hearing defects characteristic of the original *sy* mutation but do not have the digit fusions associated with *sy*, *sy^{fp}*, or *sy^{fp-2J}*. We show that

the new mutation is located on Chr 18 and that it is allelic with *sy*. We have named the mutation “no syndactylism” (*ns*), gene symbol *sy^{ns}*. Because of the disparate and non-overlapping phenotypes associated with the different spontaneous *sy* alleles, we hypothesized that the original, radiation-induced mutation, *sy*, may have disrupted two (or more) very closely linked but distinct genes, one affecting limb development, and the other the auditory/vestibular system. We present complementation test results for various hybrid combinations of *sy*, *fp*, *fp^{2J}*, and *ns* alleles in which we have examined limb morphology, vestibular behavior, and hearing ability. In addition, we present DNA marker typing data demonstrating that the original *sy* mutation is a deletion of approximately 1 cM.

Materials and methods

Mouse strains and mutations

All mice used in these studies were reared under modified barrier conditions in The Jackson Laboratory Mouse Mutant Resource (Davisson 1990). The *ns* mutation arose in August 1993 in a mixed stock derived from MK/ReJ and C57BL/6J mice; it is currently maintained by mating homozygous *ns/ns* females $\times$ *ns/+* sibs. The MK strain was derived from a cross between C57BL/6J and DBA/2J and segregates for a mutation, *Nramp2^{mk}*, that causes microcytic anaemia in homozygotes. The *Nramp2^{mk}* mutation has been eliminated from the strain carrying the *ns* mutation. The origins and strain histories of the other three mutant *sy* alleles have been described previously (Gruneberg 1956; Hummel and Chapman 1971; Lane and Hummel 1973; Sweet 1996); at The Jackson Laboratory, these alleles are maintained on the following strain backgrounds: B6C3Fe-*a/a-sy*, C3HeB/FeJ-*sy^{fp}/+*, and C.B10-*H2^b/LiMcJ-sy^{fp-2J}/+*.

Genetic mapping and deletion analysis

To map the chromosomal location of *ns*, a homozygous *ns/ns* female was mated to a CAST/Ei male. F₁ hybrids were intercrossed, and only F₂ homozygous mutants were saved for DNA typing and linkage analyses. MIT microsatellite markers were typed for the initial determination of *ns* linkage and subsequently for deletion analysis of the *sy* mutation. These markers were typed by PCR amplification (Dietrich et al. 1992; with primer pairs from Research Genetics Huntsville, AL), agarose gel separation, and ethidium bromide visualization. Size of the *sy* deletion was estimated with a (C57BL/6J $\times$ CAST/Ei) $\times$ C57BL/6J backcross mapping panel (Johnson et al. 1994). The Map Manager computer program, v2.6.5 (Manly 1993), was used to determine gene order and estimate genetic distances.

Hearing assessment by ABR threshold analysis

Hearing in mice was assessed by auditory brainstem response (ABR) threshold measurements. A computer-aided evoked potential system (Intelligent Hearing System, (IHS), Miami, Fla.) was used to test mice for ABR thresholds by previously described procedures (Noben-Trauth et al. 1997). This system, coupled to high frequency transducers, was used to generate specific acoustic stimuli and to amplify, measure, and display the evoked brainstem responses of anesthetized mice. Mice were tested with broad-band click stimuli and also with 8, 16, and 32 kHz pure-tone pips at sound pressure level (SPL) intensities from 10 to 99 decibels (dB). ABR thresholds were determined for each stimulus frequency by identifying the lowest intensity producing a recognizable ABR pattern on the computer screen. Samples of CBA/CaJ mice were tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.

Results

A new mutation with head bobbing and circling behavior was mapped to Chr 18 with an intercross with CAST/Ei. No recombinants out of 50 meioses (25 intercross progeny) were observed between the new mutation and *D18Mit123*, placing it near the *sy* locus and suggesting that it might be allelic with *sy*. Compound heterozygotes for the new mutation and the original *sy* mutation showed moderate circling and head tossing behavior but had normal digit morphology. Because of the behavioral non-complementation, we concluded that the new mutation was allelic with *sy* and named it “no syndactylism” (*ns*). Additional tests for allelism were then performed among mice with all combinations of the four known recessive mutant alleles at the *sy* locus (*sy*, *fp*, *fp^{2J}*, and *ns*). To evaluate allelism, we carefully examined test mice for foot morphology, behavior, and hearing sensitivity as measured by auditory evoked brainstem response (ABR) thresholds.

Foot morphology, behavior, and ABR thresholds for mice homozygous for each of the four *sy* mutations are presented in Table 1. *sy/sy* and *ns/ns* mice were deaf (no evoked response to 99 dB stimuli) at the earliest ages tested; they exhibited head bobbing and circling behavior typical of vestibular dysfunction. Mice homozygous for the two fused-phalanges alleles (*fp/fp* and *fp^{2J}/fp^{2J}*), however, had normal behavior and showed no sign of hearing impairment; they exhibited normal ABR thresholds for all auditory stimuli tested, even at old ages.

Foot morphology, behavior, and ABR thresholds for F₁ hybrid mice with all possible combinations of the four mutant alleles are presented in Table 2. On the basis of deafness and aberrant behavior, *sy* and *ns* appear to be alleles of the same gene, distinct from that of *fp* and *fp^{2J}*. Careful examination of the feet of homozygous mutant mice and compound heterozygotes gave a different assessment of allelism (Fig. 1, Table 2). *ns* was the only mutation that did not cause syndactylism when homozygous. On the basis of complementation test results for syndactylism, *sy*, *fp*, and *fp^{2J}* appear to be alleles of the same gene; *sy/fp*, *sy/fp^{2J}*, and *fp/fp^{2J}* mice all exhibited fused phalanges. *sy/ns*, *fp/ns*, and *fp^{2J}/ns* mice, however, had normal digit morphology, evidence that the gene causing syndactylism is distinct from the *ns* gene.

Allelic relationships among the four *sy* mutations are summarized in Table 3. *sy^{fp}/sy^{fp-2J}* heterozygotes exhibit syndactylism but have normal hearing and behavior, whereas, *sy^{fp}/sy^{ns}* and *sy^{fp-2J}/sy^{ns}* heterozygotes are completely normal. None of the three spontaneous mutant alleles completely complements the original radiation-induced *sy* mutation; *sy^{fp}/sy* and *sy^{fp-2J}/sy* heterozygotes exhibit fused phalanges but have normal hearing and behavior, and *sy^{ns}/sy* heterozygotes are deaf with a balance disorder but have normal foot morphology. These results indicate that *ns* (affecting hearing and balance) is a separate gene from that of *fp* and *fp^{2J}* (affecting digit morphology), and that both of these genes are disrupted by the original *sy* mutation.

Because *sy* was induced by irradiation (Hertwig 1942) and apparently disrupts more than a single gene, we typed markers in the vicinity of the *sy* locus on Chr 18 to see if any of them are deleted from *sy*-derived DNA. The original *sy* mutation has a complex strain history (Gruneberg 1956) and is now maintained on a B6C3Fe hybrid background. We examined B6C3Fe-*sy/sy* mice and F₁ hybrids from matings between B6C3Fe-*sy/+* mice and CAST/Ei-*+/+* mice. (Allele sizes differed between CAST/Ei and B6C3Fe-*+/+* mice for all of the marker loci tested, so that an *sy* deletion could be detected in *sy/+* F₁ hybrids by the presence only of CAST-specific alleles.) This DNA typing analysis revealed that four markers, *D18Mit124*, *D18Mit181*, *D18Mit52*, and *D18Mit205*, are deleted from the *sy* region (Fig. 2). The centromere-proximal flanking markers *D18Mit122*, *D18Mit123*, and *D18Mit238* and

the distal flanking markers *D18Mit28*, *D18Mit77*, *D18Mit78*, and *D18Mit208* are not deleted from *sy* DNA.

Discussion

Our discovery of a new spontaneous mouse mutation, *sy^{ns}*, that is allelic with *sy* but not with *sy^{fp}* or *sy^{fp-2J}* prompted us to examine more closely the genetic relationships of these four mutant alleles. Complementation tests and DNA typing results provided evidence that the original radiation-induced mutation, *sy*, is a deletion including at least two genes associated with distinct phenotypes. These results, summarized in Table 3, indicate that *fp* and *fp^{2J}* are alleles of the same gene, that *ns* is an allele of a different gene, and that both of the genes defined by these three spontaneous mutations are included in the region disrupted by the original *sy* mutation.

Our typing results for Chr 18 DNA markers revealed that the original *sy* mutation is a deletion of approximately 1 cM. The Whitehead Institute/MIT Center for Genome Research (Mouse Genomic Mapping Project, July 1996 Data Release) places all four of the deleted markers (*D18Mit124*, *D18Mit181*, *D18Mit52*, and *D18Mit205*) in a single nonrecombinant bin and the closest flanking markers separated by 1.1 cM. Therefore, the best estimate of the deletion size from the 46 intercross progeny of the MIT cross is 1.1 cM, averaging the distances from the nearest flanking markers (0–5 cM, 95% confidence interval). To verify this estimated deletion size, we typed some of the same markers on our own mapping panel derived from 144 progeny of a (C57BL/6J × CAST/Ei) × C57BL/6J backcross (Johnson et al. 1994). In this cross, no recombination occurred among *D18Mit123*, *D18Mit124*, and *D18Mit181* nor among *D18Mit52*, *D18Mit205*, and *D18Mit208*, but a single recombination event was observed between these two bins. Therefore, the best estimate of the deletion size from this cross is 0.7 cM (0–4 cM, 95% confidence interval), consistent with the 1.1-cM estimate calculated from the MIT map.

X-ray irradiation is known to induce a high frequency of deletions (Vrieling et al. 1985) that can include more than one gene. In the mouse, for example, the X-ray-induced mutation W19H has been shown to be a deletion of more than 2 cM, encompassing at least three distinct gene loci, *Kit*, *Pdgfra*, and *15H1* (Smith et al. 1991). The pleiotropy of the original *sy* mutation caused Gruneberg (1962) to caution: “The possibility should, however, not be lost sight of that *sy* (which was probably induced by X-rays) may be a small structural rearrangement (or loss of material) rather than a true point mutation.” Our allelism test results and deletion analysis verify that this suspected possibility is indeed the case, that *sy* is a contiguous gene deletion syndrome rather than a single gene mutation.

Knowledge of these genetic relationships will aid in positional cloning efforts to identify the genes responsible for the disparate phenotypes associated with the *sy* locus. The positional cloning of mouse mutations with similar syndactylism (Sidow et al. 1997) and deafness (Gibson et al. 1995) phenotypes have made important contributions to developmental biology and hearing research, respectively. Our deletion analysis refines the *sy* locus to an approximately 1-cM region of Chr 18. Candidate genes for *sy* mutations can be evaluated by testing for their presence or absence in genomic DNA from *sy/sy* mice. Analysis of the spontaneous mutations *fp* and *fp^{2J}* can be used to evaluate candidate genes that may underlie the syndactylism phenotype and analysis of the spontaneous *ns* mutation to evaluate candidate genes for the hearing and balance defects.

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Fig. 1. Hindfoot digit morphology of mice with various combinations of mutant alleles at the *sy* locus. A: *sy/sy*, B: *fp/fp*, C: *fp^{2J}/fp^{2J}*, D: *ns/ns*, E: *sy/ns*, F: *fp/fp^{2J}*, G: *sy/fp^{2J}*, and H: *ns/fp^{2J}*. Syndactylism is apparent in *sy/sy* (A), *fp/fp* (B), *fp^{2J}/fp^{2J}* (C), *fp/fp^{2J}* (F), and *sy/fp^{2J}* (G) mice, but digits are normal in *ns/ns* (D), *sy/ns* (E), and *ns/fp^{2J}* (H) mice.

Fig. 2.

Chromosome 18 microsatellite markers spanning the *sy* deletion. The markers *D18Mit122*, *D18Mit205*, *D18Mit124*, *D18Mit181*, and *D18Mit208* were typed by PCR amplification of genomic DNA prepared from spleens of B6C3-+/+ (lanes marked 1), B6C3-*sy/sy* (lanes marked 2), (B6C3 × CAST) F₁ hybrid-*sy/+*^{CAST} (lanes marked 3), and CAST-+/+ (lanes marked 4) mice. *D18Mit205*, *D18Mit124*, and *D18Mit181* were deleted from *sy*-derived DNA; no PCR product at all was obtained from *sy/sy* mice (lanes marked 2). For these same three markers, only CAST-specific products were obtained from *sy/+*^{CAST} mice (lanes marked 3). The parental origins of the *D18Mit124* product from *sy/+*^{CAST} DNA are not clearly resolved on the gel shown here; however, the lack of a product from *sy/sy* DNA is conclusive evidence for its deletion. In contrast to these three deleted loci, the closely linked flanking markers *D18Mit122* and *D18Mit208* were clearly amplified from *sy*-derived DNA. DNA size markers are shown in lane S (lower band, 100 bp; upper band, 200 bp).

Table 1

Foot morphology, vestibular behavior, and ABR thresholds of mice with different *sy* mutations. ABR thresholds are given in decibels, sound pressure level (dB SPL); 99 dB is the maximum output tested and indicates profound deafness. Threshold values for the good-hearing control strain CBA/CaJ are shown for comparison.

Mutation	Genotype	Number of mice	Age (months)	Foot morphology	Vestibular behavior	ABR thresholds(means and standard deviations)			
						click	8 kHz	16 kHz	32 kHz
CBA/CaJ, ABR	control strain	23	7–9	normal	normal	17 (3)	21 (3)	16 (4)	20 (5)
<i>sy</i>	<i>sy</i> ⁺	4	8–9	normal	normal	20 (4)	29 (6)	16 (3)	23 (3)
<i>sy</i>	<i>sy</i> / <i>sy</i>	3	2–3	syndactylism	circling	99 (0)	99 (0)	99 (0)	99 (0)
<i>fp</i>	<i>fp</i> ⁺	6	8–9	normal	normal	23 (11)	28 (11)	25 (16)	29 (18)
<i>fp</i>	<i>fp</i> / <i>fp</i>	11	5–6	syndactylism	normal	29 (15)	28 (11)	26 (14)	25 (12)
<i>fp</i> ^{2J}	<i>fp</i> ^{2J} / ⁺	3	2–3	normal	normal	23 (14)	35 (10)	20 (13)	30 (18)
<i>fp</i> ^{2J}	<i>fp</i> ^{2J} / <i>fp</i> ^{2J}	7	3–4	syndactylism	normal	26 (11)	39 (11)	29 (17)	38 (11)
<i>ns</i>	<i>ns</i> ⁺	4	8–9	normal	normal	20 (4)	25 (7)	19 (5)	33 (5)
<i>ns</i>	<i>ns</i> / <i>ns</i>	5	2–3	normal	circling	99 (0)	99 (0)	99 (0)	99 (0)

Table 2

Foot morphology, vestibular behavior, and ABR thresholds of progeny from allelism test crosses with all combinations of the four mutant *sy* alleles. For the *sy* and *ns* allele test, a total of 29 progeny with normal foot morphology and behavior were produced, but only four were tested for ABR thresholds.

Allele test	Female parent genotype	Male parent genotype	Presumed progeny genotype	Number of progeny	Age (months)	Foot morphology	Vestibular behavior	ABR thresholds (means and standard deviations)			
								click	8 kHz	16 kHz	32 kHz
<i>sy</i> and <i>fp</i>	<i>fp/fp</i>	<i>sy/+</i>	<i>fp/+</i>	9	6-7	normal	normal	19 (3)	21 (2)	16 (2)	18 (3)
<i>sy</i> and <i>fp</i> ^{2J}	<i>fp^{2J}/fp^{2J}</i>	<i>sy/+</i>	<i>fp/sy</i>	5	6-7	syndactylism	normal	28 (8)	27 (4)	18 (7)	24 (12)
			<i>fp^{2J}/+</i>	4	2-3	normal	normal	16 (3)	21 (3)	14 (3)	19 (3)
<i>sy</i> and <i>ns</i>	+/ns	<i>sy/+</i>	<i>fp^{2J}/sy</i>	5	2-3	syndactylism	normal	16 (2)	22 (3)	14 (2)	19 (2)
			+/?	29 (4)	2-3	normal	normal	15 (0)	24 (3)	20 (4)	23 (3)
<i>fp</i> and <i>fp</i> ^{2J}	<i>fp/fp</i>	<i>fp^{2J}/fp^{2J}</i>	<i>ns/sy</i>	6	2-3	normal	circling	99 (0)	99 (0)	99 (0)	99 (0)
<i>fp</i> and <i>ns</i>	<i>fp/fp</i>	+/ns	<i>fp/fp^{2J}</i>	6	3-4	syndactylism	normal	19 (2)	25 (5)	15 (4)	21 (4)
<i>fp</i> ^{2J} and <i>ns</i>	<i>ns/ns</i>	<i>fp^{2J}/fp^{2J}</i>	<i>fp/?</i>	15	7-8	normal	normal	16 (2)	24 (5)	17 (4)	21 (6)
			<i>ns/fp^{2J}</i>	10	2-3	normal	normal	16 (2)	23 (3)	16 (2)	23 (3)

Table 3

Summary of phenotypes associated with the four *sy* mutant alleles and all heterozygous combinations.

Genotypes	Phenotypes		
	syndactylism	head tossing, circling	deafness
Homozygotes:			
<i>sy/sy</i>	yes	yes	yes
<i>fp/fp</i>	yes	no	no
<i>fp^{2J}/fp^{2J}</i>	yes	no	no
<i>ns/ns</i>	no	yes	yes
Compound heterozygotes:			
<i>sy/fp</i>	yes	no	no
<i>sy/fp^{2J}</i>	yes	no	no
<i>sy/ns</i>	no	yes	yes
<i>fp/fp^{2J}</i>	yes	no	no
<i>fp/ns</i>	no	no	no
<i>fp^{2J}/ns</i>	no	no	no