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Circling Mouse: Possible Animal Model for Deafness

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Mutant mice with abnormalities are potentially useful as models for studying human defects. Here we report a group of mice with abnormal behavioral patterns. A new spontaneous mutant mouse exhibited hyperactive behavior at about seven days of age, followed by tight circling behavior. Breeding studies suggest that this mutation is caused by a single gene defect inherited in an autosomal recessive manner. Consequently, this mutation is referred to as a circling (*cir*) mouse mutation with the gene symbol *cir*. Auditory test results identified clearly the hearing loss of the *cir*, compared with wild-type mice. Pathologic studies confirmed developmental defects in cochlea and spiral ganglions that were correlated to the abnormal behavior observed in the *cir* mice. Thus, *cir* mice may be useful as a model for studying inner ear abnormalities and deafness in humans.

Hearing loss is mainly a consequence of sensory organ defects in humans. Inherited deafness affects one of 2,000 children worldwide, and a similar proportion of children suffer substantial hearing loss due to other causes, such as infection and disease of the external and middle ear (1). About 70% of inherited deafness is non-syndromic hearing loss (2). In most instances, non-syndromic deafness (DFNB forms) has a genetic origin and is inherited in autosomal recessive mode as a single gene disease (1). Inherited inner ear abnormalities in humans and mice can be grouped into several categories, including morphogenetic inner ear defects, cochleo-saccular defects, and neuroepithelial defects. The inner ear is a complex organ in terms of its development, morphology, and function. Therefore, mutant mice with inner ear abnormalities are potentially useful as models for the study of human inner ear defects.

Approximately 60 mutant loci are known to be involved in the auditory system in mice (3). Since the auditory systems are conserved between mice and humans, mouse models have been used to identify the genes involved in deafness and inner ear abnormalities. Many mouse strains with spontaneous deafness have been reported (4-8). Studies involving use of mouse models have been able to predict several human deafness genes. For example, an Myo6 gene mutation in *Snell's waltzer* mice and Myo7a gene mutation in *shaker-1* mice are correlated to autosomal forms of deafness (9, 10), respectively, leading to degeneration of Corti's organ, spiral ganglion, and stria vascularis (11, 12). Further analysis documented that *shaker-2* mice have mutations in the gene of *Myo15* encoding unconventional myosins (13-15). In addition, the genes related to autosomal forms of deafness in mice also have been mapped or identified (16-18).

We report a new spontaneous mutation in the inner ear (herein referred to as circling mice). Circling mice exhibit circling behavior, which appears at seven days of age. Homozygous adult mice have characteristic signs of Shaker-Waltzer syndrome: deafness, and circling and tossing of the head. Body weight is decreased, compared with that of heterozygous mice and clinically normal mice. One of the most notable pathologic phenotypes was near-complete loss of the organ of Corti in the inner ear. Additionally, the mutation in circling mice is transmitted by an autosomal recessive gene with 100% penetrance. On the basis of these results, circling mice are an excellent model for investigating inner ear abnormalities in humans.

Materials and Methods

Animals. Circling mice were first discovered within an ICR out-bred strain and have been maintained for 16 generations by breeding between affected siblings in the Laboratory Animal Center, Catholic Research Institutes of Medical Science, Catholic Medical College, Seoul, Korea. Circling and C57BL/6J mice were used throughout the study reported here. The C57BL/6J mice were obtained from the Korea Research Institute of Bioscience and Biotechnology. Mice were kept in a specific-pathogen-free conditioned animal care facility and were free of the following microorganisms: Sendai virus, Mouse hepatitis virus, *Mycoplasma pulmonis*, Tyzzer's organism, *Pasteurella pneumotropica*, *Salmonella* spp., *Corynebacterium kutscheri*, *Pseudomonas aeruginosa*, and *Bordetella bronchiseptica*. In circling and C57BL/6J mice, microbiological monitoring against the aforementioned microorganisms was quarterly conducted.

Mice were housed individually in plastic cages (18 × 30 × 15 cm) with bed-o'cobs bedding (The Andersons Inc., Maumee, Ohio), maintained in an animal room that was air-conditioned (temperature: 24 ± 0.5°C and humidity: 55 ± 5%) and light-controlled (12 h light, 12 h dark, with lights on/off at 7 a.m./7 p.m., respectively). All mice were allowed ad libitum access to rodent chow (Purina #5001, Bethlehem, Pa.) and tap water. Chow, bedding, and tap water were sterilized by autoclaving prior to use and supply. All animal studies were performed with the approval of the Experimental Animal Care and Use Committee of Catholic University.

Genetic analysis. Preliminary breeding data analyses of the carrier × carrier and affected × affected matings within circling mice were conducted for the appearance of normal and affected progenies. Before mating, all animals were determined to be ei-

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ther normal or affected according to their respective circling behavior. The χ^2-tests were calculated on the basis of an expected segregation ratio.

Clinical observation. Behavior tests were designed to characterize the abnormalities of circling mice. Rearing, grooming, and circling were observed, using an open-field apparatus (75 × 75 × 30 cm). Vertical and horizontal lines were drawn on the floor of the open field every 15 cm. The line intersections were counted for five minutes as an index of locomotion. Animals were weighed weekly.

Acoustic startle response (ASR) test. The ASR test was performed to examine intact ability of auditory function in circling mice at the behavior level. Two kinds of white noise bursts (80- and 120-dB sound level pressure, 80-milliseconds' duration) were used as startle stimuli to evoke ASR. Each mouse (15 weeks old) was placed in a startle chamber and was acclimated for five minutes. Following acclimation, two startle stimuli (80 or 120 dB) or no stimulus were presented in random order to animals. No-stimulus trials (null trials) were included to set the reference response of each animal. Each stimulus at each level was presented 60 times (60 trials), and the intensity of the ASR evoked by each stimulus was recorded and analyzed by use of a startle response analysis system. The ASR system was previously developed at the Department. of Psychology, Korea University, Seoul, Korea (19).

Morphologic analysis of the cochlea. The inner ear tissues of circling and C57BL/6J mice were fixed by cardiac perfusion with phosphate-buffered 2.5% glutaraldehyde and 4% paraformaldehyde. After three to four days' fixation, the tissues were decalcified in 5% nitric acid for two days and embedded in paraffin. Four-micronometer-thick tissue sections were prepared, and every tenth section was stained with H&E for light microscopic study.

Results

Clinical observations. All affected mice were born normal, but gradually manifested hyperactive behavior, starting at approximately seven days of age. At that age, shaking of the head was first observed and became more conspicuous over the next three to four days. This pattern was easily noticed from days 12 to 14, at which point mice first begin to walk. The affected mice frequently ran in tight circles, especially when placed in strange surroundings or when otherwise disturbed (Fig. 1). However, they did not show preference for any particular direction. In the open field test, a marked increase in circling activity was observed. In comparison with wild-type mice, which did not manifest circling activity, the homozygous circling mice had a rearing score of 233.25. The rearing score of wild-type animals was 56.17, whereas that of homozygous circling mice was 12.67. This suggests that mutant mice lack appreciable rearing characteristics. In terms of grooming behavior, significant difference was apparent between wild-type animals and homozygous circling mice. To test for behavioral dysfunction in circling mice, we conducted swimming tests with them and wild-type mice. All wild-type mice had normal swimming behavior, resulting in the proper orientation of mice with respect to the water surface. However, all circling mice had abnormal swimming behavior associated with lack of orientation; when placed in water, the mutants spiraled underwater and were unable to maintain the nose and tail above the water surface. Mutants needed to be rescued promptly to prevent them from drowning.

Genetics. The *cir* defect is inherited in autosomal recessive manner. Table 1 shows the results of the test crosses within the circling mice. The inter-breeding of two carriers produced an offspring ratio of approximately three normal to one affected ($0.25 < P < 0.10$). The mating of carrier and affected mice produced offspring in a ratio of approximately one normal to one affected ($0.90 < P < 0.75$). All offspring resulting from affected mouse inter-breeding were affected. These results confirmed that a single autosomal recessive gene controls the trait.

Figure 1. Phenotypic characterization of circling mice (100 days).

Body weight of the circling and heterozygous mice was measured weekly from seven to 100 days. The affected mice generally weighed 20 to 25% less than did their wild-type littermates over the period measured. Neither viability nor fertility of the circling mice was significantly lower, compared with that of heterozygous controls. Heterozygous mice have an inherently gentle nature; they were easy to handle for breeding procedures and experiments, but female *cir* mice manifested cannibalism toward these offspring. There was no further progression of the phenotype with age. The heterozygous mice appeared to move with normal gait.

Acoustic startle response (ASR) test. We initially observed that the circling mice did not respond to sharp metallic sounds, which suggested that they have impaired hearing ability. The ASR test results from two kinds of startle trials (80 and 120 dB) and null trials were compared within each animal. If animals have a normal auditory sensation, they are supposed to show ASR to the startle stimulus of 80 or 120 dB. Table 2 shows the results of the ASR test between circling mice and control mice; examples of ASR test results to each stimulus are shown in Fig. 2. The ASR recordings indicated that mice of the circling phenotype were totally deaf at all frequencies tested (Fig. 2B); non-circling siblings, heterozygous for the mutation, had thresholds in the normal range (Fig. 2A), similar to those of wild-type mice. These results suggest that *cir* mice have dysfunction of the auditory sensory system.

Pathologic study. Histologic examination of inner ear tissue

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Table 1. Results of test crosses among circling mice

Mating pairs		No. of progeny		Expected ratio			
Female	Male	Normal	Affected	Normal	Affected	χ^2	*P*
B6 (+/+)	Affected (*cir*/*cir*)	53	0	1	0	–	–
Affected (*cir*/*cir*)	Affected (*cir*/*cir*)	33	0	0	1	–	–
Carrier (*cir*/+)	Carrier (*cir*/+)	83	20	3	1	1.712	0.25–0.10
Carrier (*cir*/+)	Affected (*cir*/*cir*)	152	149	1	1	0.030	0.90–0.75

Table 2. Results of the acoustic startle response (ASR) test in circling mice (n = 12), compared with that in control mice (n = 12)

Stimulus	Wild-type	Affected (*cir*/*cir*)
80 dB	12	0
120 dB	12	0

Data are expressed as number of mice showing ASR.

specimens from adult mutant mice had abnormalities. Fifteen-week-old mice were used for this study. During dissection, the structure of the middle ear did not have any abnormalities. Figure 3A shows the typical architecture of the organ of Corti from a heterozygous mouse, with readily apparent outer and inner hair cells and a well formed tunnel of Corti. In *cir* mice, the outer hair cells were completely lost, but the supporting cells were spared (Fig. 3B). The stria vascularis appeared normal. The spiral ganglion cells were almost completely degenerated (Fig. 3D), and the Rosenthal canal was almost empty due to severe degeneration of the axons between the hair cells and the spiral ganglion cells (Fig. 3B). In a vestibular organ, such as the saccule, the utricle and semicircular canal, hair cells, and Scarpa's ganglion cells appeared intact on the basis of results of light microscopy. These abnormalities are sufficiently severe to account for the auditory deficits observed during function testing of the mice.

Discussion

Mutant mice with inner ear abnormalities are useful for studying the pathologic processes underlying inner ear defects, in addition to gaining an understanding of the process of normal auditory development and sensory transduction. These mutants also are valuable for identifying the responsible genes by positional cloning, and are used to expedite the search for genes involved in inner ear defects in humans.

A new mouse mutation (*cir* mouse), which causes circling, head tossing, and hyperactivity associated with morphologic abnormalities of the inner ear, was identified. These traits were transmitted by a single autosomal recessive gene. Body weight of the circling and control mice wase measured weekly from one to 20 weeks (date not shown). The *cir* mice were generally of lighter weight than were wild-type mice and their littermates over the period of measurement. It is possible that the *cir* mice might also have an eating disorder, contributing to the weight loss. Investigation of the hearing function, using the ASR procedure, indicated that the *cir* mutation causes total deafness. Additionally, results of pathologic study indicated that the region around the organ of Corti had abnormalities in the spiral ganglion neurons and hair cells. However, structures such as Reissner's membrane and the stria vascularis appeared to be fully developed. Abnormalities in the spiral ganglion neurons and hair cells may be the cause for loss of the auditory startling response. The circling behavior of *cir* mice was highly correlated with the vestibular system. But, the hair cells and ganglion cells appeared intact on the basis of light microscopy (date not shown).

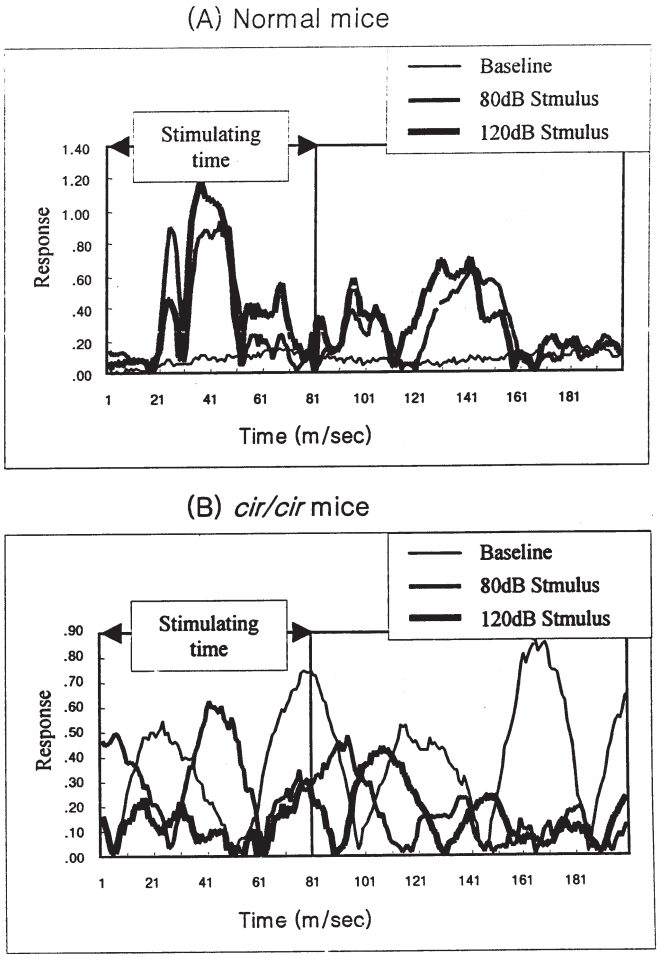

Figure 2. Acoustic startle response (ASR) test examples from control mice (A) and *cir* mice (B).

Several other mutations were reported to have phenotype and pathologic features similar to those of *cir* mice. The ames waltzer (av), bronx waltzer (bv), shaker-1 (sh-1), shaker-2 (sh-2), and bustling (bus) mice all have signs of circling behavior and inner ear defects. The bronx waltzer (bv) mutation is an autosomal recessive mutation that manifests as head tossing and circling (20). Ames waltzer (av) is a recessive mutation found in mice and causes circling, which is associated with degeneration of the inner ear neuroepithelia (21). Recently, the ames waltzer (av) defect was reported to be caused by mutation of the protocadherin gene (22). The mutation affects the inner hair cells and pillar cells in the organ of Corti of the cochlea and the maculae and cristae of the vestibular part of the inner ear (10). Shaker-1 homozygotes manifest hyperactivity, head tossing, and circling due to vestibular dysfunction. This develops together with typical neuro-epithelial-type cochlear defects involving dysfunc-

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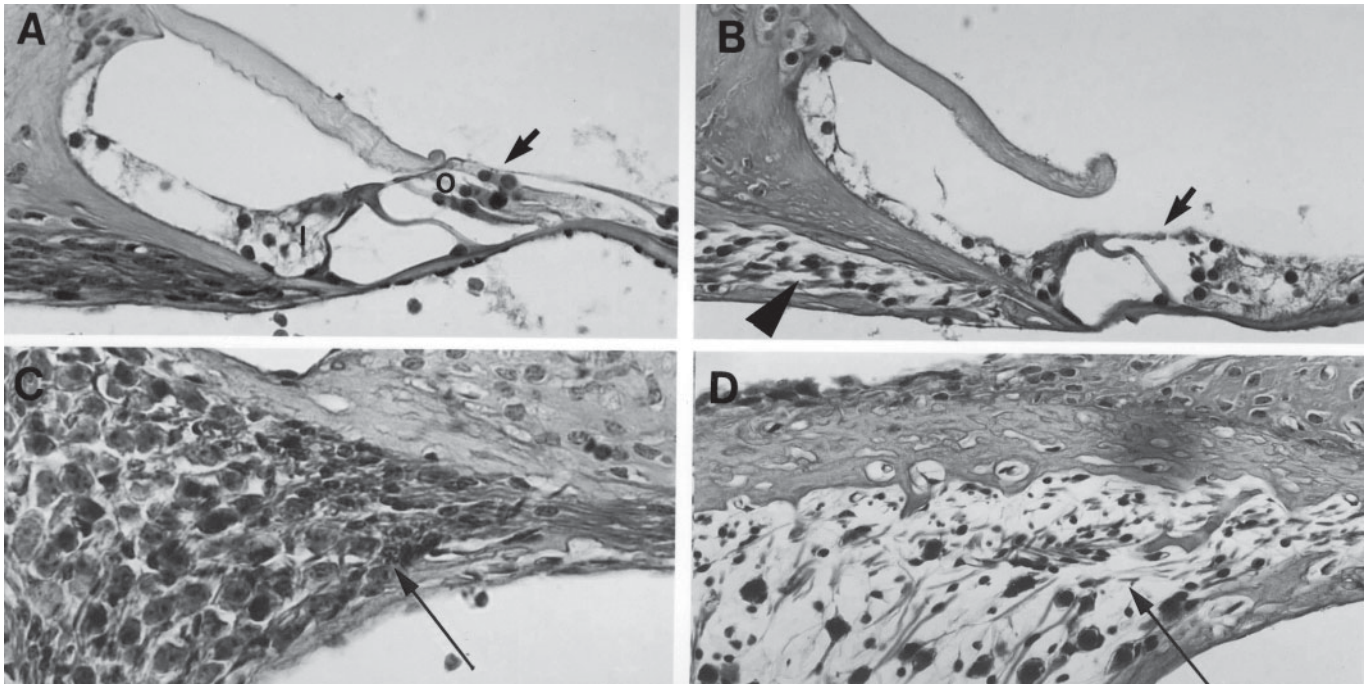

Figure 3. Histologic analysis of the cochlea from normal and circling mice at 100 days of age. (A) Normal mouse organ of Corti in the upper basal turn illustrating the two types of auditory sensory cells (O = outer hair cell, I = inner hair cell). (B) Degenerating organ of Corti in the apical cochlear turn of a circling mutant. Outer hair cells are missing. Notice remnants of organ of Corti in the apical cochlear turn (arrow) and few neuronal fibers along the Rosenthal canal (arrowhead). (C) Normal mouse cochlear duct in the upper basal turn showing major anatomic features. (D) Cochlear duct in the upper basal turn from an adult circling mutant. The spiral ganglion (arrow) has greatly reduced neuronal population. H&E stain; magnification, 200×.

tion and progressive degeneration of the organ of Corti. The *sh-1* gene encodes an unconventional myosin molecule of the type-VII family (23). Recently, the gene of the shaker-2 (sh-2) mutant mouse was identified (13). Scanning electron microscopy of the apical surface of an inner hair cell in sh-2 mouse mutants suggests that Myo15 is involved in maintenance of actin organization in hair cells of the organ of Corti (14). Bustling mouse (BUS/Idr:bus) is a mutant mouse strain that exhibits bustling/hyperkinetic behavior and functional disorders related to the vestibulocochlear system, such as rapid circling and loss of the ASR response (24). The *bus* gene was mapped on chromosome 10 and revealed an allele of the waltzer mutation (25). To verify the relationship between *cir* mice and the aforementioned mutations, further investigation involving an allelism test and genetic mapping of the *cir* gene will be made.

Our preliminary genetic mapping data revealed a *cir* gene to be on the chromosome 9 (55 to 62 cM). Furthermore, we did not observe allelism between our *cir* mice and *spinner* (*sr*) mice (chromosome 9, 64 cM) (26). Currently, it has been reported that the other two deafness /vestibular genes are located on chromosome 9: *Myo6* (44 cM) and *Tecta* (25 cM), respectively (26). The *Myo6* gene was identified from *Snell's waltzer* (*sv*) mutant mice with phenotypes similar to those of *cir* mice (21). In the case of *Tecta*, the α-Tectorin gene was inactivated by targeted deletion, and this null mouse had little changes in the structure and orientation of the hair bundles (27). Compared with these two mutants, *cir* mice had loss of outer hair cells. In contrast, outer hair cells were normally orientated in these *Snell's waltzer* and *Tecta* null mice (21, 27), which suggests that our *cir* mice are unique, compared with these previously identified mutations. Almost all mutations manifesting circling behavior have deafness that is correlated to inner ear abnormalities (12, 22-24). Thus, we have documented that the *cir* mice have circling behavior and deafness.

Mutations that affect the region around the organ of Corti, the spiral ganglion neurons, are a major cause of inner ear defects in humans. Given the importance of this type of pathologic change in humans, *cir* mice may prove to be a useful model for investigating inner ear abnormalities, in particular, to assist in identifying the gene(s) essential for normal structure and function of the inner ear.

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