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Tympanometry Assessment of 61 Inbred Strains of Mice

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Abstract

Otitis Media (OM) accounts for more than 20 million clinic visits in the United States every year. Resistance to antibiotics has hampered current management of the disease. Identification of genetic factors underlying susceptibility to OM is greatly needed in order to develop alternative treatment strategies. Genetically defined inbred mouse strains offer a powerful tool for dissecting genetic and environmental factors that may lead to OM in mice. Here we report a study of middle ear function of 61 genetically diverse inbred strains of mice using tympanometry. Of the 61 inbred strains tested, the 129P1/ReJ, 129P3/J, 129S1/SvImJ, 129X1/SvJ, A/HeJ, BALB/cJ, BUB/BnJ, C57L/J, EL/SuzSeyFrkJ, FVB/NJ, I/LnJ, LP/J, NZB/BlNJ, PL/J and YBR/Ei strains exhibited tympanograms that were statistically different from other healthy strains according to parameters including middle ear pressure, volume and compliance. These differences are most likely the result of genetic factors that, when understood, will facilitate prevention and treatment of otitis media in humans. In addition, a negative correlation between age and compliance of the tympanic membrane was discovered. This is the first report to successfully use tympanometry to measure mouse middle ear function, which has been a challenge for the hearing research field because of the mouse's tiny ear size.

Keywords

tympanometry; mouse models; middle ear; otitis media

Introduction

Otitis media (OM), the infection and inflammation of the middle ear cavity, accounts for more than 20 million annual visits to physicians in the United States, and is the most common cause of hearing impairment in children (Bernius et al., 2006; Kubba et al., 2000; McConaghy, 2001). Factors that influence susceptibility to OM include Eustachian tube dysfunction, poor mucociliary clearance, immune status, pathogens and genetic susceptibility. Several lines of evidence indicate that heredity plays an important role in OM susceptibility (Casselbrant et al., 1999; Coates et al., 2002; Goodwin et al., 2002; Harris et al., 1998). The advent of drug-resistant bacteria has sparked great interest in formulating novel methods of prevention and treatment. Development of alternative ways to treat and prevent OM is urgently needed to preserve the potency of antibiotics against pathogens, and to reduce OM morbidity and its associated costs.

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Animal models are the key to gaining insight into the pathogenesis of OM and to finding an alternative approach to combat OM. Although the chinchilla (Bakaletz et al., 1998; Giebink, 1999) and the rat (Clark et al., 2000) have been traditionally used for OM models, the development of mouse models for OM would offer significant advantages in the area of genetic research. The recent sequencing of the mouse genome has made genetic manipulation and identification comparably easier in mouse than in any other mammal. Homologies between the mouse and human genomes are well established. Availability of large numbers of mouse progeny from genetic crosses facilitates the use of new advances in high-resolution positional cloning methods to map spontaneous and chemically induced mutations. Genetically engineered mice with targeted gene mutations can also be used to validate candidate genes that may be homologs for human genes related to OM (Blake et al., 2002). The deaf mouse mutant Jeff (*Jf*) which exhibits chronic proliferative OM (Hardisty-Hughes et al., 2006) and the OM-susceptible *Junbo* mice (Parkinson et al., 2006) are two of the few mouse models available for OM studies. Pathogen-induced OM models have been extensively studied (Bikhazi et al., 1995; Ebmeyer et al., 2005; Melhus et al., 2003; Rivkin et al., 2005; Ryan et al., 1989), but the study of mutant mice has revealed genes that are difficult to identify in wild-type mice (Hardisty-Hughes et al., 2006; Parkinson et al., 2006). Our strategy is to compare many strains of inbred mice to identify genes that are differentially expressed in those mice with a predisposition toward OM or deafness. The identification of OM-related genes has and will continue to shed light on the etiology and genetic mechanisms of this disease.

Auditory brainstem response (ABR) measurement has been successfully used to evaluate hearing in mice in our previous reports (Johnson et al., 2002; Johnson et al., 2000; Johnson et al., 2001; Zheng et al., 2001; Zheng et al., 1998). However, ABR-based hearing impairment does not always signify a middle ear infection, nor does a middle ear infection always result in hearing loss. Tympanometry, a commonly used technique for the diagnosis of middle ear disease in humans, is used to assess mobility of the tympanic membrane and ossicular chain. It is a more definitive test than ABR for the diagnosis of various middle ear lesions associated with OM (Chambers et al., 1989; Fria et al., 1980; Richardson et al., 1997). Thus, establishing a baseline for using tympanometry in mice is pertinent for the identification of mouse middle ear abnormalities and genetic mouse models of OM. Here we report the tympanometric assessment of middle ear function in 61 genetically defined inbred mouse strains and find that 15 of these strains show evidence of middle ear abnormalities.

Materials and Methods

Animal Care

The care and use of animals described in this study were approved by The Jackson Laboratory (TJL) Institutional Animal Care and Use Committees (IACUC grant # DC005846). All mice were bred and maintained at TJL. The colony was monitored for specific pathogens by the TJL routine surveillance program (<http://www.jax.org/>). Prior to testing, mice were anesthetized by intraperitoneal injection with 5 mg Avertin per 10 g body weight.

Tympanometry Procedure

Tympanometry measures middle ear pressure by means of electroacoustic and manometric measurements. An MT 10 tympanometer from Interacoustics (Assens, Denmark) was used in this study. Tympanometry was performed in a quiet animal procedure room. Environmental noise was kept under 50 dB SPL. A comprehensive calibration of the sound level meter and bioacoustic simulator was performed. The physical volumes (1.5 ml, 0.5 ml, and 0.25 ml) of the tympanometer were checked. Actual mouse-ear external canal volumes (0.05 ml) were also measured by filling with saline and proved to be 30–40% of the meter-reading volumes.

The tympanogram is a graphic chart of compliance of the tympanic membrane under changing pressure conditions (Fig. 5A and B). The MT 10 measures compliance (C in ml), the equivalent volume (V in ml) of the outer ear canal, the gradient (G in ml) and the pressure (P in daPa) at maximum compliance. Gradient refers to the shape or width of the tympanometer curve. Three general types of tympanogram tracings have been described in the literature. A normal ear gives tracing type A as shown in Figure 5B (a bell-shaped curve with peak admittance occurring at or near 0 daPa). A definitively abnormal tracing is type B (a flat curve) which has a positive predictive value of 49 to 99 percent. A type C curve is bell-shaped like type A, but peaks in the negative pressure range. Type C tracings are unreliable indicators of pathology, but can be useful when correlated with other data (Onusko, 2004).

The CBA/CaJ strain has been used extensively as the standard for normal hearing in studies of mouse hearing function (Rosowski et al., 2003). The CBA/CaJ mouse strain was tested periodically to provide reference values for normal tympanograms, and to monitor the precision of the equipment and testing procedures.

Baseline Establishment and Statistical Analyses

A minimum of five age-matched mice were tested from each strain. Every mouse was repeatedly tested two or three times to check the consistency of the readings. Individual mouse tympanograms, as well as strain, age, weight, genotype and test dates were logged and subsequently entered into an electronic database. Analyses of variance (ANOVA) and covariance (COANOVA) were used to compare effects of and differences in strain, age, sex and body weight in relation to tympanometer data. T-tests were subsequently used to confirm. The JMP program (jmp.com) was used for statistical analyses.

Video Otoscopy, Histological Analysis and ABR Measurement

Video otoscopy is one of the most commonly used clinical diagnostic tools for OM, but it does not give a quantitative estimation of the disease (Jaisinghani et al., 2000). Otoscopy is very useful in diagnosing middle ear abnormalities when combined with tympanometry (Finitzo et al., 1992). To determine whether significantly different tympanometric measurements corresponded to middle ear abnormalities, we performed video otoscopy, histology, and ABR analysis on selected mice. Middle ear pictures were captured with a video otoscope from MedRx (Largo, FL), and digital images stored in a computer.

For those mice with a confirmed abnormal tympanic membrane, middle ear morphology and inflammation were investigated by histological analyses. Mouse temporal bones were dissected immediately after perfusion as previously described (Zheng et al., 2006). Slides of 5 μ m-thick mouse middle-ear tissue were H&E-stained and examined under a Nikon E600 upright microscope. Digital images were taken with a SPOT RT camera (Diagnostic, Inc., Sterling, MI, USA).

Equipment from Intelligent Hearing Systems (IHS, Miami, FL, USA) was used to perform ABR tests as previously described (Zheng et al., 1999a). Auditory thresholds were obtained for each stimulus frequency by varying the sound pressure level to identify the lowest level at which an ABR pattern could be recognized.

Online Supplemental Material

Complete tympanometry data for all 61 mouse strains tested at different ages are available online at (to be assigned by the publisher). Strains tested that gave normal readings include 129T2/SvEmsJ, A/WySnJ, AKR/J, BALB/cByJ, BXA14/Pgn, BXSB/MpJ, C3H/HeJ, C3H/HeOuJ, C3H/HeSnJ, C3HeB/FeJ, C57BL/10SnJ, C57BL/6ByJ, C57BL/6J, C57BLKS/J, C57BR/cdJ, C58/J, CAST/Ei, CBA/CaGnLeJ, CBA/CaH-T6/J, CBA/CaJ, CBA/J, CE/J, DBA/

1J, DBA/1LacJ, DBA/2J, KK/H1J, MA/MyJ, MOLD/RK, MOLF/EiJ, MRL/MpJ, NON/LtJ, NOR/LtJ, NZO/H1J, NZW/LacJ, P/J, PERA/Ei, RBF/DnJ, RF/J, RIIS/J, SEA/GnJ, SJL/Bm, SJL/J, SM/J, SPRET/Ei, SWR/J, WSB/Ei.

Results

Tympanograms of Inbred Mouse Strains

Of 61 inbred mouse strains tested here, 46 strains gave tympanogram tracings of type A (normal), 3 of type B, 3 of type C, and 9 were unclassified because of variations. None of these affected mice were circling which refutes the myth that some mice circle simply because of middle ear infections.

The tested mice ranged in age from 5–68 weeks and specific ages are listed in the online supplemental data. The mean and standard deviation of the four tympanogram parameters of CBA/CaJ are shown on Table I. Previous ABR tests showed that CBA/CaJ mice retain normal hearing well beyond one year of age (Zheng et al., 1999a). In Table I, we show that there are no significant differences in the tympanogram values for young and old groups of CBA/CaJ mice under one year of age, based on a T-test analysis ($P>0.05$).

Arithmetic means and standard deviations of middle ear tympanic readings from 61 inbred mouse strains were calculated; strains showing abnormal tympanometry are listed in Table II and in the online supplemental material. Middle ear measurements were considered abnormal if any of the mean values of C, V, G, or P from five or more mice for a given strain deviated by more than 2 SD units of the grand mean value derived from the 61 strains. The mouse strains with deviant tympanograms include 129P1/ReJ, 129P3/J, 129S1/SvImJ, 129X1/SvJ, A/HeJ, BALB/cJ, BUB/BnJ, C57L/J, EL/SuzSeyFrkJ, FVB/NJ, I/LnJ, LP/J, NZB/BINJ, PL/J and YBR/Ei mice. The mean value of each parameter describing the tympanic membrane varies among the 61 inbred strains as illustrated in the graph of mean compliance for each strain (Fig. 1). These mice were all raised and maintained under uniform, controlled environmental conditions, yet some had statistically significant abnormal compliance and gradient values. Thus our results suggest that there are underlying genetic components affecting the middle ear conditions of some mouse strains.

All of the values for 1300 measurements from the 61 strains were subjected to analysis of covariance (ANCOVA) to assess the effects of strain, age, weight and sex on middle ear pressure, volume, compliance and gradient. There were no significant effects of sex, weight or age on volume, pressure and gradient measurements gathered from all strains. In a covariance model that evaluated the effect of age on compliance, a correlation between age and compliance was observed. As the age of mice increased, the compliance of the middle ear decreased (Fig. 2; $r = -0.308$, $p < 0.00001$). However, when data collected from older mice only (21–68 weeks) were subjected to ANCOVA, the effect of age was not significant ($r = 0.05$, $p > 0.5$). Figure 3 illustrates that compliance (C) and gradient (G) were significantly different between young (5–16 weeks) and old (21–68 weeks) groups.

Detailed Analysis of Middle Ear Pathophysiology and Tympanograms in LP/J Mice

To validate our tympanometry procedure, we tested LP/J mice for middle ear abnormalities using ABR, histology, video otoscopy and tympanometry. LP/J was chosen for extensive testing because this strain has been characterized as having middle ear abnormalities (Brodie et al., 1987; Chole et al., 1983; Chole et al., 1985; Cramer et al., 1986). Forty percent (14/35) of the LP/J mice exhibited OM with hearing loss as detected by histological and ABR analyses. Means and standard deviations of tympanometric readings from a group of LP/J mice with OM (designated as LP/J-OM) and a group without OM were analyzed (Fig. 4). LP/J-OM mice

exhibited deviating tympanograms; the affected middle ears had strikingly elevated compliance and pressure readings (two standard deviations from the grand mean of all 61 strains). The compliance readings of the LP/J-OM mice were as high as 2.0 ml, which was 1.6 ml above the average of all 61 strains. Figure 5 (A and B) shows a drastic difference in the tympanograms from the affected and unaffected ears of the same LP/J-OM mouse. The abnormal compliance measurements of the LP/J-OM mice were consistent with tympanometric readings of human ossicular disarticulation and/or a tympanic membrane that has healed over a perforation but is thinner and more compliant than expected (Fowler et al., 2002). When a deviation was observed in tympanometric readings taken from a mouse, video otoscopy was used to inspect the morphology of the ear canal and tympanic membrane. Video otoscopy revealed pus lesions on the tympanic membrane (Fig. 5C). Two affected left ears from two LP/J-OM mice had profound hearing loss as assessed by ABR threshold measurements. The mean thresholds for abnormal LP/J mice were 90, 80, 70 and 95 dB SPL for the click, 8 kHz, 16 kHz, and 32 kHz stimuli, respectively. The unaffected right ears had normal ABR thresholds (50, 50, 25, 60 dB SPL for the four stimuli, respectively).

Histological sections of the middle ears from selected LP/J mice showed serous fluid and mononuclear inflammatory cells, including fluid-laden macrophages (Fig. 5E). In some cases, middle ear sections showed the presence of both neutrophils and macrophages. These findings suggest that both chronic and acute inflammation can occur in the middle ears of LP/J mice and may be correlated with the previously reported otosclerosis-like bone lesions in the LP/J middle ear (Brodie et al., 1987;Chole et al., 1983;Chole et al., 1985;Cramer et al., 1986), with ossicular disarticulation, and with mild OM.

Tympanometry measurements from affected ears of LP/J-OM mice were significantly different from the unaffected ears of LP/J mice. T-test analysis on these two groups revealed significant differences between the compliance, gradient, and pressure values ($P < 0.05$), but no significant difference in volumes was found.

Discussion

Appropriateness and Precision of Tympanometry for Assessing the Mouse Middle Ear

Tympanometry gives a quick assessment of the status of the middle ear. Tympanograms are visual, straightforward and provide readily comparable measurements. Because susceptibility to OM is influenced by a variety of factors like immunodeficiency and other innate factors, normal tympanometric readings from mice within a strain do not exclude the possibility that other mice from that particular strain may exhibit middle ear infections. Furthermore, the accuracy of the middle-ear compliance measurement depends on how accurately the ear-canal volume can be estimated. Studies in humans have shown that middle-ear compliance measurements are significantly different between newborn and adult (Holte et al., 1990;Margolis et al., 2003).

The standard deviations of the compliance measurements exhibited by the 61 inbred mouse strains are consistent with standard deviations seen in humans. When nine consecutive tympanograms were taken in each of 53 humans, using a 226-Hz tympanometer at pressures ranging from 50 daPa/s to 400 daPa/s, the mean of the differences between compliances seen between the first and ninth tests varied from 0.112 ml to 0.127 ml with standard deviations that ranged from 0.097 ml to 0.107 ml (Gaihede, 1996). Considering the number of mice tested and the genetic variability among the 45 normal mouse strains, the average standard deviation of 0.13 ml for compliance values from both ears is within the expected range. Our data are consistent with other reports in human children (Pugh et al., 2004), taking into account that human equivalent ear-canal volume is much greater than that of mice. Pugh and colleagues

reported compliance, gradient and pressure variables from 182 native Hawaiian and 177 non-native Hawaiian children.

Relationship between Age and Compliance

No change in middle ear status, as assessed by tympanometry, was detected in human adults over 60 years of age when compared to a younger cohort (Stenklev et al., 2004). In our study of mice, a negative correlation between age and compliance of the tympanic membrane was found. Alteration in the functional response of the middle ear with age is expected because the membranes become harder and stiffer with age. In humans, some studies have presented evidence of a small decrease in middle-ear compliance (Gates et al., 1990) and changes in the viscoelastic properties of the tympanic membrane (Gaihede et al., 2000) with age. Furthermore, with increased age, the human tympanic membrane becomes less vascularized, less elastic, disorganized in its collagen structure, and more rigid in the middle fibrous layer (Ruah et al., 1991).

In mice, the age effect may be explained by structural maturation of the middle ear during mouse development. There are many factors that may decrease compliance of the tympanic membrane. The elastin and collagen fibers in the lamina propria layer of the tympanic membrane and/or the ligaments that support the ossicular system could deteriorate, resulting in decreased elasticity of the tympanic membrane in aged mice. Loss of compliance in some of the affected strains identified here could be the result of a combination of OM and disorders of cartilage or ossicles of the middle ear. However, additional studies are required to confirm these possibilities. Thus conductive hearing loss may be caused by abnormal development of the ossicles such as seen in Treacher Collins syndrome (Pron et al 1993) or delayed onset disorders of cartilage or bone like otosclerosis.

Few studies have addressed aging of the mouse middle ear, but in a study that used laser interferometry to measure sound-induced umbo velocity, a significant reduction in the umbo responses was seen in 2-year-old BALB/cJ mice when compared to 12-week-olds. Umbo response deterioration suggests middle ear structural changes (Doan et al., 1996). In agreement with Doan's findings, our data show that aged BALB/c mice have significantly increased middle ear gradient and pressure (Table II). A pathogen challenge study demonstrated that mouse strain differences influence the ability to induce OM and found that BALB/c mice were the most susceptible of three strains tested (Melhus et al., 2003).

Though we undertook a large screen of inbred strains, it was not possible to screen all inbred strains and each inbred strain at all ages. However, examples from our study compared to the literature (for inbred strains not tested here) provide some useful insight and possible explanations for the observed phenotype. Loss of compliance with age could be related to structural issues as discussed above, but it could also be caused by late onset of the disease in some of the inbred strains. Conversely, some inbred mice tested at younger ages may not develop disease until later ages when opportunistic bacteria combined with an altered immune system result in disease. A survey of the literature shows that older 129S6 mice (not tested here) showed middle ear disease after a year (Rosowski et al., 2003), much older than the mice studied here in some strains. Further, C3H/HeJ mice develop consistent middle ear disease because of a compromised TLR4 receptor (MacArthur et al., 2006), but the majority develop middle ear disease after 6 months. In our study, the oldest C3H/HeJ mice tested were 6 months old; hence, our tympanometry results yielded normal parameters for this strain.

One factor that influences susceptibility to OM in human is Eustachian tube dysfunction. In certain subsets of the human population, including those affected by Down's syndrome (Brown et al., 1989) and fetal alcohol syndrome (Streissguth et al., 1985), Eustachian tube dysfunction is associated with craniofacial abnormalities. Similarly, the OM-susceptible *Junbo* mouse

shows craniofacial abnormality (Parkinson et al., 2006). However, OM in the general human population is not linked to obvious craniofacial abnormalities. In this regard, it is noteworthy that the inbred strains identified with OM in this report do not show obvious craniofacial abnormalities either; thus, other factors must influence OM susceptibility in mice and, by extension, humans. These inbred mouse strains provide an excellent model system to investigate OM-susceptibility factors, especially genetic factors.

LP/J Strain, Validation of Our Paradigm for OM Screening

The LP/J mouse strain has a genetic middle-ear condition that results in a predisposition toward abnormal middle-ear function and increased susceptibility to OM. The LP/J mouse strain exhibits bony lesions of the middle ear and otic capsules that are congruent to human otosclerosis and tympanosclerosis (Chole et al., 1985). Otosclerosis, an inherited disease characterized by hearing degradation and abnormal bone growth in ossicular fixation, affects up to 10% of humans. Deterioration and loss of cochlear hair cells were evident in LP/J mice as early as 15 weeks of age (Chole et al., 1989). The degradation of hair cells in the inner ear may be secondarily caused by middle ear infection (Cook et al., 1999). The LP/J mice we tested showed an increased susceptibility to OM, which makes this strain an excellent candidate for OM genetic research. Furthermore, abnormal tympanometric measurements in LP/J mice suggest that tympanometry is an effective screening tool for middle-ear functional abnormalities. Our tympanometric data from LP/J mice show increased compliance, gradient values and middle ear pressure for affected ears (Fig. 5A). Compliance is the most sensitive parameter and showed the most significant increase. This could be explained by tympanic membrane atrophy or ossicle chain disarticulation. Tympanic membrane atrophy has been quantitatively related to decreased hysteresis and increased compliance (Gaihede et al., 1997). In children with recurrent acute otitis media, middle ear compliance was higher than normal controls in agreement with our data. In contrast with our data, however, middle ear pressures were actually lower than controls in this human study (Ryding et al., 2002).

Only a few animal models of OM have been reported, including the chinchilla (Bakaletz et al., 1998;Giebink, 1999) and the rat (Clark et al., 2000). The mouse system offers advantages over the chinchilla and rat for genetic studies. For example, a significant difference in middle ear inflammation and effusion formation was reported between two genetically different rat strains (Clark et al., 2000). If this work had been done in the mouse, the underlying gene(s) for this difference would have been easily elucidated because of the high-resolution positional cloning methods that have been developed for the mouse. With the wealth of data available from the mouse genome project and well-developed techniques for manipulating transgenes and inducing mutations, the mouse is arguably the best-suited animal model for genetic studies.

Characteristics of Strains That Exhibited Statistically Different Tympanometry Measurements

Four of the 15 mouse strains highlighted in Table II, C57L/J (6 weeks), I/LnJ (12 weeks), LP/J (31 weeks) and YBR/Ei (60 weeks), showed elevated ABR thresholds at their respective ages (Zheng et al., 1999b). It is possible that in some mice both inner and middle ear defects coexist. Determining whether this is the case will require extensive combined inner-middle ear studies including histological examinations. We plan to undertake such studies in the future. The next step in our project is to screen mutant mice for spontaneous OM occurrence. Since we first began screening mouse models for hearing loss (Zheng et al., 1999a), many studies have followed identifying genes for deafness (Di Palma et al., 2001;Hertzano et al.) with a 181 citation record so far. This report marks the beginning of a similar project in search of OM-susceptibility genes. Mutant mouse strains that exhibit abnormal tympanometry readings will be subjected to a comprehensive pathogen challenge test. Mutant mice exhibiting OM or other middle ear abnormalities will be subjected to genetic studies. This study will generate new

mouse models that can be used to explore the underlying genetic determinants that predispose one to middle ear disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS IN THIS ARTICLE

OM	Otitis Media
ABR	auditory brainstem response
SPL	

	sound pressure level
daPa	decaPascals
C	compliance of tympanic membrane
V	equivalent volume of outer ear canal
G	gradient
P	pressure at maximum compliance

Fig 1.

The mean compliance of five or more mice from each mouse strain tested. The datapoints on this graph are taken from Table II and have been sorted from lowest C value to highest for the right ear only. The standard deviation for each mean value is represented by a positive error bar. The grand mean of all C values was 0.42 ml. There is some degree of variability among the different mouse strains and the most extreme readings were considered to be abnormal. The two outliers at the low end of the scale were NZB/BINJ ($0.13\text{ml} \pm 0.04$) and EL/SuzSeyFrkJ ($0.18\text{ml} \pm 0.31$). The outliers at the high end were 129XI/SvImJ ($0.65\text{ml} \pm 0.18$) and C57L/J ($0.65\text{ml} \pm 0.11$).

Fig 2.

ANCOVA results for compliance C of the right ear versus mouse age in weeks, for all 61 mouse strains tested. This analysis reveals that as mouse age increased from 5 to 17 weeks of age, middle ear compliance decreased ($r = -0.308$, $p < 0.00001$). Compliance and age were the only parameters in this study for which a significant correlation was found across all 61 strains. This age range for mice is roughly analogous to a human age range from 10 years to 30 years of age.

Fig 3.

Comparison of mean values for each tympanometry parameter when all mice were divided into two age groups: a young group (dark bars, age range 5–16 weeks; $n=771$) and an old group (light bars, age range 21–68 weeks; $n=631$). Parameters are as described in Figure 1 with a subscript R indicating the right ear and subscript L for the left. Regardless of strain differences, compliance (C) and gradient (G) values were higher on average in the young mice. These differences were statistically significant as indicated by the following p values: C_R, $p=3.38405E-45$; C_L, $p=9.25861E-12$; all C values combined, $p=0.00024166$; G_R, $p=5.91079E-22$; G_L, $p=0.006715133$; all G values combined, $p=1.11329E-06$. Volume (V) and pressure (P) mean values were similar in both groups. Error bars represent the standard deviation calculated for each mean.

Fig 4.

Comparison of mean values for each tympanometry parameter in a single strain of mice (LP/J) with a predisposition toward middle ear abnormalities. Fourteen of 35 ears examined from LP/J mice were found by histology and ABR analysis to have OM and hearing loss. The abnormal ears (dark bars, n=14) gave increased compliance (C), gradient (G) and pressure (P) values by tympanometry compared to the normal ears (light bars, n=21). Error bars represent the standard deviation calculated for each mean.

Fig 5.

Comparison of the abnormal and normal ears from a single LP/J mouse. Tympanograms from each ear (A) and (B) show the greatly increased compliance (static admittance) of the abnormal ear. Tympanogram data were confirmed in video otoscope images of LP/J mouse tympanic membranes from the same two ears (C) and (D). Pus lesions are evident on the affected membrane as they obscure the normal light reflex (see arrow) evident on the unaffected membrane. Histological middle ear sections from these two ears further support the diagnosis of each ear. Serous fluid and mononuclear inflammatory cells (see arrow for an example), including fluid-laden macrophages, are visible in the infected ear (E). The inset in E shows the region highlighted in E by the black rectangle magnified 40X to allow visualization of individual mononuclear inflammatory cells. The normal ear shows a clear middle ear cavity (F, arrows indicate the unaffected cavity).

Table 1

Comparing Two Age Groups of CBA/CaJ Normal Mice

Age(wks)	Ear	C(ml)	V(ml)	G(ml)	P(daPa)
21-27	R(n=5)	0.42±0.11	0.2±0.02	0.11±0.03	-82±34.09
31-38	R(n=6)	0.41±0.13	0.22±0.03	0.09±0.07	-110±24.35
21-27	L(n=5)	0.4±0.14	0.2±0.02	0.08±0.03	-102±13.6
31-38	L(n=6)	0.4±0.08	0.2±0.02	0.21±0.03	-103±24.14

Eleven mice of the age ranges given were analyzed by tympanometry and the mean values and standard deviations were calculated for each parameter. R=right ear. L=left ear. C=compliance; V=volume; G=gradient; P=pressure.

Table II

Screening Inbred Mice by Tympanometry

Strain	C _R (ml)	V _R (ml)	G _R (ml)	P _R (daPa)	C _L (ml)	V _L (ml)	G _L (ml)	P _L (daPa)
129P1/ReJ	0.41±0.05	0.22±0.01	0.07±0.02	-117.14±19.45	0.35±0.07	0.21±0.01	0.04±0.02	-109.71±18.32
129P3/J	0.50±0.13	0.26±0.02	0.10±0.04	-117.50±25.35	0.44±0.11	0.26±0.01	0.07±0.04	-130.33±23.49
129S1/SvImJ	0.52±0.14	0.29±0.07	0.12±0.05	-83.06±21.39	0.50±0.16	0.26±0.06	0.11±0.06	-77.92±29.54
129X1/SvJ	0.65±0.18	0.25±0.04	0.16±0.07	-62.46±24.71	0.52±0.15	0.27±0.06	0.13±0.05	-67.67±20.63
A/HeJ	0.41±0.10	0.27±0.09	0.13±0.06	-87.60±30.61	0.50±0.14	0.22±0.03	0.13±0.07	-87.30±28.93
BALB/cJ	0.44±0.09	0.21±0.03	0.14±0.08	-65.42±20.50	0.42±0.09	0.22±0.03	0.11±0.03	-79.08±20.65
BUB/BnJ	0.45±0.11	0.26±0.06	0.10±0.04	-95.48±43.09	0.46±0.09	0.26±0.04	0.12±0.08	-101.52±50.47
C57L/J	0.65±0.11	0.22±0.02	0.15±0.05	-103.50±13.61	0.68±0.09	0.23±0.02	0.16±0.04	-97.08±15.65
EL/SuzSeyFrkJ	0.18±0.31	0.17±0.19	0.03±0.06	-122.00±37.93	0.34±0.33	0.19±0.20	0.07±0.06	-102.40±24.07
FVB/NJ	0.24±0.06	0.16±0.02	0.04±0.02	-90.67±65.59	0.34±0.12	0.17±0.02	0.06±0.04	-112.20±24.15
I/LnJ	0.42±0.12	0.24±0.01	0.05±0.03	-130.25±21.38	0.47±0.11	0.25±0.01	0.07±0.03	-138.83±28.89
LP/J	0.53±0.23	0.23±0.03	0.10±0.07	-114.17±20.94	0.70±0.48	0.25±0.14	0.14±0.13	-99.39±32.38
N7B/BlNJ	0.13±0.04	0.17±0.01	0.03±0.04	-90.64±55.45	0.29±0.10	0.21±0.05	0.06±0.02	-105.43±36.54
PL/J	0.54±0.13	0.23±0.03	0.09±0.04	-132.85±27.99	0.53±0.14	0.23±0.02	0.09±0.04	-130.42±33.81
YBR/Ei	0.44±0.12	0.25±0.04	0.07±0.03	-133.08±23.58	0.52±0.08	0.26±0.04	0.09±0.03	-87.50±32.97
CBA/CaJ	0.31±0.06	0.21±0.05	0.06±0.02	-105.70±27.25	0.38±0.15	0.21±0.04	0.07±0.04	-107.30±24.67
Grand Mean (n=61) ^a	0.42±0.10	0.22±0.03	0.08±0.03	-106.71±16.30	0.44±0.09	0.22±0.02	0.09±0.02	-104.23±13.25

Tympanometry values were measured on each ear for at least 5 mice of each strain tested and the mean ± standard deviation calculated for each. In addition to the normal control strain CBA/CaJ, only the 15 strains that yielded abnormal tympanograms are listed in this table. Values that deviated from the grand mean by more than 2 standard deviation units were considered abnormal and are highlighted in bold and underlined. Tympanogram data for the other 45 strains whose parameters were all within the normal range are available as online supplemental material. Subscripts R and L indicate right and left ears, respectively. C, V, G and P are defined in Table I, Fig. 1, and in the text.

^a A grand mean was calculated for each value from all 61 strains of mice (5 mice per strain).