

ARTICLE

Auditory Function and Hearing Loss in Children and Adults With Williams Syndrome: Cochlear Impairment in Individuals With Otherwise Normal Hearing

JEFFREY A. MARLER,* JESSICA L. SITCOVSKY, CAROLYN B. MERVIS, DORIS J. KISTLER, AND FREDERIC L. WIGHTMAN

Hearing loss is common in school-age individuals with Williams syndrome (WS) and extensive in adults. Prior studies with relatively small sample sizes suggest that hearing loss in WS has an early onset and may be progressive, yet the auditory phenotype and the scope of the hearing loss have not been adequately characterized. We used standard audiometric tools: Otoscopy, tympanometry, air-conduction (bone conduction when available) behavioral testing, and distortion product otoacoustic emissions (DPOAEs) to measure hearing sensitivity and outer hair cell function. We tested 81 individuals with WS aged 5.33–59.50 years. Sixty-three percent of the school-age and 92% of the adult participants had mild to moderately-severe hearing loss. The hearing loss in at least 50% was sensorineural. DPOAE testing corroborated behavioral results. Strikingly, 12 of 14 participants with hearing within normal limits bilaterally had 4,000-Hz DPOAE input/output (DPOAE IO) functions indicative of outer hair cell damage and impaired cochlear compression. Our results indicate that hearing loss is very common in WS. Furthermore, individuals with WS who have “normal” hearing as defined by behavioral thresholds may actually have sub-clinical impairments or undetected cochlear pathology. Our findings suggest outer hair cell dysfunction in otherwise normal hearing individuals. The DPOAE IO in this same group revealed growth functions typically seen in groups with noise-induced damage. Given this pattern of findings, individuals with WS may be at increased risk of noise-induced hearing loss. Recommendations regarding audiological testing for individuals with WS and accommodations for these individuals in both academic and nonacademic settings are provided. © 2010 Wiley-Liss, Inc.

KEY WORDS: Williams syndrome; genetic hearing loss; noise-induced hearing loss; sensorineural hearing loss; distortion product otoacoustic emissions

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INTRODUCTION

Considerable success has been achieved in identifying the genetic causes of deafness; however, much less is known about the contribution of genetic factors to milder forms of hearing loss. This is unfortunate, given that for every child born with profound hearing loss, one or two are born with less severe but clinically significant hearing deficits [Nance, 2003]. Hearing losses described as mild can have serious consequences for typically developing children with regard to acquisition of critical vocabulary comprehension and syntax skills [Davis et al., 1986; Norbury et al., 2001], understanding speech in noisy environments [Davis et al., 1986], and development of effective attention behaviors [Bess et al., 1998]. The cognitive and educational consequences of mild hearing loss for school-age individuals with neurodevelopmental disabilities, which would be expected to be more severe, have only rarely been addressed [Evenhuis et al., 2001; Laws, 2004; Van Naarden Braun et al., 2005]. Studies of hearing disability associated with genetic disorders provide an opportunity to better understand the genetic, biological, and therapeutic issues associated with hearing loss [Tekin et al., 2001].

Studies of hearing disability associated with genetic disorders provide an opportunity to better understand the genetic, biological, and therapeutic issues associated with hearing loss.

Williams syndrome (WS) is a rare neurogenetic developmental disorder with a prevalence of 1 in 7,500 live births [Strømme et al., 2002]. WS is caused by a hemizygous 1.5 Mb deletion on chromosome 7q11.23 [Ewart et al., 1993]; so far, 25 genes have been mapped to the deleted region [Hillier

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et al., 2003]. Approximately 95% of individuals with WS have the same set of genes deleted [Bayes et al., 2003]; most of the remaining individuals have additional genes deleted. WS is characterized by specific facial features (craniofacial dysmorphism), growth deficiency, developmental delay, mild to moderate intellectual disability, characteristic cognitive and personality profiles, connective tissue abnormalities, and vascular obstructive disease, most frequently supraaortic stenosis (SVAS).

The results of recent studies from several independent laboratories suggest that WS is associated with an increase in auditory pathology [Cherniske et al., 2004; Levitin et al., 2005; Marler et al., 2005, 2008; Gothelf et al., 2006]. Chronic otitis media occurs in 50% of children with WS [American Academy of Pediatrics Committee on Genetics, 2001; Mervis and Morris, 2007] compared to 41% of children in the general population [Auinger et al., 2003]. In the general population, the occurrence of otitis media frequently diminishes in older school-age children, only to increase again in middle age [Rudin et al., 1985; Kim et al., 1993; Daly et al., 1998]. Due to the genetic disruptions in the middle-ear system in WS, otitis media and the conductive hearing loss that frequently accompanies it may persist in adulthood [Cherniske et al., 2004; Marler et al., 2008]. High-frequency sensorineural hearing loss (SNHL) or mixed hearing loss in the mild to moderate range has been reported in 60–70% of school-aged children with WS [Marler et al., 2005, 2008; Gothelf et al., 2006] compared to 7% of the general school-age population [Bess et al., 1998; Niskar et al., 1998]. Gothelf et al. [2006] hypothesized that individuals with WS are hypersensitive to noise-induced hearing loss. Finally, even after controlling for middle-ear pathology and medical conditions or ototoxic medications known to impair middle-ear health, individuals with WS may evidence surprisingly poor cochlear outer hair cell function relative to expectations for their hearing thresholds [Marler et al., 2005, 2008].

The purpose of the present study was to further characterize auditory functioning in individuals with WS. We provide data on the incidence of hearing loss in the largest sample of individuals with WS examined to date. Of considerable clinical relevance is our finding that a large proportion of the school-aged participants with WS had previously undiagnosed hearing loss (conductive, sensorineural, or mixed). In addition, we focused on the question of whether individuals with WS who had “normal” hearing nevertheless evidenced cochlear pathology. For this purpose, we studied otoacoustic emissions. Below we briefly describe these emissions, how they are measured,

and what can be inferred from these measurements, before presenting the study methods and results. We report data indicating that individuals with WS who have normal behavioral hearing sensitivity may not have normal auditory function; these individuals may have a subclinical auditory dysfunction characterized by impaired cochlear compression.¹

Otoacoustic emissions are very low-level sounds that can be measured in the ear canal when test stimuli are delivered via an in-the-ear probe. They are part of a standard test battery to evaluate cochlear function. Otoacoustic emissions are not a direct measure of perceptual hearing sensitivity, but a noninvasive, objective test of the integrity of the cochlear outer hair cells. The outer hair cells are part of the cochlear amplifier system, largely responsible for the tremendous dynamic range of hearing [Ruggero et al., 1997; Robles and Ruggero, 2001; Kemp, 2002; Bacon, 2004]. The outer hair cells are thought to

1Cochlear compression: The non-linearity of the cochlea is reflected in the non-monotonic manner with which the range of perceptible sounds is psychologically and physiologically processed. Sounds near auditory threshold (just noticeable) show a near-linear growth of loudness, whereas sounds approaching and exceeding moderate-intensity levels demonstrate loudness-growth compression. As a sound reaches high intensity levels, a linear growth of loudness returns. DPOAEs have been used to objectively demonstrate impaired cochlear outer hair cell function [Dorn et al., 1998].

have a motor capability allowing them to change their shape according to the frequency characteristics of an acoustic stimulus [Holley, 1996; Bacon, 2004]. These changes in electromotility result in an amplification or increased vibration of the basilar membrane. The amplification gain can be as much as 50–80dB, especially for low-to-mid intensity sounds [Ruggero et al., 1997]. Another phenomenon associated with the large dynamic range and frequency selectivity of hearing is cochlear compression. The mechanical responses of the cochlea to stimulus changes of low-intensity levels are linear, including those generated by the outer hair cell system. As intensity levels increase, the responses become compressed (nonlinear), with high-intensity stimuli again eliciting linear responses [Robles and Ruggero, 2001]. It is generally accepted that the outer hair cells play a primary role in modulating cochlear compression [Robles and Ruggero, 2001; Oxenham and Bacon, 2003]. Cochlear hearing losses caused by damage to the outer hair cells result in elevated behavioral thresholds, loudness recruitment (a disproportionate growth of perceptual loudness in the hearing-impaired listener), difficulty perceiving shorter-duration sounds (temporal integration), and difficulty understanding speech in a noisy environment [Oxenham and Bacon, 2003].

One clinical method of testing outer hair cell function is distortion product otoacoustic emissions (DPOAEs). DPOAEs are produced in the cochlea when two pure-tone stimuli [frequency 1 (f_1)

1

) and frequency 2 (f_2)

2

)] cause the

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et generation of non-linear intermodula-

tion tones. The intensity of one of the

tones ($2f_1 - f_2$) is a common clinical metric to evaluate cochlear integrity [Kemp, 2002].

1

f_1

2

) is a common clinical metric to evaluate cochlear integrity [Kemp, 2002].

MATERIALS AND METHODS

DPOAEs are especially helpful clinically

and The research protocol was approved by for predicting auditory sensitivity

the James Madison University Institu- cochlear health in individuals with

tional Review Board. Written consent ing thresholds 50 dB HL [Gorga et al.,

to participate was obtained from parents 1997, 2000] and are useful in

of all children, from parents of all adults ing behavioral measures in

who had legal guardians, and from all who have intellectual disabilities

[Neu-

2008].

reliable

[Kon

amplitudes

relevant

large-scale

2000,

demonstrated (e.g., by acoustic immittance testing or tympan-

Participants

ometry), very low-amplitude DPOAEs

coch-

primary

as low-

individuals

sensitivity have

Mills

Marshall

pairs

adult participants who did not have legal man et al., 2006; Hild et al.,

guardians. Written assent was obtained DPOAE recordings are stable and

from all children aged 7 years or older for individuals 7 years and older

and from all adult participants who et al., 2000].

had legal guardians. Each participant Historically, the detectability rather received a \$20 gift card for participation, than the strength of DPOAE

and parents were sent a written report has been considered clinically

summarizing the results of the evalua- [Kemp, 2002]. However, in a

tion of hearing sensitivity and auditory clinical study, Gorga et al. [1997,

function. 2005] showed that when normal mid- dle-ear status was

This study included two groups of could be interpreted as suggesting

participants. The primary group was lear pathology. DPOAEs are of

composed of 81 individuals with genet- importance to the present study

ically confirmed WS ranging in age from amplitude DPOAE levels in

5.33 to 59.50 years. Portions of the data with “normal” hearing

from 27 of these participants were also been hypothesized to reveal sub-

previously reported [Marler et al., clinical noise-induced damage [Zheng

2005]. Participants were recruited et al., 1997; Lucertini et al., 2002;

through the national Williams Syn- et al., 2007; Sisto et al., 2007;

drome Association (WSA), from an et al., 2009]. Furthermore, when

ongoing study of the development of DPOAEs are measured to single

children with WS conducted by of pure tones, with a sweep from high to

IO)
compres-
of the
further
cochlear
impairment
sensorineural
Johan-
Thus,
historically been
recent
be
in
sen-
physiological
hearing
Seixas
males, 7 females) aged 7.76–32.5 years

C.B.M., and through referrals from low stimulus intensities, the resulting geneticists and private physicians. Par- DPOAE input/output (DPOAE participants with WS were divided into two function gives insight into the groups. The School Age group (n1/443, sive, nonlinear sound processing 17 males, 26 females) ranged in age from cochlea as well as providing 5.33 to 17.92 years [mean CA (chronological age): 12.08 years, SD: 3.33 years, compression and cochlear median CA: 11.67 years]. The Adult in individuals with known group (n1/438, 20 males, 18 females) hearing loss [Dorn et al., 2001; ranged in age from 18.00 to 59.50 years nesen and Lopez-Poveda, 2008]. (mean CA: 31.83 years, SD: 11.75 years, while DPOAEs have median CA: 28.25 years). used to identify mid-to-high-frequency To test the hypothesis that indi- hearing loss [Gorga et al., 1993], individuals with WS who have normal research indicates that they may also behavioral hearing may actually have an important first round of defense subclinical auditory impairments, a attempts to identifying subclinical group of 14 typically developing sorineural hearing loss and individuals who had hearing within susceptibility to noise-induced normal limits bilaterally (TDNH; 7 damage [Lucertini et al., 2002; males, 7 females) aged 7.76–32.5 years

2Distortion product otoacoustic emissions (DPOAE): one type of sound generated by the outer hair cells of the cochlea, transmitted outwardly through the middle ear system to the external auditory meatus, where it can be measured by a sensitive microphone; elicited by presenting represents two the tones cubic in the distortion ear canal (f

product and f

2

);

(2f by 1

Àf outer 2

); the hair amplitude cell of DPOAE is reduced damage. DPOAEs are clinically useful for obtaining frequency-specific information from the cochlea, and have been reported to be sensitive to exposure to noise- induced damage.

(mean CA: 13.86 years, SD: 7.37 years, median CA: 10.67 years) was matched for gender and CA to 14 individuals with WS who also had hearing within normal limits bilaterally (WSNH; mean CA: 14.25 years, SD: 7.67 years, median: 11.08 years).

Equipment

We evaluated auditory function using two different methods in two separate settings. Most of the participants with WS (n1/469) were tested in quiet locations outside of clinic settings, typically at one of the WSA conventions. In the “convention protocol,” instrumentation consisted of a Grason-Stadler GSI-38 Auto Tympanometer, allowing the evaluation of both tympanometry and pure-tone, air-conduction behavioral responses (TDH-39 headphones). When we were able to evaluate individuals with WS in a standard clinic setting (“clinic protocol,” n1/412), hearing was tested with a Grason-Stadler GSI-61 audiometer (ER-3A insert ear-phones) and a Grason-Stadler Tymptstar. DPOAEs were elicited in both protocols using an Intelligent Hearing Systems (IHS) SmartOAE system with insert earphone (10D-OAE probe).

Procedures

Medical and hearing background. Parents of all participants with WS were interviewed about the participant’s medical and hearing background. The questions focused on the risk factors associated with hearing loss, specifically history of exposure to noise, occurrence and frequency of otitis media, other diseases of childhood associated with hearing loss, and medications known to be ototoxic.

Middle ear function. The possible presence of middle ear disorders was evaluated with otoscopic observation of the external ear canal for any tympanic membrane abnormalities and with acoustic immittance (tympanometry). Otoscopy was determined unremark-

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able when the tympanic membrane color was normal and the position was not retracted or bulging. Acoustic immittance measures used to determine middle ear function were obtained using a commercially available middle ear analyzer (Grason Stadler, Model GSI- 38). These tympanometric measures were recorded using a 226-Hz probe tone and judged within normal limits when static compliance (a measure of tympanic membrane mobility) was between 0.3 and 1.2cm³ and peak pressure in the middle ear was ± 100 daPa₃ [Alaerts et al., 2007]. In the convention protocol, participants with minimal response levels >25 dB HL₄ at 500 kHz in either ear (even in the presence of acceptable tympanometric results) were assumed to be showing a detrimental middle ear contribution to the hearing responses and therefore were not included in further hearing or DPOAE analyses. In the clinic protocol, bone-conduction testing was used to further evaluate middle-ear contributions and the presence of air-bone gaps.

Behavioral hearing sensitivity. In the clinic protocol, participants were tested in a noise-controlled environment (double-walled booth) with ambient noise levels sufficiently low to permit diagnostic assessment of hearing thresholds [ANSI, 1996]. Ambient noise levels in the convention protocol precluded the measurement or reporting of absolute thresholds; however, one of the evaluators (J.A.M. or J.L.S.) checked the stimulus levels daily to ensure that 0 dB HL stimuli across test frequencies between 500 and 8,000 Hz were audible. It is important to note that even in the convention protocol, some participants responded to test signals at 0dB HL across all test frequencies, further

3daPa: DecaPascal, a unit of measurement for pressure, during tympanometry used to provide a comparison of ear canal air pressure to ambient air pressure. 4dB HL: Decibels hearing level, a unit of sound intensity, a scale referenced to accepted standards for normal hearing and used to report audiogram information.

demonstrating that stimuli were audible even at very low intensity levels and that the convention protocol environments were sufficiently quiet. An additional demonstration of the integrity of the data is that four individuals seen during the WSA conventions were subsequently seen by independent, licensed audiologists for full audiometric evaluations. The audiological reports for these individuals indicated that their diagnostic threshold responses were within 5 dB of the responses measured in our convention-protocol environment. This is considered

well within clinical measurement error. For these reasons, we have collapsed participant responses across the two protocols and have reported behavioral hearing sensitivity data as “minimum response levels” (rather than suggesting that all are estimations of hearing threshold).

In both protocols, a standard bracketing method was used in which test tones were first presented at an a priori estimated comfortable suprathreshold level (30dB HL). If there was no response, the tone level was increased until the participant responded. At that point the tone was decreased in 10dB steps until the participant failed to respond, at which point the tone level was raised in 5 dB steps until a response occurred. This process was continued until at least two ascents and two descents had been completed.

Hearing levels for both ears were determined at frequencies 500, 1,000, 2,000, 3,000, 4,000, 6,000, and 8,000Hz for air conduction in both protocols. In the clinic protocol, bone conduction was used, when necessary, at octave frequencies between 500 and 4,000 Hz. For the School Age group, a response >20dB HL at any single frequency in a single ear measurement was considered a “fail.” For the adult group, any response at a level higher (poorer) than the normative data reported by the International Organization for Standardization [ISO, 1984] for the participant’s age was considered a “fail.” This criterion takes into account normal age-related changes in hearing sensitivity [Cruickshanks et al., 1998].

Frequency-sweep distortion product otoacoustic emissions (DPOAEs). The frequency-sweep DPOAEs were measured to pairs of primary tones, with f

1

and f

2 frequencies at a fixed f

2

/f

1

ratio of 1.22. The elicited f

2

frequencies ranged from 1 to 8kHz, in 1/8th-octave steps. The intensity levels of the primary-tone pairs were fixed across all frequencies, (intensity level of f

1

)1/465 dB SPL5 with and

L

1

L

2

(of f

2

)1/455 dB SPL. These intensity levels were chosen because of previous reports that they best discriminate between normal and impaired ears [Gorga et al., 1997]. Due to some reports of decreased reliability of the DPOAEs at very low and high frequencies [Roede et al., 1993; Siegel and Hirohata, 1994; Marshall et al., 2009], analyses were restricted to f

2

DPOAE frequencies between 1,500 and 6,000Hz. To decrease the likelihood of an error resulting from a single-frequency outlier, for each frequency of interest (1,500, 2,000, 3,000, 4,000, and 6,000Hz), we averaged the DPOAE level immediately preceding and immediately following the frequency of interest (e.g., DPOAE f

2

levels reported for 1,500Hz were derived by averaging the DPOAE f

2

levels at 1,418, 1,550, and 1,687Hz). Further analyses were then performed on these averaged frequency-sweep DPOAEs to make direct comparisons to normative data [Knight and Kemp, 2000].

A DPOAE “fail” was any single averaged DPOAE amplitude (dB SPL) within the 1,500- to 6,000-Hz range falling at or below the 5th percentile of normal-ear distributions as reported by the Gorga et al. [1997] normative study. When one or more DPOAE amplitude(s) fell at or below the 5th percentile, it was interpreted as predictive of impaired cochlear function.

Cochlear compression as measured by DPOAEs. Finally, to test the theory that hearing loss in WS may result from a physiological vulnerability to noise

polynomials) levels present in a typical environment,

with normal n1/46; typically develop- ing individuals who had hearing within

have been fit to either we tested the 4,000-Hz DPOAE IO average or individual data previously function with the 14 participants [Dorn et al., 2001; Williams and Bacon, WS who had hearing within 2005; Johannesen and Lopez-Poveda, limits (WSNH; clinical protocol, 2008]. convention protocol, n1/48) and 14 age- and gender-matched

RESULTS

normal limits (TDNH; clinical protocol, n1/411; convention protocol, n1/43).

Middle Ear

Dorn et al. [2001] found that at

significantly intensity levels is DPOAE IO func- with the 8,000 Hz. elicit amplitudes if we making it significant group reported in the

Routine otoscopic examination revealed 4,000Hz, there was a that the tympanic membrane was either reduced range of stimulus wholly or partially visualized in 74/81 over which cochlear compression participants. Of the seven participants with clearly demonstrated. significant cerumen buildup, two had tions were performed at the ear tympanometric measurements within best (lowest) high-frequency, pure-normal limits and were included in tone average minimal response levels hearing and DPOAE analyses. Our obser- measured at 4,000, 6,000, and vations confirm previous reports of exces- We predicted that we would sive cerumen build up (ear wax) in both the strongest DPOAE children and adults with WS [Cherniske used the best-ear data, thereby et al., 2004; Marler et al., 2005]. Due to a more difficult to find a growing awareness in the WS community difference. As previously

section, the

of the prevalence of hearing loss and the frequency-sweep DPOAE

importance of cerumen management, DPOAE IO function was measured

several of the participants' families to pairs of primary tones (f

1

and f

2

)

reported having excess cerumen removed with a fixed f

2

ratio at 1.22, and

prior to study participation. with the f

2

To evaluate whether certain effects of otitis media might persist into later childhood or adulthood, we analyzed middle ear compliance and pressure. It is necessary to determine that middle ear function is within normal limits prior to interpreting the DPOAE amplitudes. Tympanometry could not be tested in 4 of 81 participants. One adult and two school-age participants reported being afraid of the sensation caused by the tympanometry stimulus. In one other adult (age 14/53 years) we could not achieve a seal for either ear. This participant's audiogram (clinic protocol) showed hearing loss in the lower and higher frequencies but a threshold within normal limits at 2,000Hz. This pattern is often reported in otosclerosis and therefore is a strong indicator of middle ear dysfunction. In 11 participants (7 School Age and 4 Adult), a seal could be maintained sufficient for measurement in only one ear; for these individuals, only unilateral tympanometric results were available. As we did not have bilateral tympanometric measurements for these 15 participants and it /f

1 frequency set to 4,000Hz. The DPOAE IO was elicited with L

1 levels ranging from 75 to 35dB SPL and L

2

levels ranging from 65 to 25 dB SPL.

Statistical Analyses

Repeated measures ANOVAs, paired comparisons, and t tests were performed using the Statistical Package for the Social Sciences version 17.0 (SPSS 17.0) with $\alpha/40.05$. In order to assess cochlear compression in the WSNH group, DPOAE IO functions were fit using multilevel modeling [Moskowitz and Hershberger, 2002; Raudenbush and Bryk, 2002]. Multilevel modeling is particularly well suited to analyses of these data, as it permits simultaneous fits of group (WSNH vs. TDNH) and individual DPOAE IO functions, as well as providing a measure of the extent to which functions for individuals deviate from group or average data. We used HLM 6.0 [Raudenbush et al., 2004] to estimate second-order polynomial functions. Non-linear functions (e.g., cubic

5dB SPL: Decibels sound pressure level, unit of sound intensity, a scale referenced to 20 micro-Pascals.

is possible that the unmeasured ear was not within normal limits, the middle ear measurements from these participants were not included in any of our analyses. The results for the individuals for whom bilateral tympanometric measurements were obtained are presented in Table I. In the School Age group with bilateral data, 34/34 children had tympanometric measurements falling within normal limits. Of the Adult participants for whom bilateral data were obtained, 7 of 32 (22%) had tympanometric measurements for one or both ears falling outside the acceptable range for a healthy Type A tympanogram, indicating abnormal middle ear function (5 with Type Ad tympanograms indicating normal middle ear pressure but high compliance and 2 with Type C tympanograms indicating normal compliance but significant negative pressure). These data suggest a larger percentage of adults with WS may have middle-ear pathology than the approximately 17% expected in the general adult population [Rudin et al., 1985; Kim et al., 1993].

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0.597, These data suggest a larger

$P_{1/4} 1/40.442$, effect for Ear [$F(1,64)_{1/4} 1/41.465$, h_2

p

$1/4 0.009$]; the main $P_{1/4}$ percentage of adults with WS

0.231 , $h_2 p$ may have middle-ear pathology than the approximately 17% expected in the general adult population.

$1/4 0.022$], and the Group \hat{A} Ear interaction [$F(1,64)_{1/4} 0.029$, $P_{1/4} 0.866$, nificant. $h_2 p$

Sixty-five $1/4 0.001$] also were not sig- participants were included in the pressure measures [33 School Age (pressure data were missing for one school-age participant), 32 adult]. Neither the group effect

The pure-tone and DPOAE measure-

[$F(1,63)_{1/4} 1/40.030$, nor the Ear effect $P_{1/4} 1/40.863$, [$F(1,63)_{1/4} 1/4879$,

$h_2 p$

$1/4 0.002$]

ments from these seven Adult parti- cipants were not included in the later

$P_{1/4} 1/40.352$, $h_2 p$

hearing or DPOAE analyses.

To investigate acoustic immittance of the middle ear system, two 2 (Group: School Age, Adult) \hat{A}^2 (Ear: Right, Left) mixed-model ANOVAs were performed on compliance (cm^3) and pressure (daPa). As stated above, analyses were only performed on participants who had normal bilateral tympanometry results. Sixty-six participants were included in the compliance measures (34 School Age, 32 Adult). The Group effect was not significant [$F(1,64)_{1/4}$

$1/4 0.014$] was significant. There was a significant Group \hat{A} Ear interaction [$F(1,63)_{1/4} 1/44.640$, $P_{1/4} 1/40.035$, h_2 there p

$1/4 0.069$]. was In the School Age group, greater middle ear negative pressure in the left ear than in the right. This relation was reversed in the Adult group. However, both the confidence interval and the negligible effect size suggest that the Group \hat{A} Ear interaction is not clinically meaningful (see Table I). The lack of a significant Group effect is interesting because one might have expected decreased compliance and increased negative middle-ear pressure

TABLE I. Descriptive Statistics for Tympanometric Variables for the Evaluation of Compliance (cm^3) and Pressure (daPa) of the Middle-Ear System for Right, Left, and Averaged Ears as a Function of Group (SA, School Age). The 90% Confidence Intervals Have Been Included for the WS Data and for Tympanometry Norms* for Typically Developing Individuals

Group n Ear Mean (cm3)

WS sample (90% range)

Norms* (mean, cm3)

Norms* (90% range)

Right 0.63 0.33–0.93 WS—School Age 34 Left 0.52 0.36–0.68

Average 0.58 0.36–0.80 0.5 0.3–1.11 Right 0.77 0.46–1.08 WS—Adult 32 Left 0.68 0.51–0.85

Average 0.73 0.50–0.96 0.72 0.27–1.38

Group n Ear Mean (daPa)

WS sample (90% range)

Norms* (mean, daPa)

Norms* (90% range) Right À13 À18 to À8 WS—School Age 33a Left À24 À34 to À14

Average À19 À25 to À13 À5 À80 to 25 Right À22 À27 to À17 WS—Adult 32 Left À17 À27 to À7

Average À20 À27 to À13 76 51 to 114

aTympanometric pressure data was missing for one school-age participant. *The 226-Hz tympanometry norms used for the School Age group were those of Haapaniemi [1997] and Margolis and Heller [1987] for the Adult group.

to be more prevalent in the School Age group, becoming less pronounced in adults, as has been reported in the general population [Daly et al., 1998; Coyte et al., 1999]. The failure to find a main effect for Ear may be an artifact of the analysis method, as we included only data from individuals with bilaterally normal tympanometry results.

Auditory Function

Behavioral hearing sensitivity. Traditional behavioral audiometry could not be obtained from two School Age participants (one in each protocol). A modified play-audiometry protocol was used with these children, measuring participant response to 20 dB HL tones at octave frequencies between 500 and 8,000 Hz in a free-field environment. Both participants passed, indicating pure-tone responses sufficient for speech, but as their responses were to single “screening” frequencies rather than being presented in the standard stair-step method, their data were not included in the hearing analyses.

To explore possible differences in hearing sensitivity as a function of age, the hearing data were submitted to a 2 (Group: School Age, Adult) × 2 (Ear: Left, Right) × 5 (Frequency: 0.5-, 1-, 2-, 4-, 8-Hz) mixed-model ANOVA. Some School Age participants’ attention to task was insufficient for the length of time required to measure mid-octave frequencies, so 3,000- and 6,000-Hz data were not obtained in some cases. All participants who had tympanometric data indicating middle ear pathology or who lacked tympanometry results for both ears were excluded from the hearing sensitivity analyses. Accordingly, 59 participants (34 School Age, 25 Adult) were included in these analyses. A significant Group × Frequency interaction was obtained [$F(4,54) = 144.243$, $P = 0.005$, main effects $h^2 = 0.239$].

In addition, $F(1,57) = 12.785$, $P = 0.001$, Frequency [$F(4,54) = 424.351$, $h^2 = 0.183$], and

$h^2 = 0.183$]

$P < .001$, and

$h^2 = 0.643$]

for were significant. The main Ear ($P = 0.06$), the Group × Ear interaction ($P = 0.10$) and the Group × Ear × Frequency interaction ($P = 0.80$) were not significant.

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Post hoc paired comparisons were performed to investigate the Group × Frequency interaction. A Bonferroni adjustment was calculated to correct for multiple comparisons (0.05/10 with $P = 0.005$). Results indicated that the Adult group had significantly higher (worse) minimal response levels at 4,000 and 8,000 Hz (the two highest frequencies tested) than the School-Age group. These cross-sectional analyses support previous reports that hearing loss in WS may be progressive [Marler et al., 2005, 2008].

As most of the hearing measures (minimal response levels) were performed in the convention protocol, we have not presented the group data in a standard audiogram format. A large proportion of the participants across both age groups had higher-than-expected minimal response levels, indicating hearing loss [School Age: 20 of 34 (59%), Adult: 21 of 25 (84%)]. Furthermore, in 9 of 20 School Age (45%) and 6 of 21 Adult (29%) participants, the hearing loss was unilateral. It has been shown that typically developing children with unilateral hearing loss are at increased risk for academic failure and learning disabilities [Bess et al., 1998]. We plotted worst-ear data, as that appears to be a more accurate indicator of performance in academic settings and functional, everyday environments [Bovo et al., 1988; Bess et al., 1998]. We made the worst-ear determination using a high-frequency pure-tone average of frequencies 4,000 and 8,000 Hz. Figure 1 presents the 1,000-, 4,000-, and 8,000 Hz worst-ear data for all participants with middle-ear function within normal limits. The data are plotted in comparison to the 90th percentile for the normative data for children as reported by the Third National Health and Nutrition Examination Survey [Holmes et al., 2004] and the 90th percentile for otologically normal males as reported by the ISO [1984]. The male results from the survey are plotted since those thresholds were equal to or greater than the female

thresholds.

The hearing threshold data from all three frequencies, but especially 4,000 and 8,000Hz, indicate that the incidence of hearing loss in individuals with WS is considerably greater than that reported for the general population: 7% of school-age children [Bess et al., 1998; Niskar et al., 1998] and 46% of adults between the ages of 46 and 80 years [Cruikshanks et al., 1998]. The lack of bone-conduction measurements in the convention protocol precludes ruling out a conductive contribution in the group minimal response levels, particularly in the lower frequencies (4,000Hz). Recall that all the above analyses included only participants with Type A (normal) tympanograms. Therefore, the extent of hearing loss in the higher frequencies supports the interpretation of at least a mixed hearing loss (i.e., both conductive and sensorineural components) in these individuals. When all participants with minimal response level data are factored in (including those with unilateral middle ear pathology), the incidence of hearing loss increases [School Age: 24 of 38 (63%), Adult: 33 of 36 (92%)].

We were able to evaluate 12 individuals with WS in the clinic protocol that included bone conduction measures. Of those 12 participants, 10 were school-aged (7–18 years). The thresholds of this subset of the School Age group are plotted as an audiogram in Figure 2. Of the 10 participants, 5 had mild high-frequency SNHL and 1 had moderate high-frequency SNHL. Three of the six participants with SNHL had unilateral loss. Three of the six participants with SNHL had lower right-ear high-frequency pure-tone averages (averaged at 4,000, 6,000, and 8,000Hz) and three had lower left-ear high-frequency pure-tone averages. It is important to note that four of the six participants with high-frequency SNHL had thresholds 20 dB HL in frequency regions between 1,000 and 4,000Hz. Therefore, these four participants would have passed a standard hearing screening as performed in school settings.

Cochlear outer-hair cell integrity. Best-ear, frequency-sweep DPOAEs. Best-ear frequency-sweep DPOAE amplitudes were analyzed from the 49 participants who had [1] normal bilateral tympan-

ometry, [2] minimal response levels 20dB HL at 500 and 1,000Hz, and [3] minimal response levels 40 dB HL at 2,000, 3,000, 4,000 and 6,000 Hz in the best ear (or minimal response levels 40dB HL at 2,000 and 4,000Hz if hearing data for 3,000 and 6,000Hz were not available). These criteria were chosen because DPOAEs are most appropriate for detecting mild to mild- to-moderate hearing loss [Gorga et al., 1997, 2005] and to further minimize possible detrimental middle ear contributions to DPOAE amplitudes. Based on these criteria 10 participants (6

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Figure 1. Pure-tone minimal response levels for worst ear as a function of age for 36 children and 21 adults with WS. For comparison, worst-ear minimal response levels at frequencies 1,000, 4,000, and 8,000Hz are plotted against the 90th percentile for children [based on Holmes et al., 2004] and the 90th percentile for male adults (based on ISO [1984]). The small vertical lines identify overlapping data points.

School Age, 4 Adult) were excluded, leaving 49 (28 School Age, 21 Adult) for the analysis. Data at 2,000, 3,000, 4,000, and 6,000Hz were available for all 49 participants.

To explore the predicted DPOAE differences as a function of age, best-ear average DPOAE amplitudes were submitted to a 2 (Group: School Age, Adult) x 5 (Frequency: 1,500-, 2,000-, 3,000-, 4,000-, 6,000 Hz) mixed-model ANOVA. Neither the Group effect [$F(1,47) = 140.053$, $P = 1/40.819$, h^2 Frequency p

$1/4 0.001$] interaction nor the Group x Frequency [$F(4,44) = 140.955$,

$P = 1/40.442$, majority $h^2 p$

$1/4 0.080$] the participants was significant. In the School Age group had best-ear minimal response levels 25dB HL across all frequencies, whereas 60% of the Adult group had minimal response levels ≥ 35 dB HL for at least two frequencies. On this basis, one might have expected DPOAE amplitudes to be lower (poorer) in the Adult Group. However, it is possible that DPOAE levels were already depressed in many of the School Age participants, resulting in a significant reduction in the overall range of outer hair cell activity in both “normal” and mild-hearing loss ears in individuals with WS. This hypothesis is supported by the results of the DPOAE IO function data reported later.

The main effect of Frequency was significant [$F(4,44) = 1427.750$, $P < 0.001$, h^2 with p

$1/4 0.72$]. A Post hoc paired comparisons Bonferroni adjustment (0.05/5 with $P = 1/40.01$) indicated that for both groups the averaged DPOAE f

2

amplitudes were lowest (poorest) at 4,000 Hz, those at 3,000 and 6,000Hz did not differ significantly, and that the 3,000-, 4,000-, and 6,000-Hz averaged DPOAE f

2

amplitudes were lower (poorer) than those at 1,000 and 2,000Hz. Figure 3 plots the DPOAE f

2

amplitudes as a function of frequency for the participants with WS who had normal bilateral tympanometric results against the Gorga et al. [1997] normative data. As can be seen from Figure 3, a significant number of averaged DPOAE f₂ amplitudes at 4,000Hz were lower than the 5th percentile criterion of the Gorga et al. [1997] data. These data corroborate the behavioral results and support the presence of impaired cochlear function in the majority of participants with WS. This is an important corroboration given the intellectual disability associated with WS and possible difficulty these individuals might have with threshold estimation, an attentionally demanding task. The absence of a significant Group effect in the DPOAE amplitude levels suggests that outer hair cell function in the School Age group was not significantly better than in the Adult group, despite the significantly better hearing sensitiv-

Figure 3. Best-ear averaged DPOAE amplitudes as a function of f2 frequencies for participants with WS who had normal tympanometric results. Any 4,000-Hz DPOAE f2 amplitudes ≤ 9.23 dB SPL [the 5th percentile for normal ears as reported by Gorga et al., 1997] are predictive of impaired cochlear function. The majority of participants with WS have impaired cochlear outer hair cell function, providing further support for the behavioral hearing data. Flag 1/41 SEM.

Figure 2. Thresholds for best ear and worse ear are plotted in a standard audiogram format for 10 school-age participants with WS evaluated in the clinic protocol. Five of the 10 participants had mild high-frequency SNHL and 1 of the 10 had moderate high-frequency SNHL. The hearing loss for three of the six participants with SNHL was unilateral. Three of the six participants with SNHL had lower right-ear high-frequency pure-tone averages (averaged at 4,000, 6,000, and 8,000 Hz) and three had lower left-ear high-frequency pure-tone averages. Flag 1/41 SEM.

ity in the School Age group (see the hearing analyses above).

4,000-Hz DPOAE input/output function. To evaluate the presence of cochlear compression in individuals with WS who have hearing within normal limits (WSNH), we compared DPOAE IO functions at 4,000 Hz in 14 WS participants (12 School Age, 2 Adults) with those of 14 age- and gender-matched typically developing (TDNH) participants. For all of these individuals, response levels were 20dB across all test frequencies. We chose to evaluate 4,000Hz based on previous reports [Dorn et al., 2001] that the slope of the DPOAE I/O function at this frequency was most sensitive to the effects of hearing-loss or sub-clinical cochlear pathology (a steeper slope indicating diminished compression due to cochlear damage).

To confirm that the minimal response levels for best ear did not differ significantly across groups, we first performed a 2 (Group: WSNH, TDNH) \times 6 (Frequency: 1,000-, 2,000-, 3,000-, 4,000-, 6,000-, 8,000 Hz) mixed-model ANOVA on the best-ear hearing data. The Group [F(1,26) 1/42.857, P1/40.103, h2 interaction p

1/4 0.099] [F(5,22) 1/4 and the Group \times Frequency 1.294, P1/40.162, h2 The p

1/4 0.047] main effects were not significant. effect for Frequency was significant [F(5,22) 1/42.417, P1/40.04, h2 isons p

1/4 0.085]. with Post hoc paired comparison a Bonferroni adjustment (P 1/40.05/61/40.008) indicated that minimal response levels at 4,000 Hz were lower than those for 3,000 Hz but other frequencies did not differ significantly (P ! 0.144).

Post hoc review of individual 4,000-Hz DPOAE IO function data revealed that the lowest intensity for which all participants from both groups had responses ≥ 3 dB above the noise floor was at DPOAE f

2

levels of 50 dB SPL. To determine if there was a difference in OHC response as a function of stimulus intensity, multilevel modeling was used to describe the relation between DPOAE IO response amplitude and f

2

level (75, 70, 65, 60, 55, 50 dB SPL).

Multilevel models are particularly valuable because they not only model change at the individual level (e.g., Level 1 model/within-subject variability) but also model differences (e.g., Level 2 model/between-subject variability) in the parameters estimated at Level 1 for each individual. Examination of Figure 4, which includes both group averages and individual data, suggests that the data for both groups are best described by curvilinear functions with different shapes. We found that a second-order polynomial or quadratic function best described these data.

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Figure 4. DPOAE IO function, a physiological correlate of normal behavioral dynamic range and cochlear compression. The top graph shows the averaged fitted function for each group (TDNH, typically developing with normal hearing; WSNH, Williams syndrome with normal hearing). Group mean amplitudes are plotted as a function of DPOAE f2. Flags 1/41 SEM. The bottom graph shows individual participant data for both groups, demonstrating the small degree of group overlap at the higher- intensity DPOAE f2 amplitudes. The WSNH group had lower DPOAE levels with a precipitous drop in level as stimulus intensity decreased. Multilevel model analyses showed a significant difference between the curvilinear functions of the two groups. This difference suggested a loss of cochlear compression in the WSNH group, all of whom had minimal response levels within normal limits bilaterally across all frequencies.

The parameters of this function have theoretical importance in that the linear coefficient reflects instantaneous rate of change and the quadratic coefficient indicates the acceleration or deceleration in the DPOAE as a function of stimulus level. The Level 1 model is given by

DPOAE

ij

1/4 p

0i

p p

li δSI

ij

2

p e

ij

where SI

ij

À 75P p p

2i

δSI

ij

À 75P

represents f

2

stimulus intensity j (e.g. 70dB SPL) for participant i. The parameters p

0i

, p

li

, and p

$2i$

are the

intercept, linear and quadratic coefficients, respectively, for participant i and e

ij

is the residual error term indicating the amount of error in predicting the DPOAE response at each stimulus intensity for each participant. An arbitrary choice was made to subtract 75 from the SI, thus setting the intercept p

0_i at 75dB SPL, so that p

li

reflects the rate of change in DPOAE level at that point.

The goal of the Level 2 model was to quantify the effect of group membership on individual differences in DPOAE I/O functions by specifying a Level 2 equation for each of the Level 1 parameters:

p

k_i

$1/4 b$

k_0

$p b$

k_1

TDNH $p m$

k_i

where p

k_i

is the k th parameter (e.g., intercept) from the Level 1 equation. TDNH was coded as an indicator variable with 0 indicating WSNH and 1 indicating TDNH such that b

k_0 indicates the mean for the WSNH group and b

k_1

indicates the “effect” or mean difference for the TDNH group. Then

k_i is the difference between each individual’s parameter estimate and the group average.

The results of the multilevel model are summarized in Table II. The average DPOAE IO functions are significantly different for the two groups. The average intercept (e.g., the DPOAE level at 75 dB SPL) for the WSNH group, b

0_0

, is $\hat{A}2.3$ dB, which is 8.2 dB lower than that of the WSTD group, which has an estimated intercept of 5.9dB (i.e., b

0_0

$p b$

0_1

). The average linear coefficient, b

10

, is 1.34 or 0.9 dB greater for the WSNH group than the estimated value of 0.43 for the TDNH group. This coefficient reflects the greater initial change in WSNH DPOAE levels as signal intensity decreases from 75dB SPL. The average quadratic coefficients also differ for the WSNH (0.03) and TDNH (0.009) groups, reflecting differences in the curvature of the DPOAE IO functions. The variance components for the three parameters indicate significant variation in the individual functions, independent of group status.