Auditory Dysfunction in Stickler Syndrome

Yvonne M. Szymko-Bennett, PhD; Mary A. Mastroianni, MS; Lawrence I. Shotland, PhD; Joie Davis, CPNP, MSN; Frank G. Ondrey, MD, PhD; Joan Z. Balog, RN, MSN; Susan F. Rudy, MSN, CRNP, CORLN; Linda McCullagh, MSN; Howard P. Levy, MD; Ruth M. Libefarb, MD, PhD; Clair A. Francomano, MD; Andrew J. Griffith, MD, PhD

Objectives: To characterize the natural history and possible mechanisms of hearing loss in Stickler syndrome (OMIM 108300; or hereditary progressive arthro-ophthalmopathy) and to determine if the auditory phenotype is a useful discriminating feature for the differential diagnosis of this group of disorders.

Design: Multifamily study.

Setting: Outpatient audiology and otolaryngology clinics at the Warren Grant Magnuson Clinical Center of the National Institutes of Health, Rockville, Md.

Subjects: Forty-six affected individuals from 29 different families segregating Stickler syndrome.

Interventions: Clinical audiologic and otolaryngological examinations were performed on all individuals, including pure-tone audiometry, speech audiometry, and middle ear immittance testing. Otoacoustic emissions, auditory brainstem response, infrared video electronystagmography, and temporal bone computed tomography were performed on a subset of participants.

Results: The hearing loss was most often sensorineural in adults, and approximately 28 (60%) of the 46 adult patients had 2 or more thresholds greater than the corresponding 95th percentile values for an age-matched, otologically normal population. The hearing loss most often affected high frequencies (4000-8000 Hz) and was generally no more progressive than that due to age-related hearing loss. Type A0 tympanograms (classification using the Jerger model), indicating hypermobile middle ear systems, were observed in 21 (46%) of the 46 affected individuals. Computed tomography of the temporal bones revealed no inner ear malformations in 19 affected individuals.

Conclusions: The hypermobile middle ear systems observed in ears with normal-appearing tympanic membranes represent a novel finding for Stickler syndrome and are likely to be a useful diagnostic feature for this disorder. The overall sensorineural hearing loss in type I Stickler syndrome is typically mild and not significantly progressive. It is less severe than that reported for types II and III Stickler syndrome linked to COL11A2 (OMIM 120290) and COL11A1 (OMIM 120280) mutations, respectively, or the closely related Marshall syndrome. This difference will be a useful discriminatory feature in the differential diagnosis of this group of disorders.


STICKLER SYNDROME is an autosomal dominant disorder characterized by vitreoretinal anomalies, joint laxity, palatal clefting, facial dysmorphism, and hearing loss. Stickler syndrome is phenotypically similar to Marshall syndrome, which has led to extensive debate about their nosologic relationship. Marshall syndrome has been proposed to differ from Stickler syndrome based on the persistence of distinctive craniofacial dysmorphic features into adulthood. The hearing loss in Stickler syndrome has been reported to be variable and can be sensorineural, conductive, or mixed. The hearing loss has not been well characterized owing to this variability and the few patients studied in any one series.

Stickler syndrome is genetically heterogeneous and may be associated with mutations in any 1 of at least 3 collagen genes: COL2A1, COL11A2, or COL11A1. Type I Stickler syndrome is caused by premature termination mutations in the fibrillar collagen gene COL2A1. Type II, also called “nonocular Stickler syndrome,” “Weissenbach-Zweymueller syndrome,” or “heterozygous otospondylomegaepiphyseal dysplasia” (OSMED), is associated with missense or in-frame deletion mutations in COL11A2 and is not associated with ocular abnormalities, which makes it readily distinguishable from types I and III. Type III is caused by mutations in
SUBJECTS, MATERIALS, AND METHODS

SUBJECTS

Forty-six individuals from 29 families segregating Stickler syndrome composed the study group. The diagnosis of Stickler syndrome was based on family history and clinical evaluation by a medical geneticist (H.P.L., R.M.L., or C.A.F.). Nineteen males and 27 females, ranging in age from 9 months to 70 years (average age, 22.8 years for males and 38.8 years for females), participated in this study after receiving informed consent. This study was approved by the institutional review board of the National Human Genome Research Institute, National Institutes of Health, Rockville, Md.

CLINICAL EVALUATIONS

Otolaryngological histories were obtained and physical examinations, including pneumatic otoscopy, were performed on each subject. Audiologic evaluations consisted of pure-tone air and bone conduction audiometry, speech audiometry, and middle-ear immittance testing (tympanometry and acoustic reflex testing) in American National Standards Institute (ANSI)–approved conditions.19,20 Young children were evaluated by play or visual reinforcement audiometry according to their age. Some patients also underwent transient-evoked or distortion product otoacoustic emissions testing (tympa-nometry and acoustic reflex testing) in American National Standards Institute (ANSI)–approved conditions.19,20 Young children were evaluated by play or visual reinforcement audiometry according to their age. Some patients also underwent transient-evoked or distortion product otoacoustic emissions testing (model ILO96 Otodynamic Analyzer; Otodynamics, London, England) or auditory brainstem response testing (Nicolet Spirit; Nicolet Biomedical Inc, Madison, Wis). Six of the patients underwent video electronystagmography testing (House IR/Video ENG System, Torrance, Calif, Copyright Eye Dynamics, 1997). Nineteen of the 46 patients underwent computed tomography of the temporal bones with 1-mm axial and coronal sections.

CLINICAL DATA ANALYSIS

The type of hearing loss was classified as sensorineural, conductive, or mixed according to the European Working Group on Genetics of Hearing Impairment.21 Conductive hearing loss was defined as normal bone conduction thresholds (<20 dB) and an averaged air-bone gap of 15 dB or more for 500, 1000, and 2000 Hz. Mixed hearing loss was defined as a bone conduction threshold greater than 20 dB in combination with an averaged air-bone gap 15 dB or more for 500, 1000, and 2000 Hz. Sensorineural hearing loss was defined as an averaged air-bone gap of less than 15 dB for 500, 1000, and 2000 Hz.

The degree of hearing loss was categorized in 2 different ways: employment of commonly used age-independent clinical guidelines,22 and comparison of thresholds to age-dependent percentiles.23-24 The age-independent analysis defined degree of hearing loss as the greatest observed degree of impairment at any threshold according to the age-independent guidelines established by the World Health Organization.22 Impairment was audiometrically classified using the following pure-tone threshold ranges: normal, 0 to 25 dB; mild, 26 to 40 dB; moderate, 41 to 55 dB; moderately severe, 56 to 70 dB; severe, 71 to 90 dB; and profound, 91 to 110 dB.

The age-dependent analysis of the degree of hearing loss plotted pure-tone air conduction thresholds at 500, 1000, 2000, and 4000 Hz, and pure-tone averages (PTAs) for 500, 1000, and 2000 Hz, against corresponding 95th percentiles.23-24 Similarly, pure-tone air conduction thresholds at 8000 Hz were plotted against corresponding 90th percentile values.23 Ninety-fifth percentile values for 300,

**COL11A1** and may be distinguished from types I and II based on the appearance of the ocular vitreous in slit-lamp examination.13,14 However, at least some cases of Stickler syndrome are not linked to these 3 fibrillar collagen genes.15

Marshall syndrome is caused by splice-site mutations or genomic deletions in 54–base pair (bp) exons in the C-terminal region of **COL11A1**, whereas other types of **COL11A1** mutations cause type III Stickler syndrome.16,17 Annunen et al17 in 1999 reported that the hearing loss associated with type III Stickler syndrome or Marshall syndrome seemed to be more severe than that associated with type I Stickler syndrome. Although this genotype-phenotype correlation may provide a useful discriminating clinical feature for the differential diagnosis of these disorders, audiometric data were not shown or reported to support the authors’ conclusion.17

The pathogenesis of hearing loss in these disorders is unknown, as there are currently no published reports of radiological or histopathologic studies of temporal bones of individuals with Stickler syndrome. A previous report of a single kindred segregating the closely related Marshall syndrome indicated that the hearing loss was not associated with gross dysmorphogenesis of the osseous labyrinth.18 We have studied a series of individuals with Stickler syndrome to characterize the natural history and possible mechanisms of hearing loss in this disorder and to determine if the auditory phenotype is a useful discriminating feature for the differential diagnosis of this group of disorders.

### RESULTS

**PURE-TONE AUDIOMETRIC FINDINGS**

The Table gives the mean PTAs and air conduction thresholds and their corresponding SDs vs the age of the affected subjects. Two study subjects were infants whose hearing was evaluated by sound field audiometry and, therefore, their data could not be included in the Table. The Table indicates that the mean hearing thresholds are in the normal to mild range in the frequencies critical for speech (500, 1000, and 2000 Hz). Mean thresholds increased with age and ranged from mild to severe in the higher frequencies.

**Figure 1A** illustrates the degree of hearing loss as a function of age of affected subjects. Although at lower frequency among the oldest age groups, mild impair-
1000, 2000, and 4000 Hz were obtained from a uniformly otologically screened population, and 90th percentile values for 8000 Hz were obtained from the 1990 International Organization for Standardization standards. Ninetieth percentile values were chosen for 8000 Hz owing to a lack of established 95th percentile data for this frequency. The percentage of study subjects with pure-tone thresholds above the 95th percentile was calculated separately for men and women 25 years and older. Air conduction thresholds were used for the analysis at frequencies with no air-bone gap, whereas bone conduction thresholds were used at frequencies with air-bone gaps of 15 dB or more. Only data from subjects with complete sets of bone conduction thresholds were included. Subjects with a history of trauma, otologic surgery, ototoxic reactions, noise exposure, or concurrent medical conditions known to cause hearing loss were excluded from the analysis.

Configuration of hearing loss was classified according to the European Working Group on Genetics of Hearing Impairment. Audiometric configurations were defined as midfrequency U-shaped, 15-dB or more difference between the poorest thresholds in the midfrequencies and those at higher frequencies; low-frequency ascending, 15-dB or more from the poorer thresholds to the higher frequencies; flat, less than 15-dB difference between 250 and 8000 Hz; high frequency, 15-dB or more difference between the mean thresholds at 500 and 1000 Hz and the mean thresholds at 4000 and 8000 Hz. High-frequency hearing loss was further described as either gently or steeply sloping. Tympanometric results were categorized according to standardized values for static compliance and tympanometric peak pressure. Analytical methods included correlation analysis, contingency table analysis, and regression analysis. The chi-square test was used to assess the possible correlation between a history of otitis media and sensorineural hearing loss. A positive history of otitis media was categorized as either more than 6, 12, or 20 lifetime episodes. The presence of sensorineural hearing loss was defined as 2 or more thresholds in 1 or both ears above the 95th percentile value (for 500, 1000, 2000, or 4000 Hz) or the 90th percentile value for 8000 Hz. Cross-sectional analysis of hearing loss progression was carried out in a similar manner to that of Kustis et al. Fifthieth percentile thresholds were subtracted from observed thresholds and plotted vs age to determine the degree of progression relative to that in a normal population. Fiftieth percentile thresholds for 500 Hz to 4000 Hz were obtained from Morrell et al and 50th percentile thresholds for 250, 6000, and 8000 Hz were derived from International Organization for Standardization 1990 standards. Linear regression analysis was performed on all normally distributed adjusted threshold data (see “Clinical Data Analysis” subsection herein). Slopes of the regression lines were calculated to determine the rate of progression of hearing loss and were compared with those calculated for the uniformly otologically screened population.

Serial audiograms were available for 8 subjects and were analyzed for linear hearing loss progression according to the European Working Group on Genetics of Hearing Impairment. Progression was defined as a deterioration of 15 dB or more in the PTA or in 2 or more frequencies within a 10-year period. Pure-tone averages for the subjects with Stickler syndrome were compared with those for affected individuals of a previously described Marshall syndrome kindred and 5 affected members of a previously unreported kindred that was ascertained as part of our study. The Shapiro-Wilk test for normality was performed on both PTA distributions, and a t-test was performed to determine if a statistically significant difference existed between the 2 distributions. Parametric and nonparametric statistical analyses were performed using JMP software (SAS Institute Inc, Cary, NC).

ment was prevalent among all age groups. A lesser percentage of subjects had hearing loss in the moderate to profound categories. Profound impairment was most prevalent in the oldest age group, although these profound losses were only observed in the highest frequencies. Figure 1B shows the type of hearing loss as a function of age of affected subjects. Hearing loss in children was most commonly conductive. Normal hearing was most common in the 21- to 30-year age group, whereas sensorineural hearing loss becomes more common in the older age groups. Mixed hearing losses were present in 4 of the 7 age groups. The pure-tone audiometric configuration was classified into 1 or more of the following categories: high frequency, midfrequency U-shaped, low-frequency ascending, or flat. Figure 1C shows that high frequencies were most commonly affected, with increasing prevalence of this configuration in age groups older than 10 years. Low-frequency and midfrequency thresholds were most commonly affected in the youngest age groups. High-frequency configurations in young children were gently sloping, whereas older subjects had steeply sloping high-frequency configurations.

To identify the contribution of the underlying gene mutations to the observed sensorineural hearing loss, audiometric thresholds at 500, 1000, 2000, 4000, and 8000 Hz, as well as PTAs, were compared with those reported for an otologically screened population 25 years and older. Subjects with a history of otologic surgery were excluded from our analysis. Fourteen had undergone prior placement of 1 or more sets of tympanostomy tubes and 2 had undergone tympanoplasties (no subjects reported a history of mastoidectomy). As shown in Figure 2, approximately 60% (14 of the 24 subjects) of all affected adults with complete bone conduction data had at least 2 thresholds above the 95th percentile or, in the case of 8000 Hz, above the 90th percentile.

χ² Analysis revealed no statistically significant correlation between the number of episodes of otitis media with sensorineural hearing loss at 500, 1000, 2000, and 4000 Hz, as well as PTAs, was compared with those reported for an otologically screened population 25 years and older. Subjects with a history of otologic surgery were excluded from our analysis. Six of 32 adults were excluded from this analysis because of incomplete data, and 2 were excluded because of a history of otologic surgery (tympanoplasty). There was also no correlation between the number of episodes of otitis media with sensorineural hearing loss at 8000 Hz (P=.47, .24, and .24, for ≥6, ≥12, and ≥20 episodes per lifetime, respectively).
The subjects with types A, B, C, and AD tympanograms approximately 14 (31%) of 46 ears had type AD tympanograms with normal-appearing tympanic membranes examined by pneumatic otoscopy. Seven (15%) of 46 ears had type A0 tympanograms with normal-appearing tympanic membranes that were abnormally flaccid and thin in at least one portion or all of the membrane; pneumatic otoscopy revealed that the amplitude of motion of these latter membranes was disproportionately greater than that of the long process of the malleus.

**ANALYSIS OF HEARING LOSS PROGRESSION**

There were several anamnestic reports of progression of hearing loss among the study subjects. Longitudinal analysis revealed that 4 of 8 subjects with serial audiograms had progressive hearing loss according to the criterion proposed by the European Working Group on Genetics of Hearing Impairment (data not shown).

A strong ascertainment bias would be present in an analysis restricted to this small subset of study subjects who had previously undergone audiometric testing. Therefore, a cross-sectional analysis of age-adjusted binaural hearing thresholds was performed for subjects aged from 25 to 65 years (Figure 4). The Shapiro-Wilk test for normality revealed that the 500- and 2000-Hz adjusted threshold data were not normally distributed, thus prohibiting linear regression analysis of these data. Moreover, most of the hearing losses primarily affected high frequencies (Figure 1C). Linear regression analysis was performed only on the 4000-, 6000-, and 8000-Hz thresholds. Figure 4 shows the regression analysis of pure-tone air conduction audiometric thresholds from 4000 to 8000 Hz. Slopes of the regression lines in the cross-sectional analysis of hearing thresholds in this study were: −0.03 dB per year at 4000 Hz, 0.16 dB per year at 6000 Hz, and 0.25 dB per year at 8000 Hz, with the absolute mean value slope of 0.12 dB per year. Data of Morrell et al.21 revealed a maximum slope of approximately 2 dB per year at all of the frequencies they analyzed. Therefore, there was little, if any, progression of thresholds above that expected from normal aging from 25 to 65 years in our study.

**Figure 5** shows the PTAs of subjects with Marshall and Stickler syndromes as a function of age. A Shapiro-Wilk test revealed normal distributions for both the Marshall syndrome–affected and Stickler syndrome–affected groups, with the median PTAs for the Marshall syndrome cohort being a 50-dB hearing level, and for the Stickler syndrome cohort being a 17-dB hearing level. A test revealed that these medians were significantly different (P < .00). Linear regression analysis demonstrated that the slope of the regression line for the Marshall syndrome PTAs was significantly greater than that for the Stickler syndrome PTAs (0.61 dB and 0.13 dB, respectively). Although there is considerable variability in the Stickler syndrome PTAs, these data are consistent with the observation of Annunen et al.17 that individuals with Marshall syndrome have a more severe auditory phenotype than individuals with Stickler syndrome.
OTHER FINDINGS

Results of infrared videovestibular testing were incomplete in 6 subjects and confounded by concurrent ocular pathologic abnormalities. Vestibular symptoms and signs were infrequent and consistent with imbalance rather than true vertigo. These findings were usually attributable to the typical rheumatologic and ocular abnormalities of Stickler syndrome.

Otoacoustic emissions testing in 7 subjects revealed responses that were consistent with the degree of hearing loss. Specifically, distortion product otoacoustic emissions and transient-evoked otoacoustic emissions accurately identified auditory status between 2000 and 4000 Hz, with the most robust emissions obtained when audiometric thresholds were lower than the 30-dB hearing level. As expected, emissions were absent in subjects with thresholds above 30 dB in the range of 1000 to 4000 Hz. Temporal bone computed tomographic scans of 19 affected subjects revealed no malformations of the inner or middle ears.

We have observed that the hearing loss in Stickler syndrome is typically mild overall and sensorineural with a steeply sloping, high-frequency configuration in adults, whereas it is commonly conductive in children. The observed conductive hearing loss in children may be due to chronic otitis media or its sequelae, which commonly occurs in this population. Approximately 26 (60%) of our 44 adult subjects with Stickler syndrome had 2 or more thresholds above the 95th percentile, indicating that the sensorineural hearing loss in this disorder is an incompletely penetrant trait. These results are consistent with those of previous reports of smaller series of patients.

Type A tympanograms were a common immittance finding in our subjects and have not been previously reported for Stickler syndrome. This tympanometric finding was not significantly associated with conductive hearing loss at any frequency in our study (not shown). Hypermobility was sometimes associated with thin, visibly hyperflaccid tympanic membranes, which is a common and otoscopically detectable sequela of chronic or recurrent otitis media and/or previous tympanostomy tubes. However, 6 (21%) of 28 affected subjects with hypermobility had completely normal-appearing tympanic membranes and no history of otitis media or previous tympanostomy tube insertions. Type II collagen is known to be present in the tympanic membrane and the ossicular joints and, therefore, hypermobility may be a sequela of otitis media, a direct result of the primary collagen defect, or a combination of both of these factors. We postulate that the type A tympanograms associated with normal-appearing tympanic membranes may be due to ossicular joint hypermobility, since hypermobility is also commonly observed in other articular joints in patients with Stickler syndrome. Ossicular joint hypermobility may be a useful diagnostic feature for Stickler syndrome.

Although 4 (50%) of the 8 subjects with serial audiograms had progressive hearing loss, this ratio is likely to be an artificially high estimate due to ascertainment bias. Subjects with the most severe or progressive hearing loss were more likely to have had serial audiograms prior to their participation in our study. Our regression analysis of cross-sectional, age-adjusted hearing thresholds indicates that there is minimal progression beyond that associated with normal aging in individuals with Stickler syndrome. Therefore, the sensorineural component of the hearing loss caused by most Stickler syndrome mutations seems to be stable over long periods.

Nonprogressive hearing loss has also been reported in families with nonsyndromic deafness DFNA13 and Stickler syndrome mutations in COL11A2, although the sensorineural hearing loss associated with these mutations is more severe and appears to affect the middle and lower frequencies to a greater degree than we observed in our study subjects. In contrast, the hearing loss caused by type III Stickler syndrome and Mar-
shall syndrome mutations in COL11A1 is much more severe and progressive than that observed in our patients.3,16 Since none of our families with Stickler syndrome had ocular or craniofacial phenotypic features that were suggestive of linkage to COL11A2 or COL11A1,14,17 it is likely that most, if not all, of our subjects segregate type I Stickler syndrome (ie, mutations in COL2A1). The mild, nonprogressive sensorineural hearing loss we observed in our subjects may be used to clinically distinguish these patients from those with hearing loss linked to COL11A1 mutations17 (Figure 1A), or to the more severe, nonprogressive sensorineural hearing loss associated with COL11A2 mutations. This hypothesis is being addressed by ongoing genotypic analyses of our study subjects. Lastly, while the hearing loss attributable to the collagen mutations may be mild and nonprogressive, there will still be age-related changes that will make individuals with Stickler syndrome at risk for severe or profound hearing loss, especially in the high frequencies. The sensorineural component of the hearing loss may be directly due to recurrent or chronic otitis media in these patients. However, there was no correlation between sensorineural hearing loss and the number of episodes of otitis media in our subjects. Moreover, the hearing loss is
not associated with malformations of the osseous labyrinth as detected by temporal bone computed tomographic scans. It is possible that there are subtle structural malformations of the osseous or membranous labyrinths that may be detectable with more sensitive imaging techniques such as magnetic resonance imaging. We hypothesize that Stickler syndrome mutations affect sound transmission within the cochlea by altering mechanical properties of the cochlear partition. Collagen fibrils are thought to contribute tensile strength to the tissues in which they are expressed. Mutations affecting fibril morphology may alter their tensile strength, resulting in a range of disease phenotypes that can include osteogenesis imperfecta and Stickler syndrome. These observations are consistent with the observation of expression of Col2A1, Col11A1, and Col11A2 messenger RNA in soft tissue elements of the mouse cochlea and in earlier studies demonstrating expression of type II collagen within the cochlea. Differing effects of Marshall and Stickler syndrome mutations on auditory function may reflect differing contributions of these collagen genes to the synthesis, structure, and function of the extracellular matrix within the cochlea. These alterations could directly affect sound mechanotransduction, or they may also cause abnormal mechanical stress forces leading to cell degeneration and sensorineural hearing loss.

We are analyzing the correlation of hearing loss with extra-auditory phenotypic features such as palatal clefting, as well as the underlying fibrillar collagen genotypes in these patients. Our results and those of future studies should extend our understanding of how the extracellular matrix and its fibrillar collagens contribute to normal auditory function. They will also facilitate the diagnosis and care of patients with Stickler syndrome and its related disorders.

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Corresponding author and reprints: Andrew J. Griffith, MD, PhD, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, 5 Research Ct, Room 2A-02, Rockville, MD 20850 (e-mail: griffita@nidcd.nih.gov).

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