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Audiologic and Otologic Clinical Manifestations of Loeys-Dietz Syndrome: A Heritable Connective Tissue Disorder

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Abstract

Objective. Loeys-Dietz syndrome (LDS) is a rare genetic connective tissue disorder resulting from TGF-ß signaling pathway defects and characterized by a wide spectrum of aortic aneurysm, arterial tortuosity, and various extravascular abnormalities. This study describes the audiologic, otologic, and craniofacial manifestations of LDS.

Study Design. Consecutive cross-sectional study.

Setting. Tertiary medical research institute.

Methods. Audiologic and clinical evaluations were conducted among 36 patients (mean ± SD age, 24 ± 17 years; 54% female) with genetically confirmed LDS. Cases were categorized into genetically based LDS types 1 to 4 (*TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*, respectively). Audiometric characteristics included degree and type of hearing loss: subclinical, conductive, mixed, and sensorineural.

Results. LDS types 1 to 4 included 11, 13, 5, and 7 patients, respectively. In LDS-1, 27% had bilateral conductive hearing loss; 9%, unilateral mixed; and 36%, subclinical. In LDS-2, 38% had conductive hearing loss and 38% subclinical. In LDS-3 and LDS-4, 40% and 43% had bilateral sensorineural hearing loss, respectively. Degree of hearing loss ranged from mild to moderate. Bifid uvula was observed only in LDS-1 (55%) and LDS-2 (62%). Submucosal/hard cleft palates were primarily in LDS-1 and LDS-2. Posttympanostomy tympanic membrane perforations occurred in 45% (10/22 ears) of LDS-1 and LDS-2. There were 4 cases of cholesteatoma: 3 middle ear (LDS-1 and LDS-2) and 1 external ear canal (LDS-3).

Conclusion. Conductive hearing loss, bifid uvula/cleft palate, and posttympanostomy tympanic membrane perforation are more common in LDS-1 and LDS-2 than LDS-3 and LDS-4, while sensorineural hearing loss was present only in LDS-3 and LDS-4. These LDS-associated key clinical presentations may facilitate an early diagnosis of LDS and thus prompt intervention to prevent related detrimental outcomes.

Keywords

Loeys-Dietz syndrome, TGF-beta, connective tissue disorder, audiology, otolaryngology

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Loeys-Dietz syndrome (LDS) is a rare autosomal dominant connective tissue disorder that was first described in 2005 in 10 probands with a clinical triad of hypertelorism, bifid uvula or cleft palate, and vascular abnormalities.[1] The range of LDS manifestations is broad, but the most significant clinical characteristics of LDS found on initial presentation are the cardiovascular features, including arterial tortuosity, aneurysm, and dissection. A subset of clinical features of LDS, such as aortic root aneurysm, pectus

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deformities, scoliosis, and arachnodactyly, overlaps with that of other heritable connective tissue disorders, including Marfan syndrome, Larsen syndrome, Ehlers-Danlos syndrome, and Stickler syndrome.[2,3] However, LDS manifests distinctive clinical findings, such as craniosynostosis, hypertelorism, cleft palate/bifid uvula, cervical spine instability, club feet, thin skin, and diffuse arterial aneurysms and tortuosity.[4,5] The vascular sequelae, such as aortic dissection, occur at an earlier age and at smaller aortic dimensions than in the other connective tissue disorders, such as Marfan syndrome.[6]

The pathogenesis of LDS is related to the TGF-ß (transforming growth factor ß) signaling pathway, which has been associated with the pathogenesis of other connective tissue disorders.[7] About two-thirds of LDS cases are believed to be due to de novo mutations, which present more severely than the inherited cases.[8] Since 2014, LDS has been classified as 6 types depending on the affected gene, with each gene giving rise to different disease severity and features. Type 1 (*TGFBR1*, LDS-1) and type 2 (*TGFBR2*, LDS-2) are the most commonly reported (75%-85% of cases) and have the most severe craniofacial and aortic features, while types 5 (*TGFB3*, LDS-5) and 6 (*SMAD2*, LDS-6) represent <5% of cases. Types 3 (*SMAD3*, LDS-3) and 4 (*TGFB2*, LDS-4) have similar prevalence (5%-10%).[9] Thus far, most published studies related to the clinical phenotype of LDS focus on cardiovascular, orthopaedic, and immunologic manifestations.[5] While there have been brief mentions of hearing loss in LDS case reports,[3] there has not yet been a published audiologic and otologic phenotype description. This study describes the hearing and otologic manifestations from a cohort of 36 patients with LDS.

Methods

Subjects

Audiologic and otolaryngologic data were collected prospectively on patients with LDS at our tertiary research medical center from July 2018 to February 2020. All patients were enrolled in an observational longitudinal natural history study at the Clinical Center of the National Institutes of Health. The protocol (NCT02504853) was approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, and all subjects and/or their guardians gave informed consent/assent. Subjects were diagnosed and categorized by LDS type based on the sequence-confirmed presence of the mutations in the LDS-associated genes. This study was limited to LDS types 1 to 4, because types 5 and 6 are very recently described and low in incidence.

Audiologic Evaluation

Hearing evaluations included pure tone thresholds for air conduction from 0.25 to 8 kHz and bone conduction from 0.25 to 4 kHz, in addition to 226-Hz tympanometry. Hearing status was evaluated by 3 criteria: a 4-frequency pure tone average (PTA; 0.5, 1, 2, 4 kHz) and a 2-frequency PTA for the low frequencies (0.25, 0.5 kHz) and high frequencies (4, 8 kHz). Any PTA >20 dB HL was used as a marker of clinically significant hearing loss, as the low- and high-frequency PTAs have the potential to capture hearing loss missed by the 4-frequency PTA. PTAs ≤20 dB HL were considered normal hearing sensitivity. Degree of hearing loss was classified as mild (21-40 dB HL), moderate (41-70 dB HL), severe (71-95 dB HL), and profound (>95 dB HL) based on the PTAs. Conductive hearing loss (CHL) was defined as the presence of an air-bone gap >10 dB when air conduction was >20 dB HL and bone conduction was ≤20 dB HL. Mixed hearing loss (MHL) was present when air conduction and bone conduction were >20 dB HL in combination with an air-bone gap >10 dB. Sensorineural hearing loss (SNHL) was defined as air conduction >20 dB HL in combination with an air-bone gap ≤10 dB. A subclinical (SubC) conductive component was defined as normal hearing sensitivity by air conduction with an air-bone gap >10 dB at ≥2 frequencies.

Tympanometric measures of peak static admittance and peak pressure were interpreted according to age-appropriate normative reference ranges[10] and categorized into types A, B, and C based on the method described by Jerger.[11]

Medical Evaluation

A comprehensive head and neck examination was performed for each patient with LDS by an otolaryngologist (H.J.K.) while incorporating the patient's previous examination by geneticists from another institution, who were familiar with LDS. While no formal anthropometric or cephalometric measurement was performed, craniofacial features of interest were malar flattening and facial asymmetry. The specific palatal anomalies examined were high and narrow-arched palate, bifid uvula, and broad uvula. Relatedly, we noted the history or presence of hard or submucosal cleft palates (bifid uvula with palpable bony notch of the hard palate). The specific ophthalmologic characteristics examined were hypertelorism, exotropia, and blue sclera. Otologic manifestations included the presence of cholesteatoma, low-set auricles (the attachment of auricular helix below the lateral canthus), tympanic membrane perforation (TMP), and history of myringotomy and ventilation tube placement (MxT).

Statistical Analysis

Descriptive statistics were used to summarize demographic and clinical manifestations. Data are presented as mean or median, as appropriate, and standard deviation. Statistical analysis was conducted with Prism version 8.4.3 (GraphPad Software, Inc). Inferential statistics comparing LDS groups were made via Fisher exact test or Mann-Whitney *U* test, as appropriate, with statistical significance set at $\alpha = .05$. When statistical comparisons were made, LDS-1 and LDS-2 were combined (LDS-1/2) and compared with the combined groups of LDS-3 and LDS-4 (LDS-3/4) due to their phenotypic similarities. Comparisons in ophthalmologic, palatal, and cleft anomalies were made between the patient groups.

Results

Demographics

Thirty-seven patients with genetically confirmed LDS were seen for otolaryngologic and audiologic evaluations. One

Table 1. Patient Demographics and Craniofacial, Ophthalmologic, and Palatal Characteristics by LDS Type. [a]

				Craniofacial		Ophthalmologic			Palatal				
Type	No. of patients	Age, y, mean ± SD	Female, %	MF	FA	HT	EA	BS	HNP	Bifid	BU	S-CP	H-CP
LDS-1	11	17 ± 11	64	10 (91)	10 (91)	9 (82)	8 (73)	8 (73)	10 (91)	6 (55)	2 (18)	3 (27)	2 (18)
LDS-2	13	16 ± 9	46	11 (85)	8 (62)	11 (85)	7 (54)	7 (54)	13 (100)	8 (62)	4 (31)	3 (23)	2 (15)
LDS-3	5	37 ± 23	40	3 (60)	2 (40)	1 (20)	1 (20)	0	1 (20)	0	2 (40)	1 (20)	0
LDS-4	7	41 ± 15	71	5 (71)	4 (57)	1 (14)	1 (14)	2 (29)	5 (71)	0	1 (14)	0	0
Overall	36	25 ± 17	54	29 (81)	24 (67)	22 (61)	17 (47)	17 (47)	29 (81)	14 (39)	9 (25)	7 (19)	4 (11)

Abbreviations: Bifid, bifid uvula; BS, blue sclerae; BU, broad uvula; EA, exotropia; FA, facial asymmetry; H-CP, hard cleft palate; HNP, high and narrow-arched palate; HT, hypertelorism; LDS, Loeys-Dietz syndrome; MF, malar flattening; S-CP, submucosal cleft palate.
[a]Values are presented as No. (%) unless noted otherwise.

Table 2. Prevalence of Otologic Features by LDS Type.[a]

Type	No. of ears	LS-A	Chol-ME	Chol-EAC	TMP	MxT Hx	TMP with MxT Hx[b]
LDS-1	21	8 (38)	1 (5)	0	6 (29)	10 (48)	6/10 (60)
LDS-2	26	8 (31)	2 (8)	0	4 (15)	12 (46)	4/12 (33)
LDS-3	10	0	0	1 (10)	0	2 (20)	0/2
LDS-4	14	2 (14)	0	0	0	0	0
Overall	71	18 (25)	3 (4)	1 (1)	10 (14)	24 (34)	10/22 (45)

Abbreviations: Chol, cholesteatoma; EAC, external auditory canal; LDS, Loeys-Dietz syndrome; LS-A, low-set auricle; ME, middle ear; MxT Hx, history of myringotomy with ventilation tube placement; TMP, perforated tympanic membranes on examination.
[a]Values are presented as No. (%) unless noted otherwise.
[b]All TMPs were associated with MxTs.

pediatric patient was excluded due to a lack of audiometric behavioral testing, and a single anacusic ear was excluded from a patient with LDS-1 who had a history of trauma to the ear. The final data set consisted of 71 ears of 36 patients, 54% female, aged 5 to 67 years (mean, 24.6; SD, 16.6). The overall distribution for age and sex for all 36 patients, as well as for each LDS type, is described in **Table 1** and Supplemental Table S1 (available online). The cohort did not include any patients with LDS-5 or LDS-6. LDS-1/2 accounted for two-thirds of our cohort, and these subgroups combined were younger than LDS-3/4 ($P = .0002$).

Characteristic Physical Features of LDS

Table 1 shows the distribution of key craniofacial, ophthalmologic, and palatal features for each of 4 types of LDS in our cohort. Among the craniofacial features, malar flattening and facial asymmetry were found in all LDS types (81% and 67%, respectively). Within the 3 ophthalmologic features that we analyzed, hypertelorism was the most common ophthalmologic finding, present in 61% of our cohort. Hypertelorism and exotropia were present across all LDS types, and all 3 ophthalmologic features (hypertelorism, exotropia, and blue sclera) were more common in LDS-1/2 versus LDS-3/4 ($P = .0002, .014, .014$; odds ratios, 25, 8.3, 8.3). The prevalence of palatal anomalies such as high and narrow-arched palate, bifid uvula, submucous cleft palate, and hard cleft palate was 89% (32/36) in our LDS cohort. A high and narrow-arched palate was the most common palatal feature, occurring in 81%, and often overlapped with the other palatal anomalies. Bifid uvula was observed only in those with LDS-1 (55%) and LDS-2 (62%). A history of a submucous or hard cleft palate was found in 7 (19%) and 4 (11%) patients. All patients with a hard cleft palate and 2 with a submucous cleft palate had undergone a repair. The prevalence of submucous and/or hard cleft palates was not significantly different between LDS-1/2 and LDS-3/4 ($P = .059$), although all but 1 of the patients with cleft palate (submucous) were in the LDS-1/2 . The presence of 3 specific palatal features (high and narrow-arched palate, bifid uvula, broad uvula) was more common in LDS-1/2 than LDS-3/4 ($P = .0028, .0007, >.99$; odds ratios, 23, 34, 1).

Otologic manifestations of the 71 ears from our cohort are presented in **Table 2**. The presence or history of low-set auricles, TM perforations, and MxT was 25%, 14%, and 34%, respectively. Of the 24 ears with a history of MxT, excluding 2 ears with retained ear tubes, 45% (10 ears) had a nonhealed, perforated TM on examination. All of those cases were from patients with LDS-1 or LDS-2. A history or presence of middle ear cholesteatoma was found in 3 patients (1 LDS-1, 2 LDS-2), who had a history of MxT and palatal anomalies, including hard plate cleft and bifid uvula. Additionally, 1 patient with LDS-3 presented with a cholesteatoma in the external auditory canal, not involving the middle ear.

Table 3. Hearing Loss by LDS Type.[a]

Hearing loss	LDS-1	LDS-2	LDS-3	LDS-4
All ears	21	26	10	14
Normal	9	12	3	6
CHL	6	8	0	0
SNHL	0	0	4	6
MHL	1	0	0	0
Unspecified	0	0	2	0
Subclinical	5	6	1	2

Abbreviations: CHL, conductive hearing loss; LDS, Loeys-Dietz syndrome; MHL, mixed hearing loss; SNHL, sensorineural hearing loss.
[a]Values are presented as No. of ears.

Table 4. Degree of Hearing Loss by PTA and LDS Type.[a]

	PTA		
LDS: hearing loss	LF	4F	HF
LDS-1 (21 ears)			
Normal	15	14	15
Mild	1	3	4
Moderate	5	4	1
Severe	0	0	1
LDS-2 (26 ears)			
Normal	21	20	19
Mild	2	4	3
Moderate	3	2	4
LDS-3 (10 ears)			
Normal	10	7	4
Mild	0	3	5
Moderate	0	0	1
LDS-4 (14 ears)			
Normal	10	14	12
Mild	4	0	1
Moderate	0	0	1

Abbreviations: 4F, 4 frequencies (0.5, 1, 2, 4 kHz); HF, high frequency (4, 8 kHz); HL, hearing loss; LDS, Loeys-Dietz syndrome; LF, low frequency (0.25, 0.5 kHz); PTA, pure tone average.
[a]Values are presented as No. of ears.

Characterization of Hearing Loss in LDS

Overall, 42% (15 patients) had hearing loss identified by any 1 of the PTAs, 12 bilaterally. When those with SubC findings are included in the hearing loss group, 69% (25 patients) have abnormal audiologic results. The hearing losses were either CHL or SNHL, with the exception of 1 MHL, and the degree of loss ranged from mild to moderate across patients (**Tables 3** and **4**). CHL was limited to LDS-1/2 and SNHL to LDS-3/4. The single exception to degree and type findings was an ear with a mild sloping to severe MHL in a patient with LDS-1.

In LDS-1, 36% (4 patients) had hearing loss; 27% (3 patients) had bilateral CHL; and 9% (1 patient) had unilateral MHL. Additionally, 36% (4 patients) had SubC findings that were unilateral in 3. In LDS-2, 38% (5 patients) had CHL (3 bilaterally) and 38% (5 patients, 1 bilaterally) had SubC findings. In LDS-3, 60% (3 patients) had hearing loss bilaterally: 2 had bilateral SNHL, and 1 had high-frequency hearing loss at 6 and 8 kHz that could not be classified in the absence of bone conduction at these frequencies. One patient had a unilateral SubC finding. In LDS-4, 43% (3 patients) had bilateral SNHL and 14% (1 patient) had bilateral SubC findings.

Characterization of Tympanometry in LDS

Peak static admittance and peak pressure were normal (type A tympanogram) in 61% (43 ears). Type B tympanograms were limited to the LDS-1 (29%, 6 ears) and LDS-2 (27%, 7 ears). In LDS-1, all 6 type B tympanograms were associated with a TMP. In LDS-2, 4 type B tympanograms were associated with a TMP and 2 with MxT. One LDS-2 ear had an atypical W-shaped tympanogram. All ears with TMPs had a history of MxT. In LDS-3, 10% (1 ear) had a type C tympanogram showing negative middle ear pressure. All tympanograms for the LDS-4 group were type A.

Full details of audiometric findings are shown in the Supplemental Table S1 (available online).

Discussion

LDS is a rare autosomal dominant connective syndrome, and it shares its clinical manifestations with other congenital connective disorders, such as Marfan syndrome. However, vascular disease in LDS, including widespread arterial aneurysms and aortic rupture, tends to be more aggressive than in Marfan syndrome. This consecutive cross-sectional cohort study identified some of the key LDS-associated craniofacial, audiologic, and otologic presentations that may facilitate an early diagnosis of LDS and thus a prompt intervention to prevent related detrimental outcomes.

Abnormal audiologic findings were detected in 69% of the patients in this cohort. The degree of hearing loss ranges mostly from mild to moderate. The most common type of hearing loss was conductive, often associated with TMPs after MxT. The findings of CHL, MHL, or SubC component in 53% (19 patients) of our cohort also could be associated with connective tissue changes within the TM or the ligaments around the middle ear ossicles, as seen in other connective tissue disorders such as Ehlers-Danlos syndrome and Stickler syndrome.[12,13]

Kolla et al recently conducted a single-cell RNA sequencing analysis to study the organ of Corti development in mice and found that the *Tgfβr1* signaling pathway may play a significant role in the maturation of the outer hair cells and the maintenance of the organ of Corti.[14] With this key linkage established between the organ of Corti and the TGF-ß signal pathway, it is interesting to note the presence of SNHL only in our LDS-3/4 group, which showed milder physical phenotypes than our LDS-1/2 group. The SNHL observed in 4 of the 5 patients could not be accounted for by age and sex based on 95th percentile of normative data from the International Organization for Standardization.[15]

Overall, our cohort showed a moderate rate of submucosal and hard cleft palate defects, especially in LDS-1/2, where the rate ranged from 15% to 27%. This observation related to cleft palate defect may be physically associated with the development of eustachian tube dysfunction, as 73% of patients with a history of cleft palate had a history of ventilation tubes (MxT). The incidence of TMPs after MxT in LDS-1/2 was significantly higher (50%) than the overall rate of 16% to 18% in a large series of cleft palate cases.[16,17] This finding in our cohort could be due to multiple factors, including persistent chronic eustachian tube dysfunction and/or poor wound-healing properties associated with LDS.[5] In general, the repair process for TMP involves multiple wound-healing phases. During the early inflammatory phase, fibroblasts migrate and proliferate in the granulation tissue around the perforation site and produce extracellular matrices composed of collagen, fibronectin, and proteoglycans.[18] Throughout the process, the TGF-ß signal pathway plays an important role in the production of these extracellular matrix proteins by fibroblasts and the differentiation of the fibroblasts into myofibroblasts.[19] Therefore, the TGF-ß signaling pathway defect in patients with LDS may prevent the normal healing process of the TMP after ventilation ear tube extrusion. Interestingly, 2 of our patients with LDS had several failed tympanoplasties. Although the etiology in these cases is not known, this phenomenon may be related to the TGF-ß defects resulting in poor wound healing. On the basis of these observations, we speculate that a cartilage graft could be utilized rather than the traditional temporal fascia during tympanoplasties for patients with LDS. Due to the higher incidence of nonhealing TMP after ventilation ear tube extrusion and the lower success rate of tympanoplasty in patients with LDS, a decision to place a ventilation ear tube should be carefully considered.

In this study, 4 ears with a history or presence of cholesteatoma were identified: 3 in the middle ear and 1 in the external ear canal. Cholesteatomas can be caused by inflammatory stimuli of the subepithelial mesenchymal connective tissue when epithelial cells proliferate in the TM retraction pocket during chronic eustachian tube dysfunction.[20] We hypothesize that the relatively higher incidence of cleft palate–related chronic eustachian tube dysfunction in LDS may predispose to the formation of a retraction pocket in the TM, eventually leading to the development of cholesteatoma. Furthermore, TGF-ß family ligands and other growth and differentiation factors have been implicated in epithelial differentiation.[21] Therefore, the TGF-ß signaling pathway defect may predispose the epithelial and subepithelial mesenchymal lining of a TM retraction pocket to form a middle ear or external ear canal cholesteatoma.

Our data confirmed a high and narrow-arched palate as one of the hallmark clinical manifestations of LDS in general. Interestingly, bifid uvula, another previously known hallmark LDS feature, was present only in our LDS-1/2. Among other clinical features that we analyzed, LDS-1/2 showed a significantly higher presence of many ophthalmologic and palatal features as compared with other LDS types. While the number of patients with LDS may not have been enough to reach statistical significance, the presence of hard and/or submucosal cleft palate defect seemed to cluster more with the LDS-1/2 cohort. Of note, LDS-2 is characterized by mutations in *TGFBR2*, and it was previously shown that mice with conditional *Tgfbr2* knockout exhibited partial posterior cleft palate while the anterior hard palate was not affected.[22] This finding suggests that posterior palatal mesenchyme development is affected in LDS given the essential role for TGF-β pathway in this process.

No patients in this cohort with palatal defects had gross symptoms of velopharyngeal insufficiency, but further studies, such as fiberoptic nasoendoscopy and speech evaluation, are needed to assess the incidence of velopharyngeal insufficiency in this cohort. In addition, LDS is commonly associated with joint laxity and cervical spine malformations and/or instability. Careful perioperative management of the cervical spine should be considered when a head and neck procedure is performed in patients with LDS.

This study is the largest and most detailed published report of otolaryngologic and audiologic manifestations of patients with genetically confirmed LDS to date. Like many mammalian genetic diseases, the clinical phenotypes of LDS are diverse due to multiple factors, including variable penetrance and likely influence from genetic and environmental modifiers. Although this was a consecutive cross-sectional cohort study, significant information regarding otolaryngology history was acquired retrospectively, and no formal anthropometric or cephalometric measurement was done to precisely characterize craniofacial features. A longitudinal prospective study would be helpful to discern a more detailed natural history of otolaryngologic and audiologic manifestations in LDS.

Conclusion

LDS is an autosomal dominant connective tissue–related syndrome resulting from defects in the TGF-ß signaling pathway. From our analysis of a large cohort of patients with LDS, we have identified a set of relevant and common audiologic, otologic, and craniofacial features related to each of the 4 types of LDS included in this study. Many audiologic and clinical features were more common in LDS-1/2, especially bifid uvula, CHL, and persistent TM perforations in patients with a history of MxT. Approaches to medical and surgical management of ear pathology in LDS should be carefully considered with an understanding that the TGF-ß signaling pathway may affect the craniofacial development and wound-healing processes. The finding of SNHL—not attributable to age—in patients with LDS-3/4 warrants further investigation. It is important to conduct audiologic evaluations in conjunction with otology in this population for amplification considerations as well as medical management of middle ear disease and cleft palate. Longitudinal evaluations of patient auditory function should be conducted to inform a more accurate audiologic prognosis related to the different types of LDS.

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Author Contributions

Jun W. Jeon, design, analysis, writing, presentation; **Julie Christensen**, design, conduct, analysis, writing, presentation; **Jennifer Chisholm**, conduct, data collection, revision; **Christopher Zalewski**, conduct, statistical expertise, revision; **Marjohn Rasooly**, conduct, revision; **Caeden Dempsey**, conduct, revision; **Alaina Magnani**, conduct, revision; **Pamela Frischmeyer-Guerrerio**, conduct, revision, provision of patients, expertise; **Carmen C. Brewer**, co-senior author design, conduct, analysis, provision of patients, writing, final approval; **Hung Jeffrey Kim**, co-senior author conception, design, conduct, analysis, provision of patients, writing, final approval.

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Supplemental Material

Additional supporting information is available in the online version of the article.

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