




**ORIGINAL ARTICLE****TSPEAR variants are primarily associated with ectodermal dysplasia and tooth agenesis but not hearing loss: A novel cohort study**

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**Abstract**

Biallelic loss-of-function variants in the thrombospondin-type laminin G domain and epilepsy-associated repeats (*TSPEAR*) gene have recently been associated with ectodermal dysplasia and hearing loss. The first reports describing a *TSPEAR* disease association identified this gene as a cause of nonsyndromic hearing loss, but subsequent reports involving additional affected families have questioned this evidence and suggested a stronger association with ectodermal dysplasia. To clarify genotype–phenotype associations for *TSPEAR* variants, we characterized 13 individuals with biallelic *TSPEAR* variants. Individuals underwent either exome sequencing or panel-based genetic testing. Nearly all of these newly reported individuals (11/13) have phenotypes that include tooth agenesis or ectodermal dysplasia, while three newly reported individuals have hearing loss. Of the individuals displaying hearing loss, all have additional variants in other hearing-loss-associated genes, specifically *TMPRSS3*, *GJB2*, and *GJB6*, that present competing candidates for their hearing loss phenotype. When presented alongside previous reports, the overall evidence supports the association of *TSPEAR* variants with ectodermal dysplasia and tooth agenesis features but creates significant doubt as to whether *TSPEAR* variants are a monogenic cause of hearing loss. Further functional evidence is needed to evaluate this phenotypic association.

**KEYWORDS**

autosomal recessive deafness, ectodermal dysplasia, hearing loss, tooth agenesis, *TSPEAR*

**1 | INTRODUCTION**

Variants in the thrombospondin-type laminin G domain and epilepsy-associated repeats (*TSPEAR*) gene have been associated with two different autosomal recessive phenotypes; individuals with biallelic *TSPEAR* variants have presented either with sensorineural hearing loss (SNHL) or with a variety of phenotypes that affect ectodermal-derived structures such as skin, hair, nails, and teeth and which span a range of severity from isolated tooth agenesis to ectodermal dysplasia (ED; Delmaghani et al., 2012; Du et al., 2018; Peled et al., 2016; Sloan-Heggen et al., 2016; Song et al., 2020). This divergence of phenotypes is interesting. At one extreme, individuals have been reported to have profound hearing loss and otherwise purportedly normal features, and at another extreme have demonstrated striking ED and completely normal hearing (Delmaghani et al., 2012; Peled et al., 2016). There is currently little information available to explain such a wide divergence in *TSPEAR*-related clinical features.

The *TSPEAR* hearing loss association originates from a 2012 study which identified three consanguineous Iranian siblings affected by profound SNHL (Delmaghani et al., 2012). Whole exome sequencing (WES) for these siblings identified a homozygous c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) *TSPEAR* variant segregating with hearing loss. No other hearing loss variants were identified and the authors concluded that *TSPEAR* is a cause of nonsyndromic hearing loss. One subsequent report used panel-based

sequencing to identify additional *TSPEAR* individuals with hearing loss: two siblings with compound heterozygous variants, a synonymous c.1566G>A (p.Pro522Pro) variant with a potential impact on splicing and a frameshift c.1676\_1677delAT (p.Tyr559Cysfs\*134) variant, which together were the sole candidate hearing loss variants (Sloan-Heggen et al., 2016). Both siblings displayed bilateral SNHL and enlargement of the vestibular aqueduct (EVA). Neither of these studies reported any additional features that were evocative of ED.

In contrast to these two hearing loss reports, recent studies have described *TSPEAR* subjects with either isolated, nonsyndromic tooth agenesis or ED with tooth agenesis as a component of the subject phenotype (Du et al., 2018; Peled et al., 2016; Song et al., 2020). A 2016 report identified three separate families affected by dysmorphic facial features, scalp hypotrichosis, and hypodontia (Peled et al., 2016). These findings have been partially corroborated by subsequent studies which have identified additional individuals displaying normal hearing and nonsyndromic oligodontia (Du et al., 2018; Song et al., 2020). Of note, these reports have also identified several hearing-normal individuals with the same c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) variant initially associated with hearing loss. Based on the current literature, the majority of individuals with biallelic *TSPEAR* variants have displayed either isolated, nonsyndromic tooth agenesis or ED with tooth agenesis (Du et al., 2018; Peled et al., 2016; Song et al., 2020). The evidence around the *TSPEAR* hearing loss is less clear, raising the question of whether hearing loss

displays variable penetrance in individuals with *TSPEAR* variants, or if hearing loss has been misattributed to *TSPEAR* variants in prior studies.

There is conflicting biological evidence to suggest a role for *TSPEAR* in hearing function. Prior studies have proposed that *TSPEAR* may regulate inner ear hair cell fate decisions during development by interacting with the Notch signaling pathway (Peled et al., 2016). Additionally, *TSPEAR* exons 2–5 contain a laminin G domain, commonly found in extracellular matrix proteins, and immunohistochemistry (IHC) of healthy mouse cochlear sections suggests that *TSPEAR* may localize to the base of mammalian inner ear hair cells (Delmaghani et al., 2012; Mills et al., 2018; Mitchell et al., 2018). However, RNA-sequencing data from the gEAR portal (gene Expression Analysis Resource, umgear.org) indicates extremely low or absent expression of *TSPEAR* transcripts within murine cochlea and no studies have directly examined the effects of *TSPEAR* loss-of-function variants on hair cell morphology (Elkon et al., 2015; Scheffer et al., 2015; Waldhaus et al., 2015). While *TSPEAR* knockout mice are slated for development through the International Mouse Phenotyping Consortium, no *TSPEAR* knockout animal models have been produced thus far. Furthermore, when considering the evidence supporting a role for *TSPEAR* in hearing loss, one must consider that hearing loss is extremely heterogeneous. It is possible unidentified variants in genes other than *TSPEAR* may be causally related to an individual's hearing loss phenotype; for example, the nearby *TMPRSS3* locus has been implicated in autosomal recessive, nonsyndromic hearing loss and variants at this locus can be co-inherited alongside *TSPEAR* variants (Masmoudi et al., 2001). Lastly, hearing loss often has a significant environmental or prenatal component, with diseases such as cytomegalovirus and rubella representing the leading environmental causes of acquired childhood sensorineural hearing loss worldwide (Goderis et al., 2014; Korver et al., 2011; Korver et al., 2017).

In summary, the existing literature presents limited evidence to argue that *TSPEAR* variants are a direct cause of hearing loss, and previously identified individuals present with conflicting phenotypes of hearing loss, isolated tooth agenesis, or ED with tooth agenesis as a component of the subject phenotype. While the connection between *TSPEAR* variants and tooth agenesis or ectodermal dysplasia appears to be well-supported by previous cohort studies, we aim to clarify the association between *TSPEAR* and hearing loss phenotypes by evaluating specific variant/phenotype associations in additional individuals. In this text, we describe 13 newly reported individuals with biallelic, suspected pathogenic *TSPEAR* variants, three of whom have hearing loss phenotypes, and compare these data with previous reports.

## 2 | METHODS

### 2.1 | Editorial policies and ethical considerations

All individuals provided informed consent to be included in this study through protocols approved by their corresponding Institutional Review Boards.

### 2.2 | Cohort inclusion criteria and subject recruitment

Individuals were included in this study if they presented with biallelic, confirmed *trans*, *TSPEAR* variants. Individuals in this cohort were identified by the Mayo Clinic Department of Clinical Genetics, the Molecular Otolaryngology and Renal Research Laboratories (MORL) at the University of Iowa Carver College of Medicine, and through collaborations facilitated by GeneMatcher (Sobreira et al., 2015). For retrospectively identified individuals, clinical descriptions were abstracted from data collected by the MORL.

### 2.3 | Phenotype definitions

For all clinical descriptions, hypodontia is defined as the absence of one to five teeth, while oligodontia is defined as the absence of six or more teeth (de La Dure-Molla et al., 2019). "Isolated tooth agenesis" is used to describe a subset of cohort subjects with nonsyndromic tooth agenesis who do not meet the diagnostic criteria for ectodermal dysplasia. Individuals were classified as having ectodermal dysplasia if they presented with phenotypes affecting two or more ectodermal structures (e.g., teeth, hair, nails, or skin) (Wright et al., 2019). To ensure standard reporting between subjects, phenotypes have been described using Human Phenotype Ontology (HPO) terms when possible (Köhler et al., 2018).

### 2.4 | Subject phenotyping

Cohort individuals 1, 2, and 5 presented with hearing loss and were referred for genetic hearing loss testing; for these subjects, de-identified phenotypic information has been provided by the sequencing lab rather than the ordering clinicians. This information indicates that cohort individuals 1 and 2 presented with hearing loss and did not receive clinical or radiographic evaluation for dental phenotypes as a part of their visits with healthcare providers, while cohort subject 5 received clinical evaluation for dental phenotypes that did not include dental radiography. The remaining subjects reported in this study were phenotyped through visits with study clinicians, during which time the subjects were evaluated for ectodermal dysplasia including dental phenotypes. During this phenotyping, clinicians noted when subjects satisfied the diagnostic criteria for ectodermal dysplasia and differentiated between syndromic and isolated tooth agenesis. As a part of their clinical visits, cohort individuals 7, 8, 10, 15, 16, 17, and 18 received dental X-rays, while cohort individuals 6, 9, and 14 did not receive dental X-rays. Individuals 3, 4, 11, 12, 13, 19, 20, and 21 have been described in prior publications, but are reported in this article in order to fully detail the spectrum of phenotypes for individuals with biallelic *TSPEAR* variants. Individuals 3 and 4 did not receive dental radiography as a part of their clinical evaluation (Delmaghani et al., 2012; Sloan-Heggen et al., 2016), while the publications describing individuals 11, 12, 13, 19, 20, and 21 reported dental radiography as a part of the subject phenotyping process (Du et al., 2018;

Peled et al., 2016; Song et al., 2020). For newly identified individuals presenting with tooth agenesis, providers confirmed that all missing teeth were developmentally absent rather than extracted.

## 2.5 | Subject sequencing

Individuals were evaluated using a variety of sequencing methods, including WES ( $N = 6$ ), hearing loss gene panels ( $N = 3$ ), ectodermal dysplasia or tooth agenesis gene panels ( $N = 3$ ), and SNP array followed by Sanger sequencing ( $N = 1$ ). Sequencing methods are summarized for each newly reported case in Table 2, while sequencing chromatograms and information on variant sequencing depth are presented further in Table S2, Supporting Information. Hearing loss testing used the targeted hearing loss gene panel (OtoSCOPE v4-v6) performed at the MORL. OtoSCOPE is a comprehensive gene panel designed for nonsyndromic hearing loss and includes *TSPEAR*, but does not include the full spectrum of potential ectodermal dysplasia genes (Shearer et al., 2010; Sloan-Heggen et al., 2016). OtoSCOPE versions 4–6 include 66, 89, and 109 genes, respectively. All nucleotide changes are reported according to the GRh37 reference genome and the NM\_144991.2 *TSPEAR* transcript, which is the longest transcript and is indicated by the GTEx Portal database to be the dominant transcript in skin. Variants in *GJB2*, *TMPRSS3*, and *ATP1A3* are reported using the NM\_004004.5, NM\_024022.2, and NM\_152296.4 RefSeq transcripts, respectively. Per the clinical report, all variants were confirmed with Sanger sequencing, and all available chromatograms are included in Supporting Information.

## 2.6 | Variant classification

All variants, including those previously reported in the literature, were classified based on ACMG/AMP 2015 criteria (Table 2) (Richards et al., 2015). Alamut<sup>®</sup> Visual 2.11 (Interactive Biosoftware, Rouen, France) was used to aid in variant interpretation. The allele frequency for each variant in general population was obtained from GnomAD v2.1.1 (Karczewski et al., 2019). The AlignGVD, SIFT, MutationTaster, and PolyPhen-2 tools were used to generate in silico predictions of variant pathogenicity (Adzhubei et al., 2010; Ng & Henikoff, 2001; Schwarz et al., 2014; Tavtigian et al., 2006). CADD scores are reported for all missense variants using the GRCh37/hg19 genome assembly (Rentzsch et al., 2018). The SpliceAI, SpliceSiteFinder, MaxEntScan, and GeneSplicer tools were used to evaluate variant impact on splicing (Jaganathan et al., 2019; Pertea et al., 2001; Shapiro & Senapathy, 1987; Yeo & Burge, 2004). Frameshift and nonsense variants were evaluated for the likelihood of undergoing nonsense mediated decay using the NMDescPredictor tool (Coban-Akdemir et al., 2018). Individuals 17 and 18 were investigated using the Diagnosing Dental Defects Database (D4) and the Phenodent phenotyping tool (www.phenodent.org) (CNIL No. 908416; MES, Commission Bioéthique Collection Biologique “Manifestations bucco-dentaires des maladies rares” DC-2012-1677 and DC-2012-1002;

<https://clinicaltrials.gov>: NCT01746121 and NCT02397824) and genotyped with GenoDENT (Rey et al., 2019).

## 2.7 | Variant burden calculation

To identify *TSPEAR* gene regions that are less tolerant to variation than others, a variant concentration ( $V$ ) calculation is presented which normalizes the variant number ( $N$ ) within each exon or protein domain to the length of the gene-region in base-pairs ( $L$ ),

$$V = \frac{N}{L}$$

The score was calculated for all exons as well as for the laminin G and EAR protein domains and is intended to provide a simple metric to determine the variant concentration in these gene regions. As an additional measure of genotype/phenotype correlation, a Mann–Whitney  $U$  test used to examine the association between variant CADD score and phenotype (hearing loss vs. hearing normal,  $\alpha = 0.05$ ).

## 2.8 | Data availability

All variants described in this study are openly available for access within the ClinVar variant database (<https://www.ncbi.nlm.nih.gov/clinvar/>).

# 3 | RESULTS

## 3.1 | Cohort features

Newly reported individuals ( $N = 13$ ) are aggregated alongside previously reported individuals ( $N = 8$ ) in Table 1 to provide a comprehensive overview of reported *TSPEAR* phenotypes. When grouped by phenotype, individuals with *TSPEAR* variants fall into three broad categories: individuals with reported hearing loss (individuals 1–5), individuals with ectodermal dysplasia (with or without tooth agenesis, individuals 5–14), and individuals with isolated, nonsyndromic tooth agenesis (individuals 18, 20, and 21). Individual 5 presents with both hearing loss and ectodermal dysplasia without tooth agenesis, while individuals 15, 16, and 17 have nonsyndromic tooth agenesis accompanied by additional dental features such as conical teeth or taurodontism which do not satisfy diagnostic criteria for ectodermal dysplasia. All variants were scored according to ACMG 2015 criteria (Table 2) (Richards et al., 2015). For the three newly reported individuals with reported hearing loss, sequencing identified additional variants in the hearing-loss-associated genes *GJB2*, *GJB6*, and *TMPRSS3*, none of which were classified as benign according to ACMG 2015 criteria (Table 2, individuals 1, 2, 6). Full descriptions and family histories for each individual are reported in Appendix A.

**TABLE 1** Expanded cohort of TSPEAR individuals

Patient	Gene	Nucleotide	Amino acid	Zygoty	Phenotype										Reference	
					Hearing loss	ED	Dentition			Skin, nails, and hair			Hypotrichosis	Hypohidrosis		Miscellaneous
							Hypodontia	Oligodontia	Conical teeth	Nail dysplasia	Sparse hair					
1	TSPEAR	c.533C>T	p.Pro178Leu	Hom	Yes											Newly reported
	TMPRSS3	c.1204G>A	p.Gly402Arg	Hom												
2	TSPEAR	c.1163T>C	p.Val888Ala	Hom	Yes										Newly reported	
	TMPRSS3	c.1211C>T	p.Pro404Leu	Hom												
3	TSPEAR	c.1726_1728del(GTCinsTT <sup>a</sup> )	p.Val576Leufs*38	Hom	Yes										Delmaghani et al. (2012)	
4	TSPEAR	c.1566G>A	p.Pro522Pro	Het	Yes										Sloan-Heggen et al. (2016)	
	TSPEAR	c.1676_1677delAT	p.Tyr559Cysfs*134	Het												
5	TSPEAR	c.38delIT	p.Leu13A <sub>rgfs</sub> *38	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Newly reported	
	TSPEAR	c.589C>T	p.Arg197*	Het												
6	GJB2	c.35delG	p.Gly12Valfs*2	Het											Newly reported	
	GJB6	del(GJB6-D13S1854)	NA	Het												
7	TSPEAR	c.1915G>A	p.Asp639Asn	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Newly reported	
	TSPEAR	c.589C>T	p.Arg197*	Het												
8	TSPEAR	c.589C>T	p.Arg197*	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Newly reported	
	TSPEAR	c.83-13708_1566+248del	NA	Het												
9	TSPEAR	c.1574G>A	p.Gly525Asp	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Newly reported	
	TSPEAR	c.543-1G>A	NA	Het												
10	TSPEAR	c.942C>G	p.Tyr314*	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Newly reported	
	TSPEAR	c.1469T>A	p.Leu490Gln	Het												

(Continues)

TABLE 1 (Continued)

Patient	Gene	Nucleotide	Amino acid	Zygoty	Phenotype				Skin, nails, and hair				Reference	
					Hearing loss	ED	Dentition		Nail dysplasia	Sparse hair	Hypohidrosis	Hypotrichosis		Miscellaneous
							Hypodontia	Oligodontia						
11	TSPEAR	c.1726_1728delGTCinsTT	p.Val576Leufs*38	Hom	Yes	Yes	Yes	Yes	Yes	Yes	Yes	eczema, recurrent otitis media, Turner syndrome	Peled et al. (2016)	
12	TSPEAR	c.1726_1728delGTCinsTT	p.Val576Leufs*38	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Abnormal facial shape	Peled et al. (2016)	
13	TSPEAR	c.454_457delCTGG	p.Leu152Trpfs*29	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Abnormal facial shape	Peled et al. (2016)	
14	TSPEAR	c.1852T>A	p.Tyr618Asn	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Abnormal facial shape	Peled et al. (2016)	
14	TSPEAR	c.1915G>A	p.Asp639Asn	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Abnormal facial shape	Peled et al. (2016)	
14	TSPEAR	c.240T>G	p.Cys80Trp	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Sparse hair in childhood	Newly reported	
15	TSPEAR	c.633+2C>A	NA	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Attention disorder, large anteverted ears, prognathism, speech difficulties	Newly reported	
15	TSPEAR	c.1505delA	p.Lys502Argfs*67	Hom	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Depressed nasal bridge, developmental delay, hyperreflexia, paroxysmal dyskinesia, primary molar taurodontism, sagittal craniosynostosis, small and cupped ears	Newly reported	
16	TSPEAR	c.1726_1728delinsTT	p.Val576Leufs*38	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Depressed nasal bridge, developmental delay, hyperreflexia, paroxysmal dyskinesia, primary molar taurodontism, sagittal craniosynostosis, small and cupped ears	Newly reported	
16	TSPEAR	c.589C>T	p.Arg197*	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Depressed nasal bridge, developmental delay, hyperreflexia, paroxysmal dyskinesia, primary molar taurodontism, sagittal craniosynostosis, small and cupped ears	Newly reported	
16	ATPIA3	c.2767G>A	p.Asp923Asn	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Depressed nasal bridge, developmental delay, hyperreflexia, paroxysmal dyskinesia, primary molar taurodontism, sagittal craniosynostosis, small and cupped ears	Newly reported	
17	TSPEAR	c.1915G>A	p.Asp639Asn	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Dry skin, eczema, taurodontism	Newly reported	
17	TSPEAR	c.1331G>A	p.Arg444Gln	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Dry skin, eczema, taurodontism	Newly reported	
18	TSPEAR	c.1663C>T	p.Gln555*	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Scoliosis	Newly reported	
18	TSPEAR	c.1899dup	p.Thr634Hisfs*60	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Scoliosis	Newly reported	
19	TSPEAR	c.1726_1728delGTCinsTT	p.Val576Leufs*38	Hom	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Abnormality of the antitragus, high-arched palate, low-set ears, microcephaly, narrow forehead	Du et al. (2018)	
20	TSPEAR	c.1877T>C	p.Phe626Ser	Hom	Yes	Yes	Yes	Yes	Yes	Yes	Yes	None	Du et al. (2018)	
21	TSPEAR	c.1528C>T	p.Arg510*	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	None	Song et al. (2020)	
21	TSPEAR	c.1330C>T	p.Arg444Trp	Het	Yes	Yes	Yes	Yes	Yes	Yes	Yes	None	Song et al. (2020)	

Note: 13 newly reported TSPEAR individuals are listed alongside eight previously reported cases. The majority of these individuals segregate into three broad phenotypic categories: those who display hearing loss (#1–5), those who display ectodermal dysplasia (ED) with and without tooth agenesis (#5–21), and those who display isolated, nonsyndromic tooth agenesis (#18, 20, 21). Individual 5 presents with both hearing loss and ectodermal dysplasia without tooth agenesis, while individuals 15, 16, and 17 have nonsyndromic tooth agenesis accompanied by additional dental features such as conical teeth or taurodontism which do not satisfy diagnostic criteria for ectodermal dysplasia. Full case descriptions can be found in appendices 1 and 2.

Abbreviations: del, deletion; delins, deletion/insertion; dup, duplication; ED, ectodermal dysplasia.

\*Variants for individual 3 were originally reported as homozygous c.1726G>T and c.1728delC variants in cis, which can alternately be reported as c.1726\_1728delGTCinsTT (p.Val576Leufs\*38).

**TABLE 2** TSPEAR variant ACMG classification

Variant Patient	Gene	Nucleotide change	Amino acid	Zygosity	Variant interpretation			ACMG classification	Evidence	Sequencing method	References
					CADD score	GnomAD AF	ClinVar clinical significance				
1	TSPEAR	c.533C>T	p.Pro178Leu	Hom	17.86	0.00003668	NA	VUS	PM2, PP3, BP5	Hearing loss panel	Newly reported
	TMPRSS3	c.1204G>A	p.Gly402Arg	Hom	31	0	NA	VUS	PM2, PP3, BP5		
2	TSPEAR	c.1163T>C	p.Val388Ala	Hom	33	0	NA	VUS	PM2, PP3, BP5	Hearing loss panel	Newly reported
	TMPRSS3	c.1211C>T	p.Pro404Leu	Hom	26.1	0.00001193	Pathogenic	Likely pathogenic	PP1, PM2, PM3, PP3, PM1, BP5		
3	TSPEAR	c.1726_1728delGT	p.Val576Leufs*38	Hom	33	0	Likely pathogenic	Pathogenic	PVS1, PM2, PP3	WES	Delmagnani et al. (2012)
4	TSPEAR	c.1566G>A	p.Pro522Pro	Het	33	0.00007454	NA	VUS	PM2, PM3, PP3	Hearing loss panel	Sloan-Heggen et al. (2016)
	TSPEAR	c.1676_1677delAT	p.Tyr559Cysfs*134	Het	31	0	Pathogenic/Likely pathogenic	Pathogenic	PVS1, PM2, PP3		
5	TSPEAR	c.38delT	p.Leu13Argfs*38	Het	24.6	0.00008334	NA	Pathogenic	PVS1, PM2, PM3, PP3, BP5	Hearing loss panel	Newly reported
	TSPEAR	c.589C>T	p.Arg197*	Het	36	0.000305	Conflicting	Pathogenic	PVS1, PM2, PM3, PP3, BP5		
	GJB2	c.35delG	p.Gly12Valfs*2	Het	25.3	0.006188	Pathogenic	Pathogenic	PVS1, PS3, PS4, PM2, PP1-S, PP3, BP5		
	GJB6	del(GJB6-DJ13S1854)	NA	Het	NA	NA	Pathogenic	Pathogenic	PVS1, PS3, PS4, PP1-S, PM2, BP5		
6	TSPEAR	c.1915G>A	p.Asp639Asn	Het	28.2	0.002251	Conflicting	VUS	PM2, PM3, PP3, BS2	WES	Newly reported
	TSPEAR	c.589C>T	p.Arg197*	Het	36	0.000305	Conflicting	Pathogenic	PVS1, PM2, PP3		
7	TSPEAR	c.1915G>A	p.Asp639Asn	Het	28.2	0.002251	Conflicting	VUS	PM2, PP3, BS2	WES	Newly reported
	TSPEAR	c.1754G>T	p.Ser585Ile	Het	25.6	0.00006734	Uncertain significance	VUS	PM2, PP3		
8	TSPEAR	c.589C>T	p.Arg197*	Het	36	0.000305	Conflicting	Pathogenic	PVS1, PM2, PM3, PP3	WES	Newly reported
	TSPEAR	c.83_13708_1566-1248del	NA	Het	NA	NA	NA	Pathogenic	PVS1, PM2, PM3, PP3		
9	TSPEAR	c.1574G>A	p.Gly525Asp	Het	24.2	0.00003199	NA	VUS	PM2, PM3, PP3	WES	Newly reported
	TSPEAR	c.543-1G>A	NA	Het	23.9	0.000007119	NA	Pathogenic	PVS1, PM2, PP3		
10	TSPEAR	c.942C>G	p.Tyr314*	Het	36	0	Likely pathogenic	Pathogenic	PVS1, PM2, PP3	WES	Newly reported
	TSPEAR	c.1469T>A	p.Leu490Gln	Het	29.7	0.00005569	Uncertain significance	VUS	PM2, PM3, PP3		
11	TSPEAR	c.1726_1728delGT	p.Val576Leufs*38	Hom	33	0	Likely pathogenic	Pathogenic	PVS1, PM2, PP3	WES	Peled et al. (2016)
12	TSPEAR	c.1726_1728delGT	p.Val576Leufs*38	Het	33	0	Likely pathogenic	Pathogenic	PVS1, PM2, PP3	WES	Peled et al. (2016)
	TSPEAR	c.454_457delCTGG	p.Leu152Trpfs*29	Het	23.3	0.00004063	Pathogenic	Pathogenic	PVS1, PM2, PP3		
13	TSPEAR	c.1852T>A	p.Tyr618Asn	Het	32	0.0000199	Pathogenic	VUS	PM2, PP3	WES	Peled et al. (2016)
	TSPEAR	c.1915G>A	p.Asp639Asn	Het	28.2	0.002251	Conflicting	VUS	PM2, PP3, BS2		
14	TSPEAR	c.240T>G	p.Cys80Trp	Het	23.3	0	NA	VUS	PM2, PM3, PP3	Ectodermal dysplasia panel; Sanger sequencing	Newly reported
	TSPEAR	c.633+2C>A	NA	Het	22.7	0.000004001	NA	Pathogenic	PVS1, PM2, PP3		
15	TSPEAR	c.1505delA	p.Lys502Argfs*67	Hom	18.1	0.00001592	NA	Pathogenic	PVS1, PM2, PP3	SNP array; Sanger sequencing	Newly reported
16	TSPEAR	c.1726_1728delinsTT	p.Val576Leufs*38	Het	33	0	Likely pathogenic	Pathogenic	PVS1, PM2, PM3, PP3	WES	Newly reported
	TSPEAR	c.589C>T	p.Arg197*	Het	36	0.000305	Conflicting	Pathogenic	PVS1, PM2, PP3, PM3		

(Continues)

TABLE 2 (Continued)

Variant	Variant interpretation										
	Gene	Nucleotide change	Amino acid	Zygoty	CADD score	GnomAD AF	ClinVar clinical significance	ACMG classification	Evidence	Sequencing method	References
ATP1A3		c.2767G>A	p.Asp923Asn	Het	23.8	0	Pathogenic	Likely pathogenic	PS1, PM1, PM2, PP3		
17	TSPEAR	c.1915G>A	p.Asp639Asn	Het	28.2	0.002251	Conflicting	VUS	PM2, PP3, BS2	GenoDENT: dental anomalies panel; Sanger sequencing	Newly reported
	TSPEAR	c.1331G>A	p.Arg44Cln	Het	28.5	0.00008862	NA	VUS	PM2, PP3		
18	TSPEAR	c.1663C>T	p.Gln555*	Het	39	0	NA	Pathogenic	PV51, PM2, PM3, PP3	GenoDENT: dental anomalies panel; Sanger sequencing	Newly reported
	TSPEAR	c.1899dup	p.Thr634Hisfs*60	Het	34	0	NA	Pathogenic	PV51, PM2, PM3, PP3		
19	TSPEAR	c.1726_1728delGTCinsTT	p.Val576Leufs*38	Hom	33	0	Likely pathogenic	Pathogenic	PV51, PM2, PP3	WES	Du et al. (2018)
20	TSPEAR	c.1877T>C	p.Phe626Ser	Hom	28.5	0.0001401	NA	VUS	PM2, PP3	WES	Du et al. (2018)
21	TSPEAR	c.1528C>T	p.Arg510*	Het	37	0.0003256	Uncertain significance	Pathogenic	PV51, PM2, PP3	WES	Song et al. (2020)
	TSPEAR	c.1330C>T	p.Arg444Trp	Het	25.8	0.00001595	Uncertain significance	VUS	PM2, PM3, PP3		

Note: All variants in this study were classified according to American College of Medical Genetics (ACMG) 2015 criteria. Classifications are shown here, along with the evidence used to support each classification such as combined annotation dependent depletion (CADD) score and genome aggregation database (GnomAD) allele frequency. While numerous suspected pathogenic TSPEAR variants have been reported in the literature, these studies did not report ACMG scoring criteria. To provide a means of comparing newly reported TSPEAR variants to published TSPEAR variants, these published variants have been scored here using the available evidence. ClinVar accessed January 12, 2021; GnomAD v2.1.1 accessed January 13, 2021.

Abbreviations: AF, allele frequency; CADD, combined annotation dependent depletion; del, deletion; ins, insertion; dup, duplication; GnomAD, genome aggregation database; NA, not available; VUS, variant of uncertain significance.

\*Variants for individual 3 were originally reported as homozygous c.1726G>T and c.1728delC variants in cis, which can alternately be reported as c.1726\_1728delGTCinsTT (p.Val576Leufs\*38).

### 3.2 | Hearing loss

Individuals with hearing loss presented with variable phenotypes that included syndromic, congenital-onset SNHL and nonsyndromic adolescent-onset hearing loss. For newly reported individuals, we observed a lower prevalence of hearing loss compared to phenotypes such as isolated tooth agenesis or ectodermal dysplasia with and without tooth agenesis (3/13 individuals versus 11/13 individuals). In each of the three newly reported individuals with hearing loss, variants in other genes associated with hearing loss were also identified. Detailed descriptions of these newly reported hearing loss individuals are presented below and summarized in Table 1.

Individual 1 is an Asian-ancestry male who presented with SNHL and no features evocative of ED or tooth agenesis. The individual carries a newly reported c.533C>T (p.Pro178Leu) missense TSPEAR variant in a homozygous state (Table 1). The individual's hearing loss diagnosis is complicated by a c.1204G>A (p.Gly402Arg) homozygous missense variant in the nearby *TMPRSS3* gene. While loss-of-function variants in *TMPRSS3* have been related to autosomal recessive, childhood-onset, neurosensory deafness (Masmoudi et al., 2001), this variant is novel. The TSPEAR and *TMPRSS3* variants are currently classified as variants of uncertain significance (VUS; Table 2).

Individual 2 is an Iranian male and one of two affected children born to consanguineous parents presenting with deafness and a homozygous c.1163C>T (p.Val388Ala) TSPEAR missense variant (Table 1). This variant has not been previously reported and is currently considered to be a VUS. This individual also carries a homozygous c.1211C>T *TMPRSS3* (p.Pro404Leu) variant previously reported to be pathogenic in ClinVar, though no assertion criteria were provided for this entry (Accession number RCV000005233.4). Using the current available evidence, this *TMPRSS3* variant was classified as likely pathogenic following review in this study (Table 2).

Individuals 3–4 have been previously reported, are presented in Table 1 to provide a complete overview of TSPEAR phenotypes, and are summarized in greater detail in Appendix B. Individual 3 was reported by Delmaghani et al. to have profound sensorineural hearing loss and an otherwise normal phenotype (Delmaghani et al., 2012). WES identified two sets of homozygous TSPEAR variants, c.1726G>T and c.1728delC (which can alternatively be presented as a single c.1726\_1728delGTCinsTT, p.Val576Leufs\*38 indel). Individual 4 has been described by Sloan-Heggen et al. to have a phenotype that includes childhood onset SNHL, EVA, and proteinuria (Sloan-Heggen et al., 2016). This individual underwent gene panel-based hearing loss evaluation (OtoScope v5, 89 genes) which identified a c.1566G>A (p.Pro522Pro) TSPEAR variant which is predicted to impact splicing and a c.1676\_1677delAT (p.Tyr559Cysfs\*134) frame-shift TSPEAR variant.

Individual 5 is a Caucasian male presenting with features that include sparse hair, sparse eyebrows, dysplastic nails, cone-shaped teeth, and severe congenital SNHL. This individual's phenotype satisfies the diagnostic criteria for ectodermal dysplasia without tooth agenesis. Sequencing identified a c.589C>T (p.Arg197\*) TSPEAR variant which results in a premature stop codon, inherited alongside a likely pathogenic c.38delT (p.Leu13Argfs\*38) frame-shift variant



(Table 1). In addition to these *TSPEAR* variants, predicted to result in biallelic loss of function, the individual carries a *GJB2* variant (c.35delG, p.Gly12Valfs\*2) and a 309 kb *GJB6-D13S1854* deletion. *GJB2* is an established cause of autosomal dominant hearing loss with or without skin findings, while the *GJB6-D13S1854* deletion spans multiple gene loci and is a common cause of nonsyndromic hearing loss (del Castillo et al., 2005; Rodriguez-Paris et al., 2011; Tayoun et al., 2016). Together, the individual's *GJB2* and *TSPEAR* variants, alongside the *GJB6-D13S1854* deletion, present competing genetic candidates to explain their phenotype.

### 3.3 | Additional cohort phenotypes

Individuals with normal hearing displayed varied phenotypes that most commonly included tooth agenesis: five individuals displayed isolated tooth agenesis (nonsyndromic hypodontia or oligodontia), while seven presented with additional evidence of nail dysplasia, sweating difficulties, or sparse hair that satisfied diagnostic criteria for ectodermal dysplasia (Table 1). More detailed dental findings were reported for three individuals: individual 9 was noted to have microdontia, while individuals 16 and 17 were reported to have taurodontism. Orthopantomographs for *TSPEAR* subjects 15, 17, and 18 are detailed further in Figure 1 to illustrate a subset of *TSPEAR* dental phenotypes including oligodontia, conical teeth, and taurodontism.

Eight of twelve individuals presented with additional findings that are believed to be unrelated to their *TSPEAR* diagnosis. Various neurological findings were identified in four individuals: individual 6 displayed hypoplasia of the corpus callosum, cortical visual impairment, global developmental delay and seizures while individual 7 demonstrated movement abnormalities, profound global developmental delay, and self-injurious behavior. Individual 8 was reported to have attention deficit hyperactivity disorder and resting tremor, in addition to abnormal heart morphology, basilar artery fenestration, recurrent otitis media, strabismus and tall stature. Individual 15 was described to have attention disorder, speech difficulties, large anteverted ears, and prognathism.

Individual 16 presented with facial features that included a depressed nasal bridge, hypertelorism, and small cupped ears in addition to sagittal craniosynostosis, paroxysmal dyskinesia and developmental delays that are believed to be related to a pathogenic *ATP1A3* variant identified via WES. While subtle facial dysmorphisms (such as down slanting palpebral fissures or low columella insertion) have been reported in *TSPEAR* subjects (Peled et al., 2016), they do not match these newly reported subject descriptions and no causative link has been established thus far between *TSPEAR* variants and these phenotypes. Two remaining individuals reported with non-neurological phenotypes: individual 10 was diagnosed with Turner syndrome in addition to their *TSPEAR* diagnosis, and presented with additional features including absent Meibomian glands, conductive hearing loss associated with otitis media, and eczema. Lastly, individual 18 was

diagnosed with scoliosis. Full clinical descriptions for these individuals are available in Appendix A.

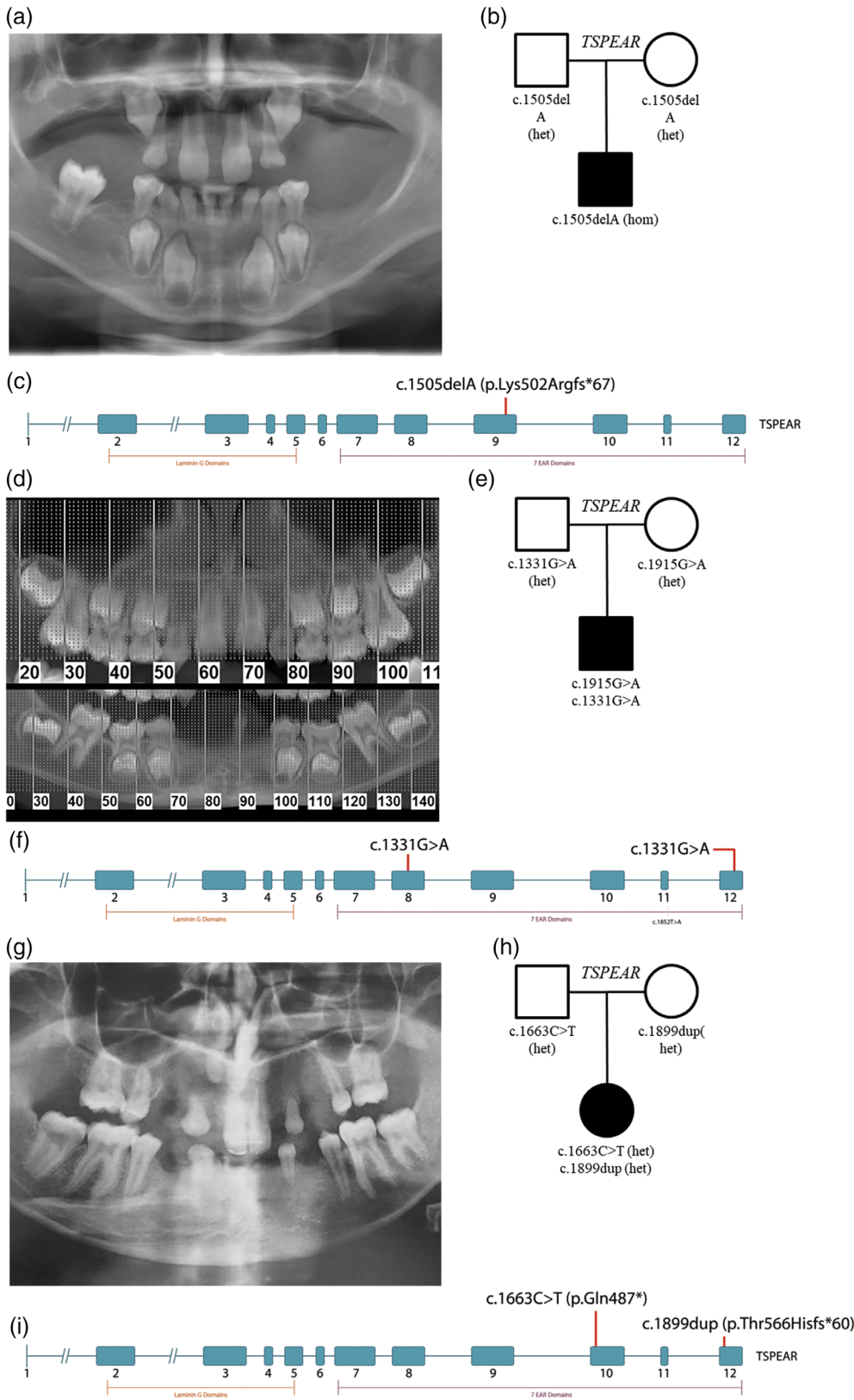
### 3.4 | *TSPEAR* structure and variant distribution

All *TSPEAR* variants were mapped using the NM\_144991.2 transcript and evaluated to determine if they concentrate within particular exons or predicted protein motifs (Figure 2). Variant burden scores, which normalize the variant concentration in each exon or predicted protein domain by the length of the region, indicate that the majority of reported variants concentrate in exons 4, 10, and 12, and within the EAR protein domains (Table S1). While variants are concentrated in particular regions of the gene, there was no observable correlation between variant location and phenotype, or between variant CADD score and presence of hearing loss (Mann-Whitney *U* test,  $\alpha = 0.05$ ,  $p = 0.91$ ).

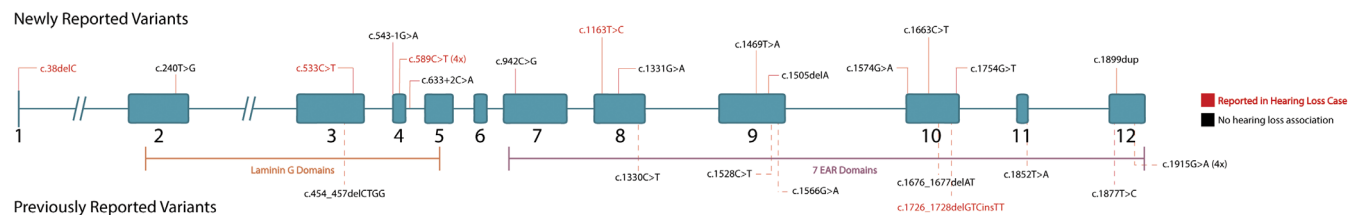
## 4 | DISCUSSION

This cohort significantly expands the number of reported individuals with *TSPEAR* alterations and confirms that the most prevalent phenotypes associated with biallelic *TSPEAR* variants include tooth agenesis (18/21 individuals) and ectodermal dysplasia with tooth agenesis (7/21 individuals). Only 3/13 newly reported individuals presented with hearing loss, and all possessed variants in additional genes that may explain the hearing loss phenotype. Two of the probands affected by hearing loss (individuals 1 and 2) do not seem to show any sign of ED despite the large body of evidence linking *TSPEAR* variants to tooth agenesis and ED. It is possible that *TSPEAR* phenotypes such as hearing loss and tooth agenesis show variable penetrance in subjects with different genetic backgrounds. For example, *FGF3* polymorphisms have been associated with variable levels of tooth agenesis in prior cohorts (Vieira et al., 2013), while multilocus inheritance has been well documented in Bardet-Biedl syndrome, where up to 23 loci have been identified that initiate or modify disease (McConnachie et al., 2020; McKusick-Nathans Institute of Genetic Medicine, 2020). While polygenic inheritance is one potential explanation for differences in *TSPEAR* phenotype penetrance, it is also possible that more subtle findings such as nail dysplasia or tooth agenesis were overlooked during clinical evaluation. The fact that hearing loss was a phenotype in less than 25% of the newly reported cases, and in each instance there was another reported plausible genetic cause for the hearing loss, weakens the evidence associating *TSPEAR* variants with hearing loss. It is reasonable to question the assertion that *TSPEAR* variants are a monogenic cause of hearing loss, or to at least recognize the reduced penetrance of this phenotype.

In light of these findings, it is worth revisiting the first studies to connect *TSPEAR* variants to hearing loss. Initial descriptions of the *TSPEAR* hearing loss association emerged from a 2012 study by Delmaghani et al. that used WES to identify biallelic *TSPEAR* variants which segregated with a SNHL phenotype (Delmaghani et al., 2012).



**FIGURE 1** Representative oligodontia phenotypes for *TSPEAR* cohort subjects. Eighteen of the cohort subjects detailed in this article presented with tooth agenesis, which varied in severity from hypodontia to oligodontia, while a subset of study individuals presented with additional features such as taurodontism and conical teeth. (a) Orthopantomographs for cohort subject 15 show the absence of 19 permanent teeth and cone-shaped canines that are characteristic of a subset of *TSPEAR* subjects detailed in this report. (b) The subject presented at 11 years of age and was born to unaffected, nonconsanguineous parents of Peruvian ancestry. Sequencing indicates that the subject is homozygous for *c.1505delA* (*p.Lys502Argfs\*67*) variants. (c) The identified deletion is located in exon 9 within the epilepsy-associated repeat (EAR) protein motif. (d) Orthopantomographs for cohort subject 17 show oligodontia with agenesis of 12, 13, 22, 23, 31, 32, 33, 41, 42, 43, as well as taurodontism of 16 and 26. (e) The subject was born to unaffected parents of European ancestry and presented for sequencing at age 9 with oligodontia, eczema, dry skin, and taurodontism. Sequencing identified two missense variants, *c.1915G>A* (*p.Asp639Asn*) and *c.1331G>A* (*p.Arg444Gln*). (f) These variants fall within exons 8 and 12, both of which form EAR protein motifs. (g) Orthopantomographs for cohort subject 18 show oligodontia with agenesis of teeth 12, 13, 14, 17, 18, 22, 23, 24, 27, 28, 31, 32, 33, 34, 38, 41, 42, 43, 44, and 48. (h) The subject was born to unaffected parents of European ancestry and presented for sequencing at age 18 with tooth agenesis and scoliosis. Sequencing indicated the subject is heterozygous for two *c.1633C>T* and *c.1899dup* variants. (i) These variants are located within exons 10 and 12 and occur within EAR protein motifs. del, deletion; dup, duplication [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 2** Variant location and gene structure. All variants associated with tooth agenesis or ectodermal dysplasia (with and without tooth agenesis, black) and variants associated with hearing loss (red) were mapped to their location within the NM\_144991.2 *TSPEAR* transcript to provide an indication of exons or protein motifs that may be intolerant to variation. Newly reported variants are shown above the diagram, while previously reported variants are shown below the diagram. Large gene deletions are not shown. The c.1726\_1728delGTCinsTT variant (p.Val576Leufs\*38) in exon 10 has been separately reported in association with both hearing loss and ectodermal dysplasia. *TSPEAR* contains a laminin G structural domain and seven epilepsy-associated repeat (EAR) domains which may mediate protein–protein interactions; both domains are indicated below the gene diagram. EAR, epilepsy-associated repeat; del, deletion; dup, duplication [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Subsequent reports in 2016 and 2018 identified individuals with the same genotype with normal hearing, which casts doubt on the original hearing loss report (Du et al., 2018; Peled et al., 2016). While no other hearing loss variants were identified in the Delmaghani study, hearing loss is an extremely heterogeneous condition with numerous genetic contributors—over 100 different genes have been associated with nonsyndromic hearing loss and it is possible that the true genetic cause was not recognized due to test limitations (Abou Tayoun et al., 2016). For example, WES has limitations in ability to detect copy number variants such as single or multiple exon deletions, which are known to be an important contributor to genetic hearing loss (Shearer et al., 2014). Additionally, negative findings on panel or WES-based approaches may simply reflect that the causal variant is not contained within the capture region, as can occur with deep intronic or noncoding variants; this is a situation potentially remedied through the use of next generation sequencing methods such as whole genome sequencing or RNA sequencing (Bodle et al., 2021; Di Scipio et al., 2020; Larrue et al., 2020). Furthermore, in the nearly 9 years since this first *TSPEAR* hearing loss publication, numerous additional hearing loss associations have emerged in the literature; variants in these hearing loss genes may have been overlooked at the time of publication (DiStefano et al., 2020). Lastly, hearing loss often has a significant environmental component, with pathogens such as cytomegalovirus accounting for a significant proportion of acquired hearing loss, particularly in underserved health systems (Goderis et al., 2014; Korver et al., 2017; Sloan-Heggen et al., 2016). These same limitations may apply to the second study to identify individuals with *TSPEAR* variants and nonsyndromic hearing loss: a 2016 report describing siblings with childhood onset, nonsyndromic, bilateral hearing loss, EVA, and compound heterozygous *TSPEAR* variants (Sloan-Heggen et al., 2016). These siblings were sequenced using a hearing loss gene panel—no other hearing loss variants were identified. These two reports represent the only cases of *TSPEAR*-related hearing loss that have been described in the literature. While these cases did not identify any alternate genetic explanations for subject hearing loss, they represent the minority of publications to date and there is an emerging consensus in the literature that the primary phenotypes associated

with *TSPEAR* variants are tooth agenesis and ED rather than hearing loss.

While we have identified new individuals in this study who present with biallelic *TSPEAR* variants, these individuals carry variants in other hearing loss genes such as *TMPRSS3* that may present more compelling explanations for their hearing loss phenotype. Our ability to more comprehensively explore the cause of hearing loss for these individuals is limited by an absence of complete clinical histories; the hearing loss descriptions collected in this article have been abstracted from limited clinical reports, and more complete information on the degree of hearing loss is therefore unavailable. Similar limitations apply to normal hearing cohort subjects, as dental radiographs and complete clinical histories were only available for a subset of these individuals. This makes it difficult to comprehensively evaluate how variable tooth agenesis phenotypes are in *TSPEAR* subjects, and complicates the identification of more subtle *TSPEAR*-associated phenotypes such as nail dysplasia. Despite this, this article strengthens evidence around the ED and tooth agenesis *TSPEAR* phenotypes, adds additional variants to the literature, and delivers insight into gene function. Missense variants appear to concentrate in exons 4, 10, and 12, and within the EAR domain of *TSPEAR*, indicating that these regions may have greater significance for normal gene function. The sub-RVIS score, a measure of tolerance to variation within protein domains and exons, lists exons 4, 9, and 10 as the least tolerant in this gene and describes exon 8 and 12 as the most tolerant, in partial agreement with results from this study (Gussow et al., 2016). Additionally, exons 7–12 correspond to the EAR protein motif, which is believed to mediate protein/protein interactions (The UniProt Consortium, 2019). If *TSPEAR* interacts with *NOTCH1*, as has been previously suggested by Peled et al., the EAR domains may be the primary protein regions responsible for accomplishing this (Peled et al., 2016).

Lastly, this cohort presents several additional occurrences of the c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) variant which has been consistently reported in the literature, almost entirely within individuals of Middle-Eastern ancestry (Delmaghani et al., 2012; Du et al., 2018; Peled et al., 2016). This variant is absent from the

GnomAD database, and while this change can also be reported as two separate c.1726G>C (p.Val576Leu) and c.1728delC (p.Lys577Serfs\*37) variants in *cis* with each other, neither variant has been observed as of the GnomAD v3 release (Karczewski et al., 2019). This c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) variant may be highly prevalent in Middle-Eastern populations given its consistent observance in individuals of Middle-Eastern ancestry; the GnomAD database does not currently contain a separate population category for Middle-Eastern individuals and these individuals are instead classified as “other” ( $N = 3070$  exomes and 1077 genomes). The Greater Middle East (GME) Variome Project (Scott et al., 2016), which reports whole exome data for 2497 individuals across the GME, also does not report any individuals with this same indel variant, although these sample sizes are low in comparison to the GnomAD database. Collectively, this delineates a continued need for the increased representation of diverse populations within publicly-available sequencing databases.

In conclusion, this cohort study significantly expands the body of reported individuals with *TSPEAR* variants, and brings into question whether loss-of-function of *TSPEAR* is a monogenic cause of hearing loss. Future work, featuring either larger *TSPEAR* cohorts or experimental approaches to analyze gene function will be needed to conclusively examine the putative *TSPEAR* hearing loss association.

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#### CONFLICT OF INTEREST

IMW and KMc are employees of GeneDx, Inc.

#### AUTHOR CONTRIBUTIONS

BB collected and analyzed patient variant data. EWK conceived and directed the study. BB, AF, FPV, and EWK performed the variant interpretation and wrote the article. CJN, RJS, JAH, AA, and ABZ providing editing suggestions for the article. NS, TR, KM, and IMW provided genetic data and assisted with variant interpretation. KM and IMW facilitated contacts between study authors. CJN, TR, BL, JS, KS, EA, DC, AW, AC, SL, SG, CR, CL, DBV, BCL, MLD, AA, JAH, BD, FCR, VS, BG, ABZ, and RJS provided clinical and phenotypic relevant information included in the article. All authors reviewed and approved the final submitted text.

#### DATA AVAILABILITY STATEMENT

All variants described in this study are openly available for access within the ClinVar variant database (<https://www.ncbi.nlm.nih.gov/clinvar/>).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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## APPENDIX: NEWLY REPORTED PHENOTYPIC DESCRIPTIONS

All newly reported individuals with hearing loss are detailed in the main text as individuals 1, 2, and 5. The clinical histories for the remaining individuals are detailed below. The main text and Table 1 also reference individuals who have been previously published in other studies; these cases are summarized in Appendix B.

Individual 6 is a European ancestry male who displays phenotypic features such as nail dysplasia, conical teeth, and hypodontia that are believed to be connected to the observed TSPEAR variants and that satisfy diagnostic criteria for ectodermal dysplasia with tooth agenesis. In addition, the individual presented with nystagmus and brachydactyly, as well as significant neurological features including global developmental delay, hypoplasia of the corpus callosum, and cortical visual impairment. WES detected a maternally inherited, c.1915G>A (p.Asp639Asn) missense variant and a paternally inherited c.589C>T (p.Arg197\*) nonsense variant. This individual has a brother with cone-shaped teeth and tooth agenesis, while a maternal aunt was noted to have a supernumerary tooth and a paternal grandfather was reported to have tooth agenesis.

Individual 7 is a European ancestry male presenting with a phenotype that includes oligodontia, conical teeth, and poor sweating. This phenotype satisfies the diagnostic criteria for ectodermal dysplasia with tooth agenesis. This individual also displayed strabismus in addition to significant neurological features such as intellectual disability, movement disorder, dissociative disorder, profound developmental delay, and self-injurious behavior. This individual harbors a c.1915G>A (p.Asp639Asn) variant in addition to a c.1754G>A (p.Ser585Ile) missense variant which is located at the 3' end of exon 10 and is predicted to disrupt splicing. Exon 10, if skipped, will result in an out-of-frame product. This individual's family history was otherwise uninformative.

Individual 8 is a male who presents with oligodontia, nail bed pitting, and thin nails. This phenotype satisfies the diagnostic criteria for ectodermal dysplasia with tooth agenesis. Additionally, this individual's phenotype consists of complex congenital heart disease, left strabismus, attention deficit hyperactivity disorder, tall stature, fenestration of the basilar artery, bilateral resting tremor, and recurrent otitis media infections. More specifically, this individual's heart disease encompasses coarctation of the aorta, a bicuspid aortic valve, an atrial septal defect, and patent ductus arteriosus. This individual has an identified c.589C>T (p.Arg197\*) nonsense variant which results in a premature stop. In addition, this individual has a c.83-13708\_1566+248 deletion that encompasses exons 2–9 of *TSPEAR*. This individual did not have similarly affected relatives.

Individual 9 is a European ancestry male who was referred for cone-shaped teeth, and who also displayed dysplastic nails, hypopigmented hair, sparse hair, sparse eyebrows, hypodontia, and misshapen teeth. This individual was also reported to “turn red” when hot, which suggests possible hypohidrosis. This phenotype satisfies the diagnostic criteria for ectodermal dysplasia with tooth agenesis. This individual's family history was otherwise uninformative. WES detected a c.1574G>A (p.Gly525Asp) paternally inherited missense alteration and a maternally inherited c.543-1G>A splicing variant which affects the –1 nucleotide of intron 3. This is predicted to result in the loss of a splice acceptor site, and the formation of an out of frame product due to skipping of exon 4.

Individual 10 is a three year old female of European ancestry who presented with hypotrichosis, conical teeth, eczema with mottling of skin, absence of the Meibomian glands, abnormal nail growth, hypohidrosis, and conductive hearing loss associated with otitis media. This phenotype satisfies the diagnostic criteria for ectodermal dysplasia without tooth agenesis. WES detected two novel compound heterozygous variants in *TSPEAR*, a c.942C>G (p.Tyr314\*) nonsense change and c.1469T>A (p.Leu490Gln) variant that is predicted to activate a cryptic splice acceptor in the center of exon 9. Variant segregation was confirmed by parental testing. Her diagnostic evaluation was complicated by incidental detection of variant Turner syndrome with a karyotype of 46,X,i(X)(q10)(46)/45,X(4). Her infant sister was subsequently found to have the same *TSPEAR* genotype and similar clinical features without Turner syndrome.

Individual 14 is a European ancestry male initially evaluated at 18 months and presenting with ectodermal dysplasia and conical teeth. The individual's hearing was normal and, upon initial evaluation, he also displayed sparse hair which resolved later in childhood. This phenotype satisfies the diagnostic criteria for ectodermal dysplasia without tooth agenesis. Ectodermal dysplasia panel-based sequencing identified compound heterozygous *TSPEAR* variants: a paternal c.240T>G (p.Cys80Trp) variant and a maternal c.633+2C>A variant with a possible effect on exon 4 splicing. The family history for this individual was otherwise uninformative.

Individual 15 is a European ancestry male presenting at age 11 years with oligodontia, conical-shaped canine teeth, attention disorder, anteverted ears, prognathism, and speech difficulties. Audiometric testing confirmed that the individual has normal hearing. The

individual was sequenced using a SNP array which identified multiple regions of homozygosity, one of which overlaps the *TSPEAR* locus. Targeted Sanger sequencing of the chromosome 21 region containing *TSPEAR* revealed the presence of likely pathogenic, homozygous c.1505delA (p.Lys502Argfs\*67) variants. While the parents are both of Peruvian ancestry, there was no reported history of consanguinity and the individual's family history was otherwise uninformative.

Individual 16 is an African American ancestry male who presented at age 7 years with oligodontia, conical teeth, primary molar taurodontism, sagittal craniosynostosis, and dysmorphisms that included hypertelorism, small and cupped ears, and a depressed nasal bridge. This individual also presented with a neurological phenotype that includes paroxysmal dyskinesia and developmental delays, which are believed to be caused by a pathogenic *ATP1A3* variant (c.2767G>A, p.D923N). This particular variant has been identified in several probands with variable features that included rapid-onset dystonia parkinsonism, alternating hemiplegia, and developmental delays (Anselm, Sweadner, Gollamudi, Ozelius, & Darras, 2009; Roubergue et al., 2013; Zanotti-Fregonara et al., 2008). Whole exome sequencing identified additional findings in *TSPEAR*: a c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) and a c.589C>T (p.Arg197\*) in *trans* which present a compelling explanation for the subject's dental phenotypes. Audiology evaluation indicated normal hearing. The subject's parents were unaffected, but his brother (who did not receive genetic testing) was reported to have missing permanent teeth as well.

Individual 17 is a 9-year-old European ancestry male presenting with ectodermal dysplasia, oligodontia, and conical teeth. He also displayed taurodontism and had no reported history of hearing loss. Sequencing identified compound heterozygous *TSPEAR* variants: c.1915G>A (p.Asp639Asn) and c.1331G>A (p.Arg444Gln) missense changes, the latter of which occurs near the end of the exon 8 and is predicted to create a weak splice acceptor site. The individual's family history was otherwise uninformative.

Individual 18 is a European ancestry female who presented at age 18 with oligodontia and severe scoliosis. This individual carries a c.1663C>T (p.Gln555\*) nonsense variant. Additionally, this individual harbors a c.1899dup (p.Thr634Hisfs\*60) variant which is predicted to create an out-of-frame product. This individual had no relevant family history and their hearing was not impacted.

## APPENDIX: SUMMARY AND SCORING OF PREVIOUSLY REPORTED *TSPEAR* CASES

Previously reported *TSPEAR* cases are described below. For the original manuscripts detailing these individuals, please refer to Delmaghani et al. (2012), Peled et al. (2016), and Du et al. (2018). Subject numbering refers to their order in Table 1 of the main text.

Individual 3 is an Iranian-ancestry male who was reported by Delmaghani et al. to have bilateral, profound SNHL, as confirmed by audiology (Delmaghani et al., 2012). No other clinical features or variants of interest were reported. This individual was initially reported to have biallelic c.1726G>T and c.1728delC *TSPEAR* variants, which can



alternatively be presented as biallelic c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) complex variants. This individual was born to consanguineous parents and had two affected brothers presenting with the same *TSPEAR* variants.

Individual 4 has been briefly described as part of a large hearing loss study, but is being reported here in greater detail (Sloan-Heggen et al., 2016). This individual is a Caucasian male with a phenotype that includes childhood onset SNHL, enlargement of the vestibular aqueduct, and proteinuria. The individual carries two *TSPEAR* variants: a c.1676\_1677delAT (p.Tyr559Cysfs\*134) deletion, and an additional c.1566G>A (p.Pro522Pro) missense variant affecting the last nucleotide of exon 9. Splicing prediction tools delivered conflicting reports for this variant—Alamut Visual tools (SpliceSiteFinder, MaxEntScan, and GeneSplice) suggested a reduction in splice donor strength, while the SpliceAI predicted a lesser impact on splice donor efficiency (DS\_DL=0.4613) (Jaganathan et al., 2019). Disrupted splicing at exon 9 and subsequent skipping of the exon is predicted to result in an out-of-frame product. The individual has a sister who is similarly affected with bilateral SNHL and EVA.

Individuals 11–13 have been previously reported by Peled et al. (2016). These individuals presented with hypodontia, hypotrichosis, and subtle facial dysmorphisms that included a long oval face, square chin, down slanting palpebral features, low insertion of columella, and thick lips. These phenotypic features satisfy the diagnostic criteria for ectodermal dysplasia with tooth agenesis. Individual 11 is of Middle-Eastern ancestry and carries two c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) *TSPEAR* frameshift

variants, while individual 12 is of Middle Eastern ancestry and is compound heterozygous for the same c.1726\_1728delGTCinsTT (p.Val576Leufs\*38) variant and a c.454\_457delCTG (p.Leu152Trpfs\*29) deletion. Individual 13 is of Jewish ancestry and is compound heterozygous for c.1852T>A (p.Tyr618Asn) and c.1915G>A (p.Asp639Asn) missense variants and was less severely affected. Individual 11 was born to consanguineous parents and had an affected cousin and uncle, while the family history for individuals 12 and 13 was uninformative.

Individuals 19–20 were previously reported in a study by Du et al. (2018). Individual 19 was noted to have oligodontia, microcephaly, and facial dysmorphisms including a narrow forehead, increased hair growth on the forehead, a high arched palate, low set ears, and minimal striation at the border of the ear lobules and antitragus and was homozygous for c.1726\_1278delGTCinsTT (c.1726\_1728delGTCinsTT\*) *TSPEAR* variants (Du et al., 2018). Individual 20 is homozygous for c.1877T>C (p.Phe626Ser) variants and was observed to have nonsyndromic tooth agenesis—no other clinical features were identified. Both individuals were born to consanguineous parents. Family history was otherwise uninformative for individual 19, while individual 20 had a similarly affected brother and father.

Individual 21 is a male of Korean ancestry who was reported by Song et al. to have nonsyndromic oligodontia (Song et al., 2020). This individual inherited a paternal c.1528C>T (p.Arg510\*) nonsense variant and a maternal c.1330C>T (p.Arg444Trp) variant. The family history was otherwise uninformative.