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De novo mutations are a common cause of genetic hearing loss

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Abstract

Purpose: *De novo* mutations (DNMs) are a well-recognized cause of genetic disorders. The contribution of DNMs to hearing loss (HL) is poorly characterized. We aimed to evaluate the rate of DNMs in HL-associated genes and assess their contribution to HL.

Methods: Targeted genomic enrichment and massively parallel sequencing were used for molecular testing of all exons and flanking intronic sequences of known HL-associated genes, with no exclusions on the basis of type of HL or clinical features. Segregation analysis was performed and previous reports of DNMs in PubMed and ClinVar were reviewed to characterize the rate, distribution, and spectrum of DNM in HL.

Results: DNMs were detected in 10% (24/238) of trios for whom segregation analysis was performed. Overall, DNMs were causative in at least ~1% of probands for which a genetic diagnosis was resolved, with marked variability based on inheritance mode and phenotype. DNMs of *MITF* were most common (21% of DNMs), followed by *GATA3* (13%), *STRC* (13%), and

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AUTHOR CONTRIBUTIONS:

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ETHICS DECLARATION:

Clinical data was collected as approved by the University of Iowa Institutional Review Board (IRB) #201602772. A waiver of informed consent was granted as this is a retrospective study with limited clinical data. To ensure anonymity of patients, patient information is deidentified included providing ages in ranges and not identifying sex of patients. Our ethical approval does not allow deposition of clinical data into a public repository. This study was performed in accordance with the Declaration of Helsinki.

SUPPLEMENTARY INFO:

Supplementary Figure S1. Overview of review strategy.
Supplementary Table S1. Genes targeted in OtoSCOPE v4-v9
Supplementary Table S2. OtoSCOPE versioning data and number of probands run on each version of the panel.
Supplementary Table S3. *De novo* mutations detected in OtoSCOPE cohort.
Supplementary Table S4. *De novo* mutation rate per gene among screened trios and OtoSCOPE cohort.
Supplementary Table S5. PubMed search string.
Supplementary Table S6. ClinVar search string.
Supplementary Table S7. *De novo* mutations previously reported in PubMed and ClinVar in 105 HL-associated genes reviewed in this study.
Supplementary Table S8. Contribution of *de novo* mutations to pathogenic and likely pathogenic variation in the Deafness Variation Database for all genes reviewed.

ACTG1 (8%). Review of reported DNMs revealed gene-specific variability in contribution of DNM to the mutational spectrum of HL-associated genes.

Conclusion: DNMs are a relatively common cause of genetic HL, and must be considered in all cases of sporadic HL.

Keywords

Deafness; hearing loss; genetic variant; de novo mutation

1. INTRODUCTION:

Mendelian inheritance dictates that half of an individual's genetic material is derived from sperm and half is derived from the oocyte. However, each individual carries a small number of *de novo* mutations (DNMs) which arise during gametogenesis or postzygotically.[1] *De novo* single-nucleotide variants (SNVs) arise at an estimated rate of $1.0–1.3 \times 10^{-8}$/base pair/generation, equivalent to roughly 62–74 DNMs per diploid genome, of which 1–2 are coding; indels and copy number variants (CNVs) occur *de novo* at a lower rate, approximately 5.9 and 0.16 per diploid genome respectively.[2–4]

DNMs exhibit a greater propensity towards pathogenicity than transmitted variants, as they are subjected to less stringent selective pressures. Consistent with this observation, there is growing appreciation of substantial contributions of *de novo* SNVs, indels, and CNVs to a myriad of genetic disorders including autism spectrum disorder and hereditary cancer syndromes,[5,6] and *de novo* origin of a genetic variant is considered strong evidence of pathogenicity under American College of Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines for variant interpretation.[7,8] The overall contribution of DNMs to a given disorder may be predicted based on the size of genes which contribute to the disease phenotype, the tolerability of variation within contributing loci, and the frequency at which variants arise *de novo* within the loci.[9] Recurrent observation of DNM at any site at a rate higher than expected based on genome-wide rates suggests a predisposition to errors in DNA repair or replication resulting in DNM, the mechanisms of which are variable and include failure of DNA repair pathways, deamination of methylated CpGs and subsequent C>T transversion, slipped strand mispairing, and genomic rearrangements.[1]

DNMs are a well-documented cause of both non-syndromic hearing loss (NSHL) and syndromic hearing loss (SHL), the latter of which may present initially as NSHL due to delayed onset or detection of the non-auditory phenotype (so-called NSHL mimics).[10] The genetic etiology of HL is diverse, comprising at least 224 known genes, of which disease-causing DNMs have been detected in at least 52, most frequently in genes with autosomal dominant inheritance and syndromic phenotypes such as *TCOF1*, *KMT2D*, and *NF2* (Supplementary Table S1). Previous studies of HL cohorts report conflicting rates of DNM, likely due to differences in selection criteria, cohort size, and incomplete segregation analysis. The findings of Baux et al. (2017), Cabanillas et al. (2018), and Guan et al. (2021) suggest that 4–19% of probands with HL harbor disease-causing DNMs.[11–13] Conversely, He et al. (2017) failed to detect a single case of DNM among 26 probands in

whom targeted genomic enrichment and massively parallel sequencing (TGE+MPS) of 143 deafness-associated genes was performed.[14]

We hypothesized that DNMs were an underrecognized etiology of HL and leveraged our laboratory's clinical cohort to retrospectively assess the prevalence of HL due to DNM. We performed genetic testing on 5957 probands. Parental testing was performed for 238 unique probands in whom a likely genetic diagnosis was resolved, leading to identification of 24 DNMs in 15 deafness-associated genes. Review of DNMs in our cohort and the literature revealed the substantial contribution of *de novo* changes to pathogenic variation in HL-associated genes. DNMs contribute substantially to genetic deafness and must be considered in its etiology when molecular testing reveals potentially causative variants that appear incompatible with the family history.

2. MATERIALS AND METHODS:

2.1 Subjects

Records were examined for all unique proband-parent trios referred to the Molecular Otolaryngology and Renal Laboratories (MORL) for comprehensive genetic testing from inception January 2012 to June 2021. No exclusions were made on the basis of age, age of onset, phenotype, family history, or previous genetic testing. This study was approved by the Institutional Review Board of the University of Iowa and performed in accordance with the declaration of Helsinki. Our ethical approvals do not allow deposition of patient data into a public repository.

2.2 Targeted genetic testing, bioinformatic pipeline, and variant interpretation

Probands' testing was performed using OtoSCOPE panel (version 4–9), a custom TGE+MPS panel, as previously described.[15] Each version of the platform included all known NSHL and NSHL mimic genes at the time of design (Supplementary Table S1–S2). Bioinformatic analysis was performed using a local installation of Galaxy software running on a high-performance computing cluster at the University of Iowa.[16] In brief, reads were mapped to the reference GRCh37 with Burrows-Wheeler Alignment,[17] duplicates were removed with Picard, variants were called with GATK,[18] and copy number variant analysis and annotation were performed using a custom toolset.[19,20] A multidisciplinary expert panel, including geneticists, clinicians, bioinformaticians, and genetic counselors, reviewed results in the context of available phenotypic data to assess pathogenicity according to ACMG specifications for genetic HL and resolve a genetic diagnosis when possible.[8,21]

2.3 Familial testing and bioinformatic analysis of *de novo* mutations

Familial testing was recommended for families in which a likely genetic diagnosis and/or a likely pathogenic (LP) or pathogenic (P) variant was identified on OtoSCOPE to aid in family counseling, confirm variants are in *trans*, or to resolve variants of uncertain significance in accordance with expert specifications of the ACMG variant interpretation guidelines for genetic HL.[8] Segregation analysis was performed using Sanger sequencing for SNVs and indel variants, and with OtoSCOPE upon clinician request or in cases where CNV analysis was indicated. Candidate DNMs were identified in families in which a proband's

variant was not detected in either parent using Sanger sequencing or OtoSCOPE. To confirm parental relationships and rule out sample contamination or swap and gonosomal mosaicism for candidate DNMs, trio sequencing data were analyzed using Vcfcompare and Varscan[22] with subsequent visual inspection of the alignment in the integrative genomics viewer (IGV).[23] Over 1000 high quality variants were assessed in each of the 23 trios. Parental DNA was insufficient to perform TGE+MPS testing for one trio and Sanger sequencing of 16 novel, ultra-rare and rare variants identified in the proband was used instead to confirm parental relationships.

2.4 Mutational spectrum of *de novo* mutations in HL-associated genes

We reviewed previously reported DNMs within the PubMed database and ClinVar database. Records were extracted in March 2022 (Supplementary Figure S1, Supplementary Table S5–S6). We additionally reviewed available records of prior TGE+MPS studies of HL populations which reported segregation analysis and mined relevant references for additional studies for inclusion. Records were reviewed to extract previously reported DNM and mosaic variants, including SNVs, indels, and small CNVs in HL-associated genes. Variants in which segregation analysis showed a mosaic or *de novo* origin were considered for inclusion. CNVs/structural variants encompassing multiple genes and SNVs/indels in which the genomic coordinates of the variant could not be determined were excluded. Studies concerning DNM/mosaicism in neoplastic processes, cutaneous disorders, or any other phenotype clearly unrelated to HL-associated phenotypes were excluded from analysis.

3. RESULTS:

3.1 Genetic testing

During the study period, a probable or definitive genetic diagnosis was resolved in 2508 of 5957 (42%) probands tested using OtoSCOPE. Among probands with a positive diagnosis, 63% were diagnosed with ARNSHL, 15% with ADNSHL, 23% with syndromic HL (including NSHL mimics such as Usher syndrome and deafness-infertility syndrome), 1.6% with X-linked HL, and <1% with mitochondrial HL, consistent with previous reports from our laboratory.[15] Clinical correlation and segregation analysis were recommended for all probable and definitive genetic diagnoses. Two parental samples were obtained for 246 of 2508 (9.8%) unique probands for segregation analysis. Eight families were excluded from subsequent analyses due to non-segregation of the variant with HL, or recessive variants confirmed to be in *cis.* A positive diagnosis was confirmed for 238 families, of which 147 (62%) had no reported family history of HL. Non-transmission of a candidate variant consistent with DNM was seen in 24 of 238 (10%) probands, and in 19 of 147 (13%) probands with no reported family history of HL (Figure 1).

To confirm parental relationships and rule out sample mix-up and parental post-zygotic mosaicism, parental samples were tested using OtoSCOPE followed by bioinformatic analyses using Varscan and Vcfcompare and manual inspection using the integrative genomics viewer (IGV). Over 800 high quality single nucleotide variants were assessed in each of the 23 trios. All variants other than the *de novo* mutations followed a Mendelian inheritance. Parental DNA samples for Proband 19 were not sufficient to perform

TGE+MPS, thus Sanger sequencing of 16 novel, ultra-rare and rare variants detected in the proband was performed instead and was consistent with true parentage. No high confidence reads for any of the DNMs were detected in parental samples excluding parental gonosomal mosaicism. The mean read depth at each DNM was 625X with a coverage ranging between 145X and 1207X (Supplementary Table S3).

3.2 Diagnosis by phenotype and gene

All probands with a DNM had either no family history of HL or a phenotype clinically inconsistent with affected family members, and most exhibited subtle or no syndromic features (Table 1, Supplementary Table S3). The rate of diagnoses involving a DNM differed by phenotype and gene (Figure 1A,1B, 1E, Supplementary Table S4). DNMs accounted for 7 of 27 (26%) autosomal dominant nonsyndromic HL (ADNSHL) diagnoses and 11 of 17 (65%) autosomal dominant syndromic HL (ADSHL) diagnoses but only 2 of 138 (1.4%) autosomal recessive nonsyndromic HL (ARNSHL) and 3 of 54 (5.6%) autosomal recessive syndromic HL (ARSHL) diagnoses. To calculate the minimum rate of diagnoses involving a DNM in ADNSHL and ADSHL, we considered the whole MORL cohort with a positive diagnosis (2508). DNMs accounted for a minimum of 1.8% (7 of 382) of ADNSHL diagnoses and 5.6% (11 of 195) of ADSHL diagnoses, and 1% (24 of 2508) of diagnoses overall.

3.3 *De novo* mutation rate

The rate of DNM detection differed by gene (Supplementary Table S4). With only one trio tested for each, DNM rate for *AIFM1, NR2F1*, *SIX1* and *TCOF1* genes was 1 in 1 allele (100%) for *AIFM1* and 1 in 2 alleles (50%) for *NR2F1, SIX1,* and *TCOF1*. The most significant rates for DNM were for *MITF, GATA3*, and *ACTG1* at 42% (5/12), 38% (3/8), and 33% (2/6), respectively. Considering all patients within the complete MORL diagnostic cohort (5957 probands), DNMs were most prevalent in the following genes: *NR2F1* (1 in 444 tested alleles, 0.2% DNM), *MITF* (5/7724, 0.07%), and *GATA3* (3/6564, 0.05%) (Supplementary Table S4).

3.4 Mutational spectrum

We detected a wide spectrum of DNMs including 2 unique CNVs, 15 SNVs (9 missense, 5 nonsense, 1 synonymous), 4 frameshift indels and 1 inframe indel (Figure 1C, 1D). Of SNVs, C>T transitions were most common (53%), followed by T>A transversion (20%). In addition to variants previously reported as P and LP in *GATA3*, *GJB2, KCNQ4*, *MITF, MYO7A, SIX1, STRC, TCOF1*, and *WFS1*, we identified 8 novel DNMs in *ACTG1*, *AIFM1*, *ATP2B2*, *CDH23*, *GATA3*, *MITF*, *MYO6*, and *NR2F1* (Table 1). 8 of 22 (36%) DNMs in this cohort arose at the site of a previously reported DNM (in *GATA3, GJB2, MITF, SIX1,* and *TCOF1*) or were recurrent within our cohort (in *STRC*) (Table 2).

To clarify the contribution of DNMs to pathogenic variation in HL-associated genes, we systematically reviewed previous reports of DNMs within 105 genes encompassing common and rare etiologies of syndromic and non-syndromic HL. A total of 795 records from PubMed and 417 records from ClinVar were reviewed to extract a total of 594 unique previously reported DNMs and mosaic variants after exclusion of large structural/

copy number variants, variants in which complete segregation analysis was not reported, and variants with unspecified or clearly irrelevant phenotypes (Supplementary Figure S1, Supplementary Table S5–S7).

The *de novo* mutational spectrum is gene-specific and broadly recapitulates the spectrum of P-LP variants classified in the Deafness Variation Database (DVD) (Figure 2A–B).[21] Striking variability was observed in the contribution of DNMs to pathogenic variation by gene, most notably in genes involved in autosomal dominant HL-associated syndromes (Figure 2C, Supplementary Table S8). Examining genes in which >2 DNMs are reported, the contribution of DNMs to the P-LP variant pool is greatest in several ADSHL genes: *NR2F1* (59% of P-LP variants reported *de novo*), *ATP6V1B2* (50%) *ACTB* (48%), and *ACTG1* (44%), followed by *SOX10* (36%), *SIX1* (28%), and *MITF* (27%). The variability in DNM contribution to P-LP variants is generally robust to correction for coding region size (Figure 2D).

ACTG1 and *GJB2* exhibited a high number of reported DNMs but are associated with both syndromic and nonsyndromic phenotypes, and multiple modes of inheritance in the case of *GJB2. ACTG1* is associated with both Baraitser-Winter cerebrofrontofacial syndrome type 2 (BWS2) and DFNA20/26,[24] while *GJB2* is associated with both DFNB1 and a spectrum of autosomal dominant disease phenotypes ranging from nonsyndromic DFNA3 to keratitis-ichthyosis-deafness syndrome.[25] In these genes, the contribution of DNMs to P-LP variation varies dramatically by phenotype. Although the majority of P-LP variants in *GJB2* cause DFNB1, all DNMs were reportedly associated with DFNA3 or syndromic disease. In contrast, the contribution of DNMs to DFNA20/26 P-LP variants is approximately half that of BWS2, with 31% of DFNA20/26 variants reported arising *de novo*.

Among recessive hearing loss, DNMs were almost exclusively reported in genes associated with syndromic phenotypes. Only one ARNSHL-associated DNM was identified in our review: *LOXHD1* NM_144612.7:c.6355del (NP_653213.6:p.Ala2119fs).

Variant classification—Following segregation analysis, two DNMs in *ACTG1* remained VUS under ACMG guidelines for variant interpretation in HL; the remainder of DNMs were classified as LP or P (Table 1, Supplementary Table S3).[8] Given 1- phenotype consistency with DFNA20/26 in the affected probands, 2- *in silico* predictions suggesting both variants are damaging, 3- absence of the variants in population databases, 4- prior detection of both variants in additional affected probands within our cohort, and 5- the high contribution of DNMs to the LP/P variant pool in *ACTG1*, we applied the PS2_Moderate rule resulting in reclassification to LP.

4. DISCUSSION

In this study, we identified likely causative DNMs in 15 HL-associated genes in 10% (24/238) of trios, with substantial variability in the rate of DNMs by gene and inheritance pattern. Remarkably, DNMs were found to be causative in ~13% (19/147) of probands with no reported family history of HL and ~26% (7/27) of probands with ADNSHL. Expanding this analysis to the complete OtoSCOPE cohort (2508 probands with genetic

diagnoses), DNMs are causative in at minimum ~2% of probands with ADNSHL and ~6% with ADSHL. These data mandate consideration of DNMs in the evaluation of all cases of sporadic HL.

Baux et al. (2017), Cabanillas et al. (2018), van Heurck et al. (2021), and Guan et al. (2021) likewise performed molecular testing of HL cohorts, and detected DNMs in 4 of 99 (4.0%), 4 of 21 (19%), 9 of 34 (26%), and 27 of 191 (14%) of probands with positive diagnoses, respectively.[11–13,26] These results are roughly concordant with the rate of DNMs in our cohort, though differences in inclusion criteria, methodology, and sample size limit direct comparisons. Among these cohorts and that of our laboratory, 10 of 12 (83%) DFNA20/26 (*ACTG1*-associated NSHL) cases were due to DNMs.[11–13,26] Moreover, our review of previously reported DNMs revealed that 44% of P-LP *ACTG1* variants have been reported as DNM, and the contribution of DNM to *ACTG1* is markedly higher than other causes of ADNSHL even after restriction to analysis of only NSHL-associated variants (Figure 2C, Supplemental Table S7–S8). The contribution of DNMs to the documented P-LP variant spectrum of DFNA20/26 is comparable to that of *SOX10, MITF, COL2A1*, which all result in syndromic phenotypes and are well-documented to frequently arise from DNMs. Likewise, at least ~6% of DFNA82 (*ATP2B2*) cases in our cohort are due to DNMs, and 19% of unique P-LP variants in *ATP2B2* are reported *de novo* (Figure 2C, Supplementary Table S4 and S8). This is concordant with the first descriptions of *ATP2B2* as an ADNSHL-associated gene, wherein a DNM was causative in 2 of 5 reported probands.[27] In stark contrast, most ADNSHL genes exhibited markedly lower contribution of DNM. Only ≤2% of P-LP variants in *KCNQ4, MYO6*, and *TECTA* have been reported *de novo* (Figure 2C–D, Supplementary Table S8).[21]

The contribution of DNM to any disease phenotype is strongly related to the impact of the disease on reproductive fitness.[1] Concordant with strong selective pressures against transmission of pathogenic variants in genes associated with multisystem disorders, we identified a larger contribution of DNMs to the P-LP variant spectrum of genes associated with syndromic HL and non-auditory phenotypes, such as *COL2A1, NR2F1, SOX10* and *MITF*. Nonsyndromic hearing loss in contrast has a modest impact on reproductive fitness which may be context or culture dependent.[28] Interestingly two NSHL-associated genes, *ACTG1* and *ATP2B2* exhibited a significant prevalence of DNMs within our cohort at a minimum of ~12% and 5.6%, respectively (Supplementary Table S4). Since *ACTG1* is also associated with syndromic HL: Baraitser-Winter cerebrofrontofacial syndrome type 2 (BWS2) that exhibits a highly variable expressivity,[29] the genetic fitness of carriers might be further decreased. Variability in phenotypic expression or subtle non-auditory features which evade clinical detection, but impact fitness might therefore account for the increased contribution of DNMs to the P-LP variant spectrum of *ACTG1*. The mechanisms underlying an increased contribution of DNMs to the P-LP variant pool in *ATP2B2*-associated NSHL are uncertain as no other syndromic phenotype are associated with this gene to date.

Under ACMG/AMP recommendations for sequence variant interpretation in HL, *de novo* origin of a variant is considered strong evidence of pathogenicity and can lead to reclassification via application of the PS2 criterion.[8] However, DNMs associated with conditions with high genetic heterogeneity such as ADNSHL (generally characterized by

downsloping high frequency HL) can only satisfy the weaker PS2_Supporting criterion unless the DNM is detected in other probands, substantially limiting this criterion's utility in classifying variants. This poses a substantial barrier to classification of DNMs in *ACTG1*, as most DNMs have not been observed recurrently (Figure 2E), precluding application of PS2_Moderate or greater. Consistent with this limitation, 2 DNMs in *ACTG1* could not reach the LP classification despite their absence in gnomAD, *in silico* predictions consistent with damaging missense variants, and phenotypic consistency with DFNA20/26.

Given compelling evidence for a prominent role of DNMs in DFNA20/26 (*ACTG1*) and DFNA82 (*ATP2B2*), we recommend modification of the PS2 criterion in ACMG/AMP recommendations for sequence variant interpretation, such that a single DNM detected in *ACTG1* or *ATP2B2* satisfies PS2_Moderate, rather than PS2_Supporting. Our laboratory and others have reported DNMs in other genes frequently associated with ADNSHL, such as *KCNQ4, MYO6*, *TECTA,* and *WFS1*. Given the low contribution of DNMs to the P-LP variant pool in these genes (≤~2% of P-LP variants reported *de novo*) and the genetic heterogeneity of NSHL, additional study is warranted to consider the appropriateness of extending this recommendation to additional ADNSHL genes.

This study is limited by biases inherent to our retrospective review strategies. First, we only examined probands who received a likely genetic diagnosis with OtoSCOPE and for whom segregation analysis of both parents was pursued, limiting analysis to the segregation of candidate variants detected on TGE+MPS panel. Samples were available from both parents of just 238 of 2508 (9.5%) probands who received a probable genetic diagnosis, limiting the scope of this review. Families may be more likely to pursue segregation analysis if testing suggests an unexpected diagnosis, such as ADNSHL in families with no prior history of HL. Iterative improvements of OtoSCOPE introduce further bias in the genes in which DNMs can be detected, with 2 important NSHL mimic genes – *MITF* and *GATA3* – first included in versions 7 and 8 of the panel respectively. Lastly, we restricted our review of the *de novo* mutational spectrum to confirmed DNMs detected in our cohort and records which we identified in the PubMed and ClinVar databases, for a limited gene set. The contribution of DNM to genetic variation in HL-associated genes is undoubtedly greater than can be determined with such techniques. Conclusions about the role of DNM in recessive hearing loss are greatly limited by the paucity of available data. Systematic reporting of comprehensive trio sequencing of large HL cohorts is necessary to thoroughly clarify gene-specific contributions of DNM to the mutational spectrum of HL-associated genes.

5. CONCLUSION

DNMs are a common cause of HL, accounting for at least ~1% of all genetic diagnoses, ~2% of ADNSHL, and ~6% of ADSHL. These findings mandate consideration of a *de novo* etiology in probands with sporadic HL and the development of gene-specific criteria for interpretation of DNM detected in probands with HL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY:

As this is a retrospective review of clinical data with limited chance for harm to patients, informed consent was not obtained. To ensure anonymity of patients in this retrospective clinical study, patient information is deidentified. Age groups are provided in ranges and sex of patients is obfuscated or not provided. Our ethical approvals do not allow deposition of patient data into a public repository. Requests for additional data will be honored as permitted by our ethical approval.

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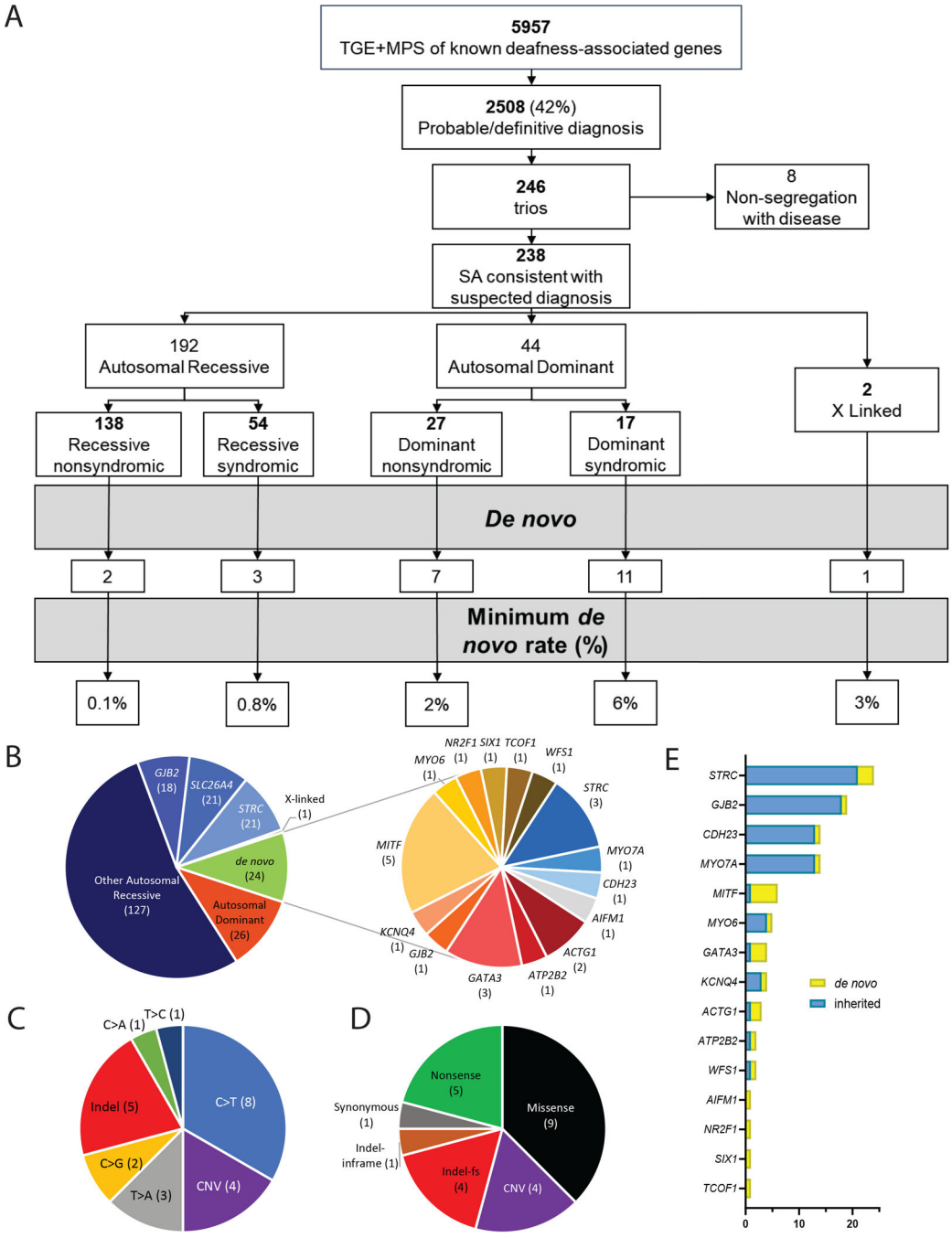

Figure 1.

Summary of familial testing results of 246 unique probands with hearing loss. A) Review of design and rate of *de novo* mutation (DNM) by diagnosis type. Variants in 8 probands were in *cis* or did not segregate with the disease phenotype, and were excluded from subsequent analysis. For the remainder, diagnoses were classified based on the expected or known pattern of inheritance of the variants in question. B) Summary of hearing loss diagnoses due to common genetic causes (*GJB2*, *SLC26A4*, *STRC*), uncommon recessive, dominant, and X-linked hereditary etiologies, and DNM within the cohort. C-D) Nucleotide change

and mutation types among DNMs in this cohort. E) Number of probands with hearing loss diagnoses due to inherited vs. *de novo* variants among trios examined.

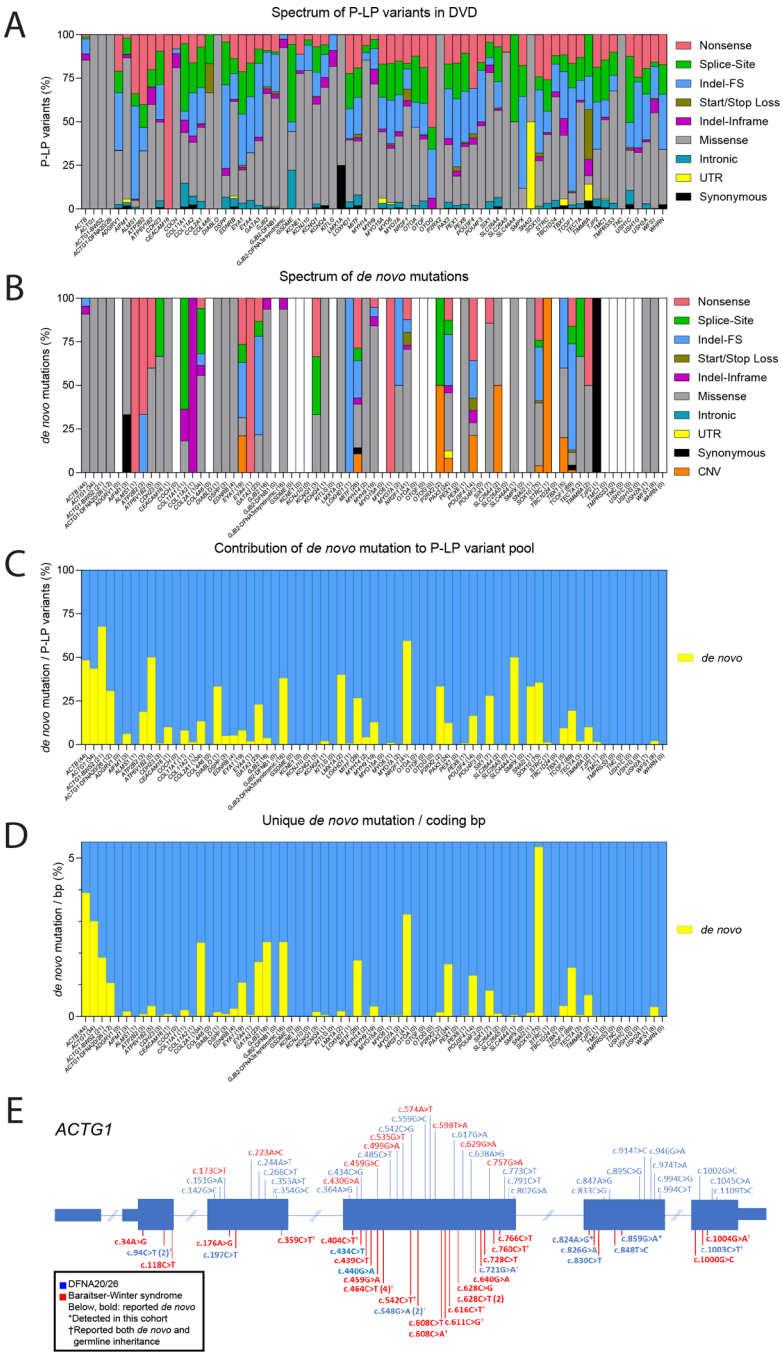

Figure 2.

The pathogenic and likely pathogenic variant spectrum and contribution of DNM to genetic hearing loss is gene specific. A) P-LP variant spectrum of genes reviewed in this study, using variants previously classified LP or P in the DVD. B) The mutational spectrum of DNMs detected in this cohort and previously described in the PubMed and ClinVar database broadly recapitulates gene-specific P-LP variant spectra. C) The contribution of DNM to P-LP variation varies by gene and phenotype. D) Rate of unique DNM per coding bp. E) Distribution of reported DNMs and P-LP variants in *ACTG1* (NM_001199954.2), showing

reports of DNM throughout the gene with few sites of recurrent DNM. DNM: *de novo* mutation. LP: likely pathogenic. P: pathogenic. DVD: Deafness Variation Database. To account for DNMs not reported in the DVD, the contribution of DNM to the P-LP variant pool was calculated in the following fashion: [reported causative DNMs / (P-LP variants in DVD v9 + reported causative DNMs unlisted in DVD)].

Table 1.

Summary of *de novo* mutations detected in the OtoSCOPE cohort. Minor allele frequencies are obtained from the Genome Aggregation Database v2.1.1.

Gene	Genomic coordinates	*De novo* variant	2nd variant	HL onset	HL severity	Extra-auditory features	Maximum MAF (population)	References, PMID or ClinVar accession (*n de novo*)	Unrelated probands with variant detected on OtoSCOPE, *n*	ACMG Criteria Applied	ACMG Classification	Diagnosis
ACTG1	17:79478113:T>C	c.824A>G, p.His275Arg		5–10 years	Mild sloping to moderately severe	NR	ND	This study	1	PM2, PP3, PS2_Mod*, PS4_Sup	LP*	DFNA20/26
ACTG1	17:79478078:C>T	c.859G>A, p.Val287Met		10–20 years	Mild sloping to severe	NR	ND	SCV002318769.1	1 (segregates w/ HL in affected cousin)	PM2, PP3, PS2_Mod*, PS4_Sup	LP*	DFNA20/26
AIFM1	X:129270058:C>T	c.1267G>A, p.Val423Ile		Congenital	NR	Peripheral neuropathy, cerebellar ataxia and atrophy, optic atrophy, developmental delay	ND	This study		PM2, PP3, PP4, PS2	LP	CMTX4
ATP2B2	3:19292243:T>A	c.2155A>T, p.Lys719Ter		Congenital	Mild	Speech, fine motor, and developmental delay	ND	This study		PM2, PS2_Mod*, PVS1	P	DFNA82
CDH23	10:73558246:A>T	c.6965A>T, p.Asp2322Val	c.6050–9G>A	Congenital	Severe to profound	Delayed motor milestones, vestibular hypofunction	ND	This study		PM2, PM3_Sup, PP3, PS2_M	LP	Usher syndrome type 1D
GATA3	10:8100570:G>T	c.544G>T, p.Glu182Ter		Congenital	Mild	NR	ND	This study		PM2, PS2_Sup, PVS1	P	HDR syndrome
GATA3	10:8100727:TC>T	c.708delC, p.Ser237GlnfsTer67		<5 years	Mild to moderate	NR	0.0008970% (NFE)	26282285, 17210674	1	PM2, PP4, PS2_Sup, PS4_Sup, PVS1_Strong	P	HDR syndrome
GATA3	10:8100727:T>TC	c.708dupC, p.Ser237AlafsTer29		Congenital	Moderate to severe	NR	0.006339% (AFR)	15830275, 30396722		PM2, PP4, PS2_Sup, PS4_Sup, PVS1_Strong	P	HDR syndrome

Gene	Genomic coordinates	*De novo* variant	2nd variant	HL onset	HL severity	Extra-auditory features	Maximum MAF (population)	References, PMID or ClinVar accession (*n de novo*)	Unrelated probands with variant detected on OtoSCOPE, *n*	ACMG Criteria Applied	ACMG Classification	Diagnosis
GJB2	13:20763170:C>T	c.551G>A, p.Arg184Gln		Congenital	Moderate	NR	ND	24945352 (2), 21868108 (2), 20937258 (1), 12111646, 11439000, 20096356, 20442751, 21040787, 21510145, 22281373, 22925408, , 25162826, 27481527, 27534436, 29140768, 29921236	4	PM2, PP1_S, PP3, PS3_Sup, PS2_VS, PS4_M	P	DFNA3
KCNQ4	1:41285565:G>A	c.853G>A, p.Gly285Ser		Congenital	Moderate	"Mild facial dysmorphism"	ND	10025409, 18786918, 23750663, 25116015, 10369879 (variant at same codon)		PM1, PM2, PM5, PP1_S, PS2_Sup, PS3_Sup, PS4_Sup	P	DFNA2A
MITF		Whole-gene deletion		Congenital	Profound	Temporal bone anomaly		Whole-gene deletion: 34142234 (1) Partial deletion: 17627390	2	PM2, PP4, PS2_Mod, PS4_Sup, PVS1	P	WS2A
MITF	3:69988313:C>G	c.647C>G, p.Ser216Ter		Congenital	Profound	Temporal bone anomaly	ND	This study		PM2, PS2_Sup, PVS1	P	WS2A
MITF	3:70001021:G>C	c.939G>C, p.Lys313Asn		Congenital	Profound	NR	ND	27889061		PM2, PM5, PP3, PP4, PS2_Sup, PS4_Sup	LP	WS2A
MITF	3:70005611:CGAA>C	c.970_972del, p.Arg324del		Congenital	Profound	Retinal pigmentation defect, delayed motor milestones	ND	34142234 (4), 18510545 (1), 30936914 (1), 20485200, 20478267, 23787126, 24194866, 26781036, 27889061, 29115496, 29531335,	2	PM2, PM4_Sup, PP1_S, PP4, PS2_VeryStrong, PS3_Sup, PS4_M	P	WS2A

Gene	Genomic coordinates	*De novo* variant	2nd variant	HL onset	HL severity	Extra-auditory features	Maximum MAF (population)	References, PMID or ClinVar accession (*n de novo*)	Unrelated probands with variant detected on OtoSCOPE, *n*	ACMG Criteria Applied	ACMG Classification	Diagnosis
								30978479, 30936914				
MITF	3:70014048:G>A	c.1230G>A, p.Thr410=		Congenital	Profound	Temporal bone anomaly	ND	21438779, 29115496, 29986705	1	PM2, PP1_S, PP4, PS2_Sup, PS3_Sup, PS4_Sup	P	WS2A
MY06	6:76591463:A>T	c.2344A>T, p.Lys782Ter		<5 years	Mild	NR	ND	This study		PM2, PS2_Sup, PVS1	P	DFNA22
MY07A	11:76873960:T>TC	c.1623dup, p.Lys542GlnfsTer5	c. 2267G>C, p.Arg756Pro	Congenital	Severe To Profound	Delayed motor milestones	0.003268% (SAS)	10930322, 27743452, 30459346		PM2_Sup, PM3, PP1, PP4, PS2_M, PVS1	P	Usher syndrome type 1B
NR2F1	5:92929351:C>T	c.1075C>T, p.Gln359Ter		Early childhood	Moderate rising to normal	Global developmental delay, hypotonia, hooded eyes, esotropia	ND	This study		PM2, PP4, PS2, PVS1_Strong	P	BBSOAS
SIX1	14:61115580:G>A	c.328C>T, p.Arg110Trp		Congenital	Mild sloping to severe	Temporal bone anomaly	ND	33436522 (1), 15141091, 24164807	1	PM2, PM5_Strong, PP3, PP4, PS2_Mod, PS4_Sup	P	Branchio-oto-renal syndrome
STRC		*STRC-CATSPER2* deletion	*STRC-CATSPER2* deletion	Congenital	Mild to moderate	NR		24963352		PM3_VeryStrong, PS2_M, PVS1	P	DFNB16
STRC		*STRC-CATSPER2* deletion	*STRC-CATSPER2* deletion	Congenital	Mild to moderate	NR		24963352		PM3_VeryStrong, PS2_M, PVS1	P	Deafness-infertility syndrome
STRC		*STRC-CATSPER2* deletion	*STRC-STRCP1* conversion	Congenital	Mild to moderate	NR		24963352		PM3_VeryStrong, PS2_M, PVS1	P	DFNB16
TCOF1	5:149754535:GC>G	c.1303del, p.Gln435ArgfsTer58		Congenital	Mild	Macrocephaly	ND	22317976 (1)		PM2, PP4, PS2_Mod, PS4_Sup, PVS1	P	Treacher-Collins syndrome
WFS1	4:6303911:G>A	c.2389G>A, p.Asp797Asn		<5 years	Mild to moderate	NR	ND	25250959, 29447883	2	PM2, PM5, PP1_S, PP3, PP4, PS2_M, PS4_Sup	P	DFNA6/14/38

ND: not detected. NR: not reported. A more detailed description of these variants is available in Supplemental Table CMTX4: Charcot-Marie-Tooth, X-linked 4. HDR syndrome: hypoparathyroidism-deafness-renal disease syndrome. WS2A: Waardenburg syndrome 2A. BBSOAS: Bosch-Boonstra-Schaaf optic atrophy syndrome.

[*] These variants expertly curated with PS2_Moderate applied.

Variants annotated with NM_001199954.2/NP_001186883.1 (*ACTG1*), NM_004208.4/NP_004199.1 (*AIFM1*), NM_001001331.4/NP_001001331.1 (*ATP2B2*), NM_022124.6/NP_071407.4 (*CDH23*), NM_001002295.2/NP_001002295.1 (*GATA3*), NM_004004.6/NP_003995.2 (*GJB2*), NM_004700.4/NP_004691.2 (*KCNQ4*), NM_001354604.2/NP_001341533.1 (*MITF*), NM_001368865.1/NP_001355794.1 (*MYO6*), NM_000260.4/NP_000251.3 (*MYO7A*), NM_005654.6/NP_005645.1 (*NR2F1*), NM_005982.4/NP_005973.1(*SIX1*), NM_001135243.1/NP_001128715.1(TCOF1), NM_006005.3/NP_005996.2 (*WFS1*).

Table 2.

Sites of recurrent *de novo* mutation detected in this cohort, predisposing sequence context features, and total *n* probands which displayed confirmed *de novo* origin of the variant.

Gene	*De novo* variant	Probands	Predisposing features	References (PMID)
GATA3	c.708delC, p.Ser237AlafsTer29	1	Heptanucleotide poly-C	This study
GATA3	c.708dupC, p.Ser237GlnfsTer67	1	Heptanucleotide poly-C	This study
GATA3	c.708insT, p.Ser237GlnfsTer67	1	Heptanucleotide poly-C	30143558
GJB2	c.551G>A, p.Arg184Gln	6	CpG site	20937258, 21868108, 24945352, this study
MITF	c.970_972delGAA, p.Arg324del	7	Trinucleotide GAA repeat	34142234, 18510545 30936914, this study
MITF	Whole-gene deletion	2		34142234, this study
STRC	*STRC-CATSPER2* deletion	3	High homology region	This study
SIX1	c.328C>T, p.Arg110Trp	2	CpG site	33436522, this study
TCOF1	c.1303delC, p.Gln435ArgfsTer58	2	Hexanucleotide poly-C sequence	22317976

Variants were annotated on transcripts NM_001002295.2 (*GATA3*), NM_004004.6 (*GJB2*), NM_001354604.2 (*MITF*), NM_005982.4 (*SIX1*), NM_001135243.1 (*TCOF1*).