Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene *ATOH1*

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Abstract

Variants in more than 150 genes are responsible for inherited hearing loss, with different causal alleles in different populations. The Israeli Jewish population includes communities of diverse geographic origins, revealing a wide range of deafness-associated variants and enabling clinical characterization of the associated phenotypes. Our goal was to identify the genetic causes of inherited hearing loss in this population, and to determine relationships among genotype, phenotype, and ethnicity. Genomic DNA samples obtained from informative relatives of 88 multiplex families, all of self-identified Jewish ancestry, with either non-syndromic or syndromic hearing loss, were sequenced for known and candidate genes for hearing loss using the HEar-Seq gene panel. The genetic causes of hearing loss were identified for 60% of families sequenced. One gene was encountered for the first time in human hearing loss: *ATOH1* (Atonal), a basic helix-loop-helix transcription factor responsible for autosomal dominant progressive hearing loss in a five-generation family. Our results demonstrate that genomic sequencing with a gene panel dedicated to hearing loss is effective for genetic diagnoses in a diverse population. Comprehensive sequencing enables well-informed genetic counseling and clinical management by medical geneticists, otolaryngologists, audiologists, and speech therapists. Comprehensive sequencing can also be integrated into newborn screening for deafness.

KEYWORDS

Next-generation sequencing, Massively parallel sequencing, Diagnostics, Hearing, Deafness

INTRODUCTION

Hearing loss is a leading cause of disability worldwide, with an estimated 466 million people suffering from a loss of greater than 40dB.[1-3] Hearing loss can have dramatic effects on communication, levels of education, and psychosocial development; it is responsible for a subsequent decline in quality of life, particularly in an increasingly older population.[4,5] Determining the causes of hearing loss is crucial for clinical management, genetic counseling, and potential prevention. More than 150 genes harbor variants causing non-syndromic hearing loss,[6-8] and hundreds of genetic syndromes include hearing impairment, in the form of syndromic hearing loss.[9] Virtually every population harbors both deafness-causing alleles that appear worldwide and population-specific ones that are undetected in other regions.[10]

The genetic heterogeneity of the Jewish population of modern Israel is reflected in the genetic causes of its traits. This population is made up of communities that differ with respect to geographic origin, spoken language, and traditions. Ashkenazi Jews from Europe and North America, Sephardi Jews from North Africa (Morocco, Algeria, Libya, and Tunisia) and southern Europe (Italy, Greece, and Turkey), and Mizrahi (Eastern) Jews from the Middle East (Iran, Iraq, Syria, Yemen, and Egypt) and have genetic origins traced to the Jews who lived in the Middle East 4000 years ago, dispersing with the Babylonian exile in 586 BCE.[11,12] In the intervening centuries, endogamy within each of these communities led to high frequencies of recessive genetic traits, many due to community-specific founder mutations.[13,14,15]

GJB2 variants are the most prevalent cause of hereditary hearing loss worldwide and are responsible for ~30% of deafness in Jewish families.[14,16,17] Hence in Israel, routine genetic testing has been for the two most common pathogenic variants, *GJB2* c.35delG and *GJB2* c.167delT. For hearing loss not explained by these alleles, high-throughput sequencing using hearing-loss-dedicated gene panels offers the opportunity to identify other disease-causing variants in hundreds of genes.[18,19,15,20]

The goal of this project was to identify the genetic causes of hearing loss in Israeli Jewish families with more than one affected relative (i.e. multiplex families) and to determine the number of genes responsible for hearing loss in the Israeli Jewish population as a whole. The long-term goal is to apply these results to development of guidelines for the molecular diagnosis of deafness in this population.

MATERIALS AND METHODS

Participants

The Ethics Committee of Tel Aviv University, the Helsinki Committee of the Israel Ministry of Health, and the Human Subjects Division of the University of Washington approved the study. Probands with hearing loss and their relatives were recruited from medical genetics clinics throughout Israel. All probands had a positive family history of hearing loss. Participants were asked about their medical history, including family history of relevant symptoms, consanguinity, degree of hearing loss, age of onset, symmetry of hearing loss, hearing aids or cochlear implant use, tinnitus, exposure to ototoxic drugs and/or noise, pathological conditions of the inner ear, and vestibular function. Hearing loss could be non-syndromic or syndromic, stable or progressive, and pre-lingual or post-lingual in onset. Probands or their parents gave written informed consent and provided a blood sample for DNA extraction. *GJB2* and *GJB6* were evaluated by Sanger sequencing, and probands with hearing loss due to *GJB2* were so advised and not sequenced with the gene panel.[14] After these steps, 188 individuals from 88 multiplex families were evaluated with the HEar-Seq gene panels. Hearing controls from each Israeli Jewish ethnic group were identified from healthy couples undergoing genetic screening at the Rabin and Sheba Medical Centers and from the National Laboratory for the Genetics of Israeli Populations (https://en-med.tau.ac.il/nlgip).

Genomics

Genomic DNA was sequenced using HEar-Seq gene panels containing between 178 and 372 genes. Details of the panels are indicated in Table S1 and Methods in the Supporting Information section.[15,20,21] For each version of the panel, genomic coordinates (hg19) were submitted to eArray (Agilent Technologies) to design cRNA oligonucleotides to cover exons and flanking introns. Molecular barcodes were assigned, and 96 samples were multiplexed and sequenced in a single flow cell of the Illumina HiSeq 2500 with 105bp paired-end reads. Genotypes of family members were determined by panel sequencing or Sanger sequencing. Genotypes of controls were evaluated by Sanger sequencing or restriction enzyme analysis.

A bioinformatics pipeline was designed for analysis of panel data. Data were collected locally in the laboratory and processed on a Dell PowerEdge R920 server. Samples were processed from real-time base-calls (RTA1.8 software [Bustard]) and converted to qseq.txt files. Following demultiplexing, the reads were aligned to the reference human genome (hg19) using Burrows-Wheeler Aligner (0.7.9a).[22] PCR duplicates were removed by SAMtools 0.1.19 (http://samtools.sourceforge.net/). Indel realignments and base quality score recalibration were carried out, and genotypes called and filtered,

with the Genome Analysis Tool Kit (GATK; v3.0-0; broadinstitute.org/gatk) with recommended parameters.[23] Large insertions, deletions, and inversions were identified with Pindel 0.2.5[24] and BreakDancer 1.1.4.[25] Mis-alignments were corrected by aligning to data from 850 in-house exomes. CNVs were called using CoNIFER,[26] XHMM[27] and the King lab in-house CNV detection pipeline.[28] Variants were annotated using an in-house pipeline with respect to location (exonic, near splice, intronic, UTRs) and predicted function (frameshift, inframe indel, nonsense, missense, silent, and potential splice altering, cryptic splice, and regulatory effect). Allele frequencies were obtained from the gnomAD browser. GERP, PolyPhen2, SIFT, and Mutation Taster scores were included. Potential splice altering and cryptic splice variants were predicted using our splice variant prediction pipeline, based on the NNSPLICE and MaxEnt algorithms.[29,30] Variants were classified using the criteria of the American College of Medical Genetics (ACMG) criteria[31,32] and deposited in ClinVar (www.ncbi.nlm.nih.gov/clinvar/).

Protein analysis

For Western blot and degradation assays for ATOH1 analysis, cDNA for human wild-type *ATOH1* or mutant *ATOH1* c.1030delC was cloned into the expression vector pCDT-N3xFLAG3xHA. HEK293T cells were transfected and treated with cycloheximide. HA tags were used to detect proteins. Similarly, for Western blot and transactivation assays for MITF analysis, cDNA for mouse wild-type *Mitf* or mutant *Mitf* c.1190delG was cloned into the vector pcDNA3.1. HEK293 cells were transfected with wild-type or mutant plasmid, a pGL3 luciferase construct containing the tyrosinase promotor sequence, and a CMV-pRL Renilla vector. Luciferase signals were normalized to Renilla signals and results expressed as fold change over empty vector. Three biological replicates were performed. Western blots were visualized using the Odyssey infrared imaging system. Additional details are provided in the Supporting Information Methods.

RESULTS

Genetic causes of hearing loss were identified for 60% (53/88) of the families evaluated by the HEar-Seq gene panel (Supporting Information Table S1). These genetic diagnoses involved 57 different causal alleles in 27 different genes (Supporting Information Table S2 and Figure S1). Most of the responsible alleles (32 of 57, or 56%) had not been previously reported (Table 1). These diagnoses expanded the total number of genes known to be responsible for inherited loss in the Israeli Jewish population from seven[14] to 32 (Supporting Information Table S3). Founder mutations from each of the ancestral Jewish communities contributed to these diagnoses (Supporting Information Table S4). For example,

CEACAM16 c.703C>T, p.(Arg235Cys) was responsible for recessive hearing loss in family DF301, of Jewish Iranian ancestry (Supporting Information Figure S1), and subsequently identified in multiple families of the same ancestry evaluated in clinics.

Panel sequencing revealed involvement of a new gene for human hearing loss. *ATOH1* (Atonal) encodes a basic helix-loop-helix transcription factor that is essential for neuronal development in the cerebellum.[33] Heterozygosity for any of several different variants of *Atoh1* leads to hearing deficits in mice, some with syndromic features.[34,35] *ATOH1* was included on the panel because mutations in the mouse ortholog lead to hearing loss. In an Iraqi Jewish kindred, *ATOH* c.1030delC co-segregated over five generations with progressive non-syndromic hearing loss (Figure 1A, B). This variant causes a frameshift that alters the last ten residues of the normally 354-amino acid protein and adds six residues to its length before a stop. Western blot analysis of wild-type and mutant ATOH1 proteins revealed a significantly slower rate of degradation for mutant compared to the wild-type protein (multiple t-test with Holm-Sidak correction, time course 0 to 5 hours, two biological replicates) (Figure 1C, D).

For genes responsible for syndromic hearing loss, different variants in the same gene revealed new relationships of genotypes to phenotypes. For example, variants in *MITF* cause autosomal dominant Waardenburg type 2A and Tietz albinism/deafness syndromes, both of which are highly heterogeneous clinically (Figure 2A, B). In family DF311, three relatives heterozygous for *MITF* c.935T>C, p.(Leu312Pro) had congenital albinism and severe to profound sensorineural hearing loss. (Hearing loss of DF311.02 is due to a variant in *CDH23*.) Leu312 is a completely conserved residue in the middle of the MITF basic helix-loop-helix (bHLH) domain. A proline at this position would likely break the helix and preclude proper DNA binding and possibly preclude dimerization as well. In family DF219, three relatives heterozygous for *MITF* c.981insC, p.(Leu327fs9*) demonstrated the same hearing loss and albinism signs. A frameshift at residue 327 would lead to truncation in the middle of the bHLH domain and loss of normal protein function. In contrast, in family DF186, three relatives heterozygous for *MITF* c.1190delG, p.(Gly397fs15*) also demonstrated congenital sensorineural hearing loss but no pigmentation signs other than hair whitening of the mother in her twenties. Truncation due to frameshift at residue 397 is distal to the bHLH domain, so its consequences to protein function were unknown. A transactivation assay of the protein encoded by *MITF* c.1190delG indicated that the transcriptional potential of the mutant protein is greatly impaired compared to that of wild-type MITF (Figure 2C).

GATA3 variants present an analogous story. *GATA3* variants are responsible for an autosomal dominant syndrome including hypoparathyroidism, sensorineural deafness, and renal dysplasia (HDR), for which different alleles are associated with a wide spectrum of phenotypes.[36] Two families in our

series reflect this heterogeneity (Figure 3). In family HL738, the proband (DF738.01) and his mother, heterozygous for *GATA3* c.681ins35, p.(Glu228fs37*), demonstrated congenital severe-to-profound hearing loss and kidney dysplasia. Unfortunately, medical records for the sister and niece of the proband were not available. In contrast, in family HL769, all relatives heterozygous for *GATA3* c.829G>A, p.(Asp277Asn), demonstrated severe-to-profound hearing loss, but no renal or parathyroid problems. The possibility of undetected renal abnormalities seems unlikely, as the proband (DF769.03) and her oldest brother are in their 30s and their mother is age 58. It is possible that this variant of *GATA3* causes only nonsyndromic hearing loss and that *GATA3* could be added to the group of genes that can cause either syndromic or non-syndromic hearing loss: *SLC26A4*,[37] *MYO7A*,[38] *CDH23*,[39] and *WFS1*.[40]

A final example is *TECTA*, which can be responsible for dominant or recessive nonsyndromic hearing loss. Families HL277, DF183, and DF303 harbored different missenses mutations of *TECTA*, leading to hearing losses that differed in both mode of inheritance and pattern of hearing thresholds (Supporting Information Figure S1). For *TECTA*, audiogram shapes are not correlated with mode of inheritance, but rather with the location of the variants.[41,42] Relationships between genotypes and phenotypes for other genes are summarized in the Supporting Information Results and Figure S2.

DISCUSSION

Genetic diagnoses for hearing loss will play an increasing role in treatment of both congenital and later onset hearing loss. Success of cochlear implant may depend on the genetic cause of the hearing loss.[43] The clinical application of gene therapy for some forms of hearing loss may prove feasible,[44,45] but its application depends on correct genetic diagnosis. Gene panel-based sequencing increased the yield of genetic diagnoses from 23%[14] to 60% of familial hearing loss in the Israeli Jewish population. The analysis revealed 57 different pathogenic variants in 27 genes, with most variants not previously reported, and increased the number of genes known to cause hearing loss in the Jewish population from seven to 32.

Yield from the HEar-Seq gene panel compares favorably with whole exome sequencing (WES). Costs of WES have decreased in recent years, but far fewer patients can be sequenced simultaneously with high coverage by WES than with a gene panel. This approach has proven effective for genetic diagnosis of other conditions as well; for example, inherited eye disorders.[46] Also, while WES is valuable for gene discovery, it increases the frequency of incidental findings,[47] which can introduce legal, ethical, and social dilemmas. Nevertheless, for families not solved with a gene on the panel, WES is the next step in searching for a genetic diagnosis.

The discovery that mutation of *ATOH1* can cause human hearing loss adds to understanding the role of this transcription factor in mammalian hearing. ATOH1 is crucial for the development and differentiation of inner-ear hair cells[48] and is first expressed in the nascent organ of Corti. Loss of *Atoh1* in mice causes hearing impairment, cerebellar and cochlear malformations, and death,[33] while conditional deletion of *Atoh1* leads to lack of differentiated inner ear hair cells,[49] and the naturally occurring mutation Atoh1 p.Met200Ile causes hearing loss, progressive cerebellar atrophy, and trembling.[34] The *ATOH1* mutation of family HL263 yields a protein with an abnormal C-terminus associated with an abnormally slow degradation rate. This is consistent with previous observations that *Atoh1* protein stability is regulated by its interaction with the E3 ubiquitin ligase Huwe1 at phosphorylation sites S328 and S329 (human S331 and S342).[50,51] In this context, a mouse with mutation at the phosphorylation site Atoh1 S193 was shown to have late-onset deafness.[35] In wild-type mice, expression of *Atoh1* ceases by the end of the first postnatal week. Induction of *Atoh1* at the neonatal stage causes formation of immature ectopic hair cells, with randomized stereocilia orientation and reduced basolateral measured currents.[52,53] Atoh1 is also known to positively auto-regulate its own expression.[54] We hypothesize that the human *ATOH1* mutation increases the stability of the protein through decreased degradation, resulting in increased enhancement of its own expression. This untimely expression may generate immature ectopic hair cells that interfere with development of normal hair cells.[55,56]

Pathogenic variants in *CLPP*, *USH2A,* and *SOX10*, which cause syndromic hearing loss, were detected in children prior to onset of additional symptoms. Early diagnosis of syndromic hearing loss in these children enables advance planning for education and treatment.

Our results are informative for genetic counselors, medical geneticists, audiologists and otolaryngologists caring for families with inherited hearing loss. Genetic diagnosis can be integrated with history, physical examination, and audiometry to guide management of the patient with hearing loss. The results can also assist in developing guidelines for genetic screening of newborns with possible hearing loss, in Israel and elsewhere.

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FIGURE LEGENDS

FIGURE 1 *ATOH1 c.1030delC* and age-related hearing loss in a five-generation family. A, Pedigree of family HL263 with progressive sensorineural hearing loss. Filled symbols represent individuals with hearing loss. V represents the variant allele and N the normal allele. B, Hearing thresholds of family members of various ages heterozygous for the mutation. C, Western blot of ATOH1 protein extracted from HEK293T cells transfected with wild type or mutant ATOH1, after 1-5 hours treatment with 1mM cycloheximide. D, Quantification of the results of part C. Statistical test was repeated measures ANOVA with post-hoc Holm-sidak correction for multiple comparisons.

FIGURE 2 *MITF1* variants associated with hearing loss and Waardenburg Syndrome Type 2A / Tietz Syndrome in three families. A, Pedigrees of families DF311, DF186, and DF219, indicating variation in syndromic features. B, Hearing thresholds by age, reflecting severe to profound hearing loss in all affected individuals. C, Transactivation assay using a tyrosinase promoter and luciferase reporter revealing that the transcriptional potential of protein encoded by *MITF* c.1190delG is greatly impaired compared to the wild-type protein. Statistical test was one-tailed student's t-test.

FIGURE 3 *GATA3* variants associated with nonsyndromic and syndromic hearing loss in two families. A, Pedigrees of families HL738 and HL769. B, Hearing thresholds by age, reflecting severe to profound hearing loss in all affected individuals.

SUPPORTING INFORMATION

Table S1. Genes on HEar-Seq panels

Table S2. Variants identified by HEar-Seq in Israeli Jewish families

Table S3. All variants leading to hearing loss in Israeli Jewish Families in the study

Table S4. Founder mutations in Israeli Jewish families

Figure S1. Pedigrees, audiograms and genotypes of families with hearing loss solved by HEar-Seq. N = wild type; V = variant.

Figure S2. Distribution of hearing loss variants in Jewish communities of different ancestries, including Jewish Ashkenazi, Mizrahi, Sephardi, and mixed.

Figure S3. Transcriptional activity of the *MITF* c.1190delG, p.(Gly397fs15*) variant. Western blot analysis showed a similar level of expression of the wild type MITF and mutant version.

ACKNOWLEDGEMENTS

The authors wish to thank all the families for their participation in this study. This research was supported by a grant from the National Institutes of Health/NIDCD R01DC011835 (K.B.A., M.C.K., M.N.K.), the Intramural Program at the NIDCD, DC000059 (M.W.K.), the Israel Science Foundation (grant no. 3499/19) within the Israel Precision Medicine Partnership Program (K.B.A.), the Ernest and Bonnie Beutler Research Program of Excellence in Genomic Medicine (K.B.A.), and the Hedrich Charitable Trust (K.B.A.).

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

K.B.A. and M.-C.K. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Z.B., S.G, T.W., K.B.A. and M.-C.K. were responsible for the concept and design. Z.B., S.G., T.W., F.T.A.M., S.T., O.I., M.K.L., M.B., W.C., S.C., M.B.-C., N.D.-F., A.A.-R., R.C., L.K., A.O.A., M.S., D.G., N.L., M.F., B.D., M.M., M.S., H.V., H.P., R.S., N.S., N.Z., H.B.-F., A.S., O.H., R.H., D.A.-N., N.R-S., O.M., E.S., A.P., M.K., M.S., L.B.-S., E.P., D.L., N.S., M.W.K., M.-C.K., K.B.A. acquired, analyzed, and/or interpreted the data. Z.B., F.T.A.M., M.B.-C., W.C., E.S., M.-C.K. and K.B.A. drafted the manuscript. S.G., O.I., M.K.L., N.S. and M.-C.K. performed the statistical analysis. All authors approved the final version of the manuscript.

ETHICS APPROVAL

The Ethics Committee of Tel Aviv University, the Helsinki Committee of the Israel Ministry of Health, and the Human Subjects Division of the University of Washington approved the study.

DATA AVAILABILITY STATEMENT

Novel variants are available at ClinVar (www.ncbi.nlm.nih.gov/clinvar/).

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TABLE 1 New genes and alleles discovered by the HEar-Seq gene panel in Israeli Jewish families with hearing loss

Gene	Family	Genomic coordinates (hg19)	cDNA	Effect	Allele freq in hearing controls	Allele freq in unrelated deaf	ClinVar ID	ACMG criteria
ATOH1 NM_005172.1	HL263	chr4:94751102	c.1030delC	His344fs17*	0/724	1/386	813817	PP3, PP1, PM2, PM4
ATP2B2 NM_001001331.2	DF328	chr3:10420936	c.1033C>T	Gln345*	0/770	1/386	sub	PP3, PP1, PP4, PM2, PM4
CABP2 NM_001318496.1	DF326	chr11:67289435	c.250G>A	Glu84Lys	0/770	1/386	sub	PP3, PP1, PP4, PM2, PM3
CEACAM16 NM_001039213.3	DF301	chr10:73565593 Founder allele	c.703C>T	Arg235Cys	0/470	4/386	236048	PP3, PP1, PP4, PM2
CLPP NM_006012.2	DF313	chr19:6361758 chr19:6361914	c.173T>G c.233G>C	Leu58Arg Arg78Pro	2/510 0/416	1/386 0/416	500291 813818	PP1
COCH NM_004086.2	HL1103	chr14:31355200	c.1159C>T	Leu387Phe	0/520	1/386	236036	PP3, PP1, PP4, PM2
COL11A2 NM_080680.2	HL1140	chr6:33152074 chr6:33138676	c.967insC c.3385G >A	Thr323fs17* Gly1129Arg	0/470 0/402	1/386 1/386	813820 813821	PP3, PP1, PP4, PM2, PM3, PM4
EYA4 NM_172105.3	HL21	chr6:133783474	c.441delC	Tyr148fs49*	0/390	1/386	236032	PP3, PP1, PP4, PM2, PM4
EYA4 NM_172105.3	DF312	chr6:133844297 - 133844299	c.1720_1722 delTACinsAAA	Tyr574Lys	0/446	1/386	sub	PP3, PP1, PP4, PM2
GATA3 NM_002051.2	HL738	chr10:8100707	c.681ins35	Glu228fs37*	0/586	1/386	236031	PVS1, PM2, PP3, PP1, PP4
GATA3 NM_002051.2	HL769	chr10:8106009	c.829G>A	Asp277Asn	0/440	1/386	813823	PP3, PP1, PM2
MITF NM_198159.2	DF311	chr3:70001035	c.935T>C	Leu312Pro	0/530	1/386	547531	PM2, PP3, PP1, PP4
MITF NM_198159.2	DF186	chr3:70014025	c.1190delG	Gly397fs15*	0/448	1/386	236050	PP1, PP3, PP4, PM2, PM4
MITF NM_198159.2	DF219	chr3:70005649	c.981InsC	Leu327fs9*	0/430	1/386	813825	PP1, PP3, PP4, PM2, PM4
MYO6 NM_004999.3	DF305	chr6:76538307	c.238C>T	Arg80*	0/442	1/386	178957	PP3, PP1, PP4

MYO6 NM_004999.3	HL1133	chr6:76568683	c.1452insT	Asn485*	0/800	1/386	sub	PP3, PP1, PP4, PM2, PM4
MYO6 NM_004999.3	HL1274	chr6:76568710	c.1473del3insC	skip exon 14, Glu461fs13*	0/618	1/386	236034	PP3, PP1, PP4, PM2, PM4
MYO6 NM_004999.3	HL158	chr6:76624636	c.3765delC	Cys1256fs28*	0/326	1/386	813826	PP3, PP1, PP4, PM2, PM4
MYO15A NM_016239.3	HL72	chr17:18054799	c.7751_8224del 3446ins23	Gln2583fs19*	0/530	2/386	236039	PP3, PP1, PP4, PM2, PM4
MYO15A NM_016239.3	DF327 DF317	chr17:18069748 Founder allele	c.9861C>T	Gly3287Gly	0/402	2/386	228276/ 45777	PP3, PP1, PM3
PCDH15 NM_001142769.1	HL1134	chr10:56287598	c.146T>C	Val49Ala	0/540	0/386	450626	PP3, PP1, PM2
SLC26A4 NM_000441.1	HL1132 HL1327	chr7:107312627	c.349C>T	Leu117Phe	7/1000	7/386	43555	PP3, PP1, PM2, PM3, PS4
SOX10 NM_006941.3	HL971	chr22: 38379660	c.125_132del8	Leu42fs21*		1/386	813829	PP3, PM2, PVS1
STRC NM_153700.2	HL927	chr15:43,892,353 -43,910,998 (min)	del entire gene	-		2/386	sub	PP3, PP1, PP5, PP4, PM4, PM1, PM3, PVS1
TECTA NM_005422.2	DF303	chr11:120979969	c.248C>T	Thr83Met	0/440	2/386	813831	PP3, PP1, PP4, PM2
TECTA NM_005422.2	HL277	chr11:121000866	c.2887G>A	Ala963Thr	0/540	1/386	236059	PP3, PP1, PP4
TECTA NM_005422.2	DF183	chr11:121058558	c.6017A>G	Asp2006Gly	0/390	1/386	236033	PP3, PP1, PP4, PM2
TJP2 NM_001170414.2	DF180	chr9:71704982 - 71840362 dup	dup exons 1-6	-	0/384	1/386	236035	PP3, PP1, PP4, PM2, PM4, PM1, PS3
TMC1 NM_138691.2	DF193	chr9:75263573	c.15insA	Val6fs12*	0/490	2/386	236041	PP3, PP1, PP4, PM2, PM4
TMC1 NM_138691.2	HL1159	chr9:75369733	c.674C>T	Pro225Leu	0/466	1/386	424807	PP3, PP1, PP4, PM2, PM3

Supporting Information

METHODS

Gene panels. Genomic DNA was sequenced using the HEar-Seq gene panels containing: 244 protein-coding genes (HEar-Seq1) capturing 3,981 exons and 2 miRNAs, with a total target size of 1.43 Mb that included 40 bp of flanking intronic bases;[1] 281 genes (HEar-Seq2) capturing 4,419 exons and 3 miRNAs, with a total target size of 1.85 Mb, including 40 bp of flanking intronic bases;[2] and 372 genes (HEar-Seq3) capturing 6,222 exons and 3 miRNAs, with a total target size of 1.93 Mb, including 10bp of flanking intronic bases;[3] 178 genes (HEar-Seq4) capturing 3134 exons and one miRNA, with a total target size of 808 kb that included 25 bp of flanking intronic bases (Supporting Information Table S1).

ATOH1 analysis. For the western blot analysis, the pCDT N3xFLAG3xHA was generated by a modification of pCIG (pCAGGS-IRES-nucEGFP vector; a gift from Terry P. Yamaguchi, NCI**).** 3xFLAG and 3xHA tags were cloned at the N-terminus, and IRES-nucEGFP was replaced with IRES-tdTomato. The N-3FLAG3xHA tags nucleotide sequence is as follows:
ATGGACTACAAAGACGATGACGACAAGGATTATAAGGACGATGACGATAAAGATTACAAGGATGACGACGATAA
GCTGAGCTCGTACCCATATGACGTTCCAGACTACGCGTATCCGTACGACGTTCCGGATTACGCTTACCCTTACGAC
GTACCTGACTACGCTCCACCTGATATCATCGAT— and its amino sequence is as follows:
MDYKDDDDKDYKDDDDKDYKDDDDKLSSYPYDVPDYAYPYDVPDYAYPYDVPDYAPPDIID--.

HEK293T cells were transfected with p-CDT with the wild-type (ATOH1-WT) or the c.1030delC *ATOH1* variant (ATOH-MUT) adjacent to an IRES-tdTomato fluorescent protein tag in the presence of cycloheximide (CHX). The H7 anti-HA antibody was used to detect the NHA-tagged ATOH1. For the protein degradation assay, HEK293T cell cultures growing in 60 mm dishes were transfected with pCDT with ATOH1-WT or ATOH1-MUT using Xfect Transfection Reagent (Clontech). At day 3 after transfection, cell cultures were treated with 1 mM cycloheximide **(**Sigma**)**, and were harvested in cold PBS after 0, 1, 2, 3, and 5 hrs. RIPA buffer supplemented with protease inhibitor cocktail cOmplete Mini (Roche) and phosphatase inhibitor cocktail PhosSTOP (Roche) was used to lyse the cells. After clarification, the supernatant was divided into two portions – one for BCA protein assay (Thermol) to quantify the total amount of protein, and the other for gel separation using NuPAGE 4-12% SDS-Bis-Tris gel (Invitrogen). After gel separation, the protein was transferred into the cellulose membrane using an iBlot apparatus (Invitrogen) based on the manufacturer's protocol. The western blotting was carried out on the iBind Flex apparatus. The primary antibody, H7 anti-HA antibody (Sigma), or anti-beta-actin (Sigma), was used

in a 1:5,000-10,000 dilution. The secondary antibody, horseradish peroxidase (HRP) conjugated anti-mouse IgG antibody (Jackson Immunoresearch Lab), was used in a 1:5,000-10,000 dilution. For ECL visualization, Clarity Western ECL substrate A and B solutions (Bio-Rad) were used, and DBio Blue sensitive X-ray film (Vita Scientific) was used for image capture. After X-ray film exposure and development, the bands on the film were scanned at highest resolution (600x600 dpi) on the desktop scanner. ImageJ (NIH) was used to measure the intensity of the bands. A similar area with no band was used as background for subtraction. The reading of beta-actin bands was further used for standardization of the Atoh1 band reading. For the final presentation, the reading was normalized against the reading at 0 hrs.

MITF analysis. For the transactivation assay, HEK293T cells (2×10^4) were grown for 24 hours in a 96-well plate in 100 µL DMEM + 10% FBS. Cells were transiently co-transfected with an empty pcDNA3.1 construct or pcDNA3.1 containing mouse wild-type Mitf (Mitf-WT) or the c.1190delG Mitf mutation (Mitf-MUT), a pGL3 basic luciferase construct containing the tyrosinase promotor sequence and a CMV-pRL Renilla control vector. 24 hours post transfection the Dual-Glo Luciferase Assay (Promega) was performed according to the protocol with a Modulus II microplate reader (0.5 sec integration time). Luciferase signals were normalized to corresponding Renilla signals and results expressed as fold change over empty vector. Three biological replicates were performed. For immunoblotting, the cells were lysed and sampled 24 hours post transfection in sample buffer, boiled for 5 min and 20 µL of each sample were analyzed on 8% SDS-poly-acrylamide gels that were transferred to nitrocellulose membranes. Membranes were incubated overnight with antibodies for MITF (C5, Thermo Scientific) and β-actin (13E5, Cell Signaling) and then secondary antibodies (Anti-rabbit IgG and Anti-mouse IgG, Cell Signaling). Results were visualized using the Odyssey infrared imaging system.

RESULTS

One of the major advantages of early diagnosis is that it enables caregivers to predict whether the subjects under study are likely to develop symptoms other than hearing loss. Pathogenic variants were detected in genes known to be involved in SHL, with variable onset of symptoms relative to the hearing loss.

The novel recessive variants in *CLPP*, c.173T>G, p.(Leu58Arg) and c.233G>C, p.(Arg78Pro), were identified in compound heterozygosity in Family DF313 (Supporting Information Figure S1) in siblings

under the age of three. *CLPP* is associated with Perrault syndrome, characterized by sensorineural hearing loss (SNHL) and infertility in both females and males[4]. These siblings have congenital profound hearing loss and auditory neuropathy but no other symptoms. Due to the young age of the DF313 children, it is not clear whether they are pre-symptomatic for infertility or if the compound heterozygote variants lead to NSHL.

A novel dominant variant, *SOX10*, c.125_132del8, p.(Leu42fs21*), was detected in an adult, individual HL971 (Supporting Information Figure S1), with congenital profound NSHL, which he inherited from his Ashkenazi Jewish mother. The *SOX10* gene is known to be involved in Waardenburg syndrome WS2E[5] and WS4C,[6] as well as in Kallmann syndrome.[7] However, neither the proband nor his mother initially reported any additional symptoms besides deafness. Upon detecting the above variant and asking the proband about his olfaction, we discovered that both he and his deaf mother have anosmia, which is characteristic of Kalmann syndrome.

In Family HL149, an *USH2A* c.3368A>G, p.(Tyr1123Cys) variant[8] was detected in compound heterozygosity with the *USH2A* c.240_241insGTAC variant, which is a known pathogenic variant associated with SHL[9] (Supporting Information Table S2). The mother, in her early thirties, has been diagnosed with USH2A syndrome, is homozygous for c.240_241insGTAC, and has a sloping mild-to-severe hearing loss with mild retinitis pigmentosa (RP). Her compound heterozygous son, subject 149.06, three years old, had a sloping mild-to moderate hearing loss, similar to his mother, but no signs of RP at this young age. As *USH2A* variants are involved in either non-syndromic RP or in USH2A, it is likely that this child will develop RP later in life[8].

Founder mutations play a major role in hearing loss in a portion of the Israeli Jewish families (Supporting Information Table S4). For example, *SLC26A4* c.349C>T, p.(Leu117Phe), is included in genetic screening for diseases in the Ashkenazi Jewish population. *SLC26A4* variants may cause Pendred syndrome or nonsyndromic hearing loss with enlarged vestibular aqueduct (EVA). The pathogenicity of *SLC26A4* c.349C>T, p.(Leu117Phe) has been controversial. No families homozygous for this variant have been reported, although heterozygosity without a second detected *SLC26A4* variant has been seen in deaf patients with EVA.[10-12] SLC26A4 p.(Leu117Phe) protein is normally distributed at the apical membrane of thyroid follicular cells, and iodide transport, thought to involve pendrin, is not affected by this variant[10]. Nevertheless, the segregation of the c.349C>T variant with deafness in seven families, alongside its ethnicity-specific high-prevalence rates, suggests pathogenicity with a strong founder effect in the Ashkenazi Jewish population.[13]

Another example of a founder mutation is the *OTOF* splice variant c.5193-1G>A (ClinVar 48256), leading to the skipping of exon 42, resulting in a non-frameshift deletion of 33 amino acids. This variant was detected in one Jewish Syrian family. A carrier rate of 0.8% was found among 368 hearing controls of the same ethnic group, all heterozygotes. This variant was absent in 700 hearing controls of all other Jewish ethnicities and was not found in gnomAD, supporting the conclusion that it is a founder mutation among Syrian Jews.

Our results reveal novel Ashkenazi Jewish pathogenic variants, as well as variants associated with specific Jewish ethnicities and absent in others (Supporting Information Figure S2; Table S2). Certain pathogenic variants are present in high prevalence among all Jewish ethnicities, e.g., *STRC* del, which is characteristic of congenital, mostly moderate SNHL.

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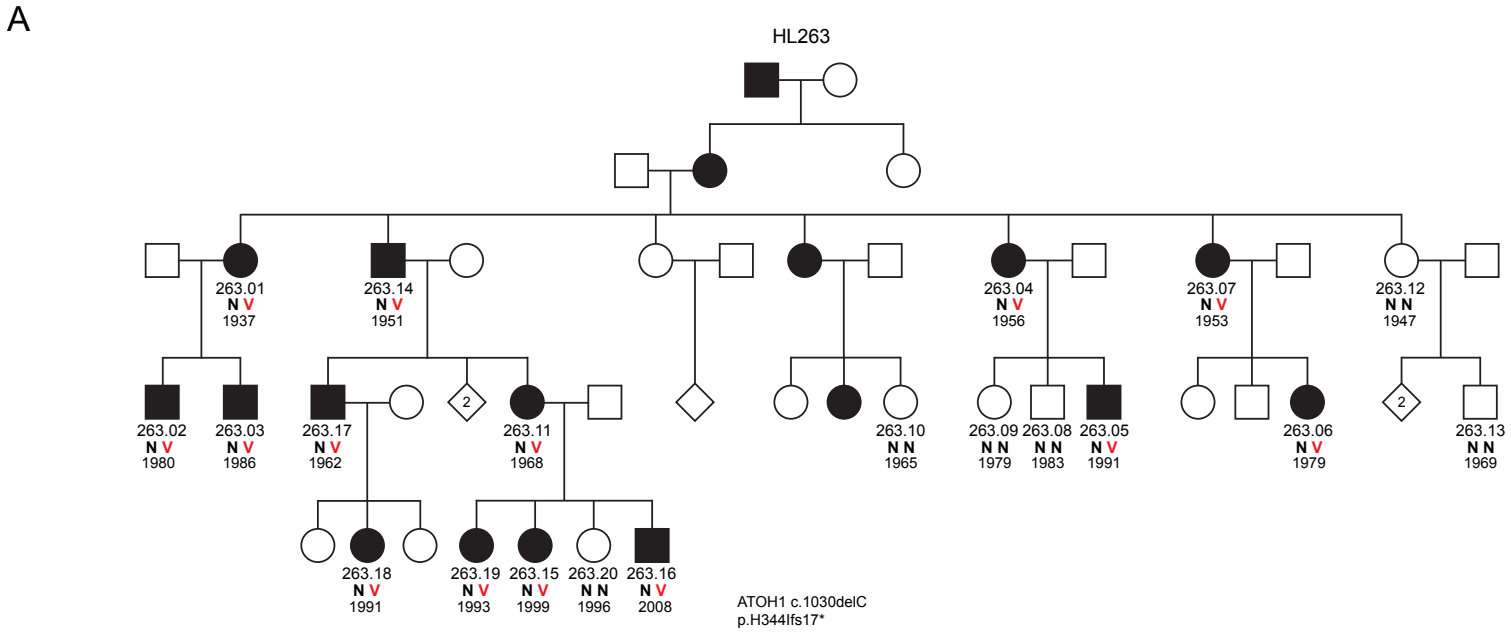
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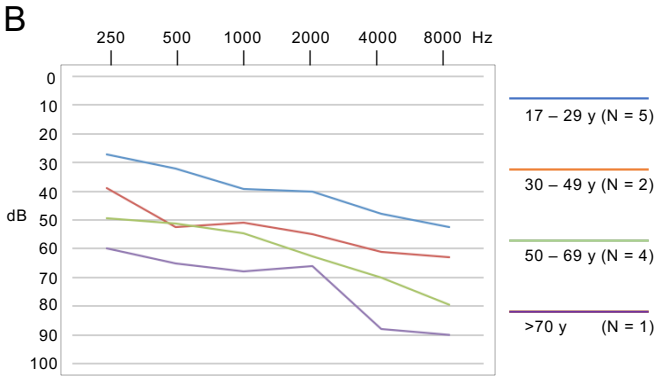
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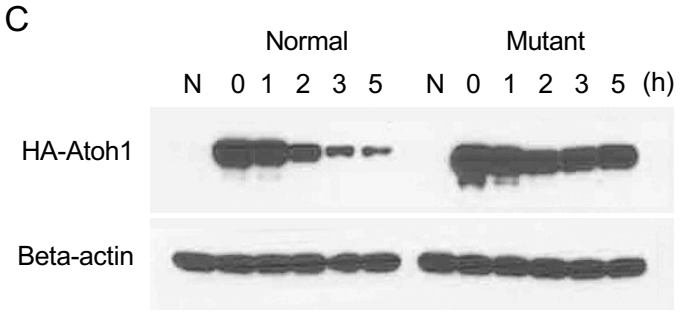
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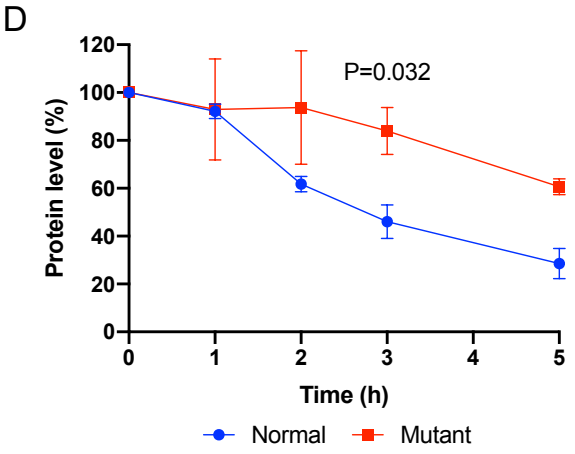
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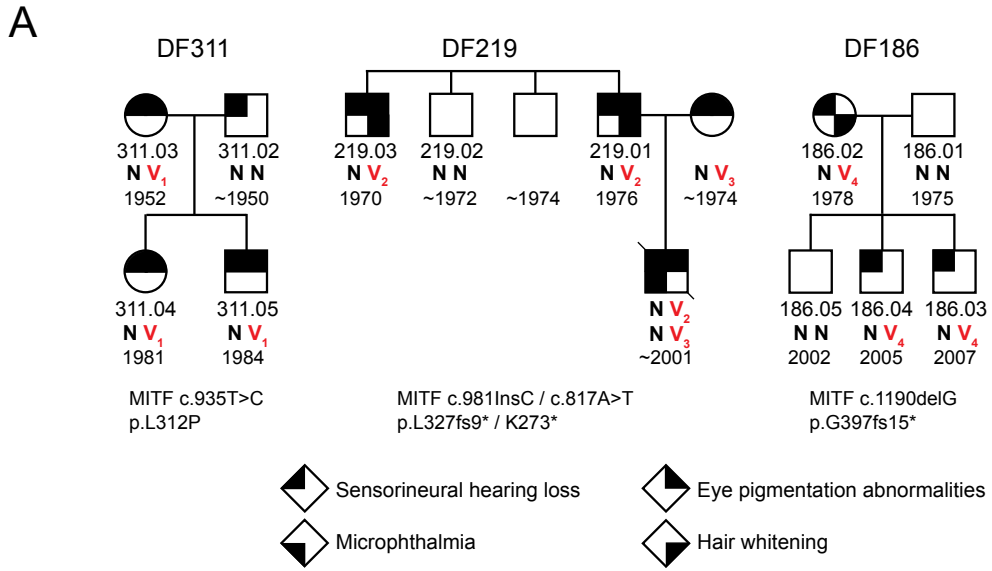
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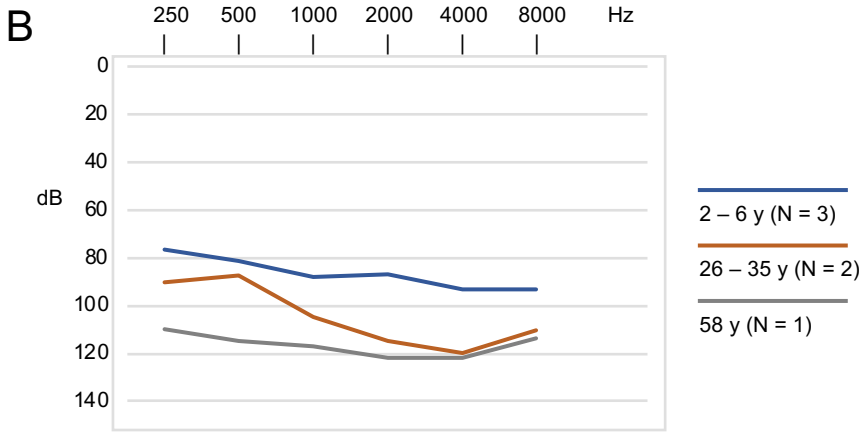
Hair whitening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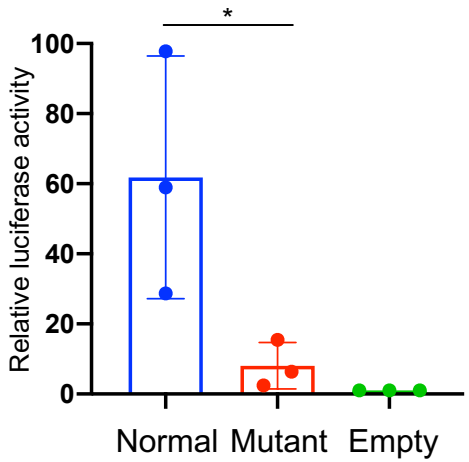
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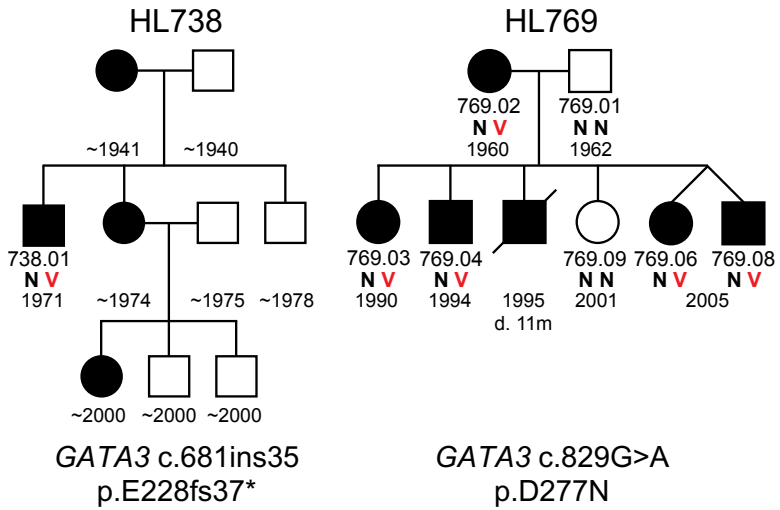

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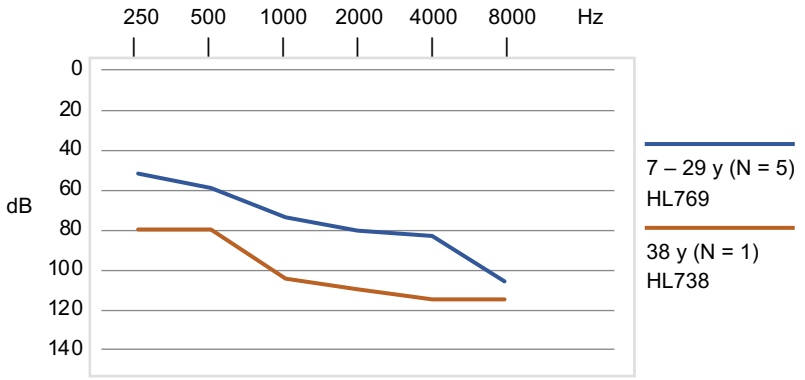
GATA3 c.829G>A
p.D277N

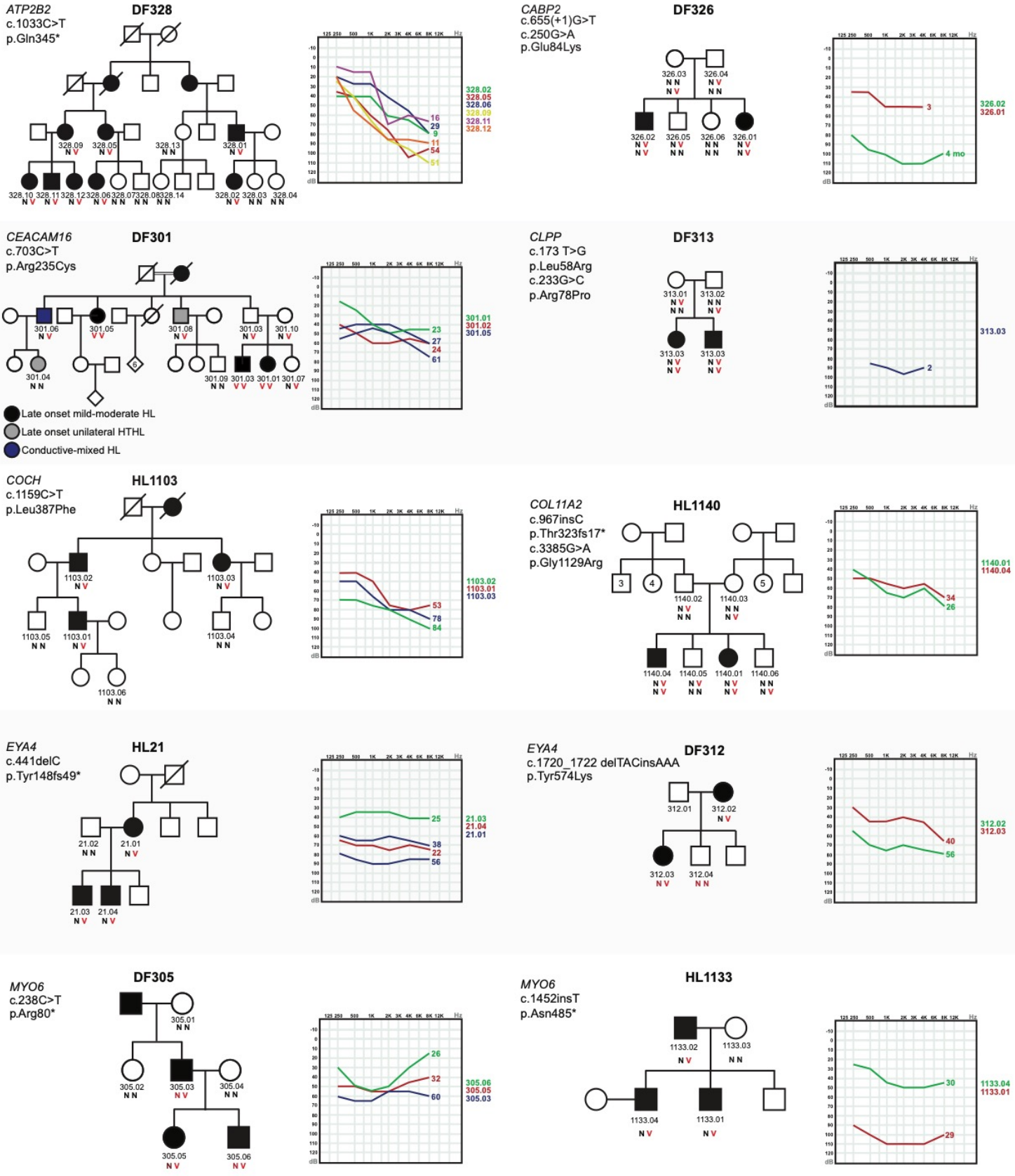
B



Supporting Information

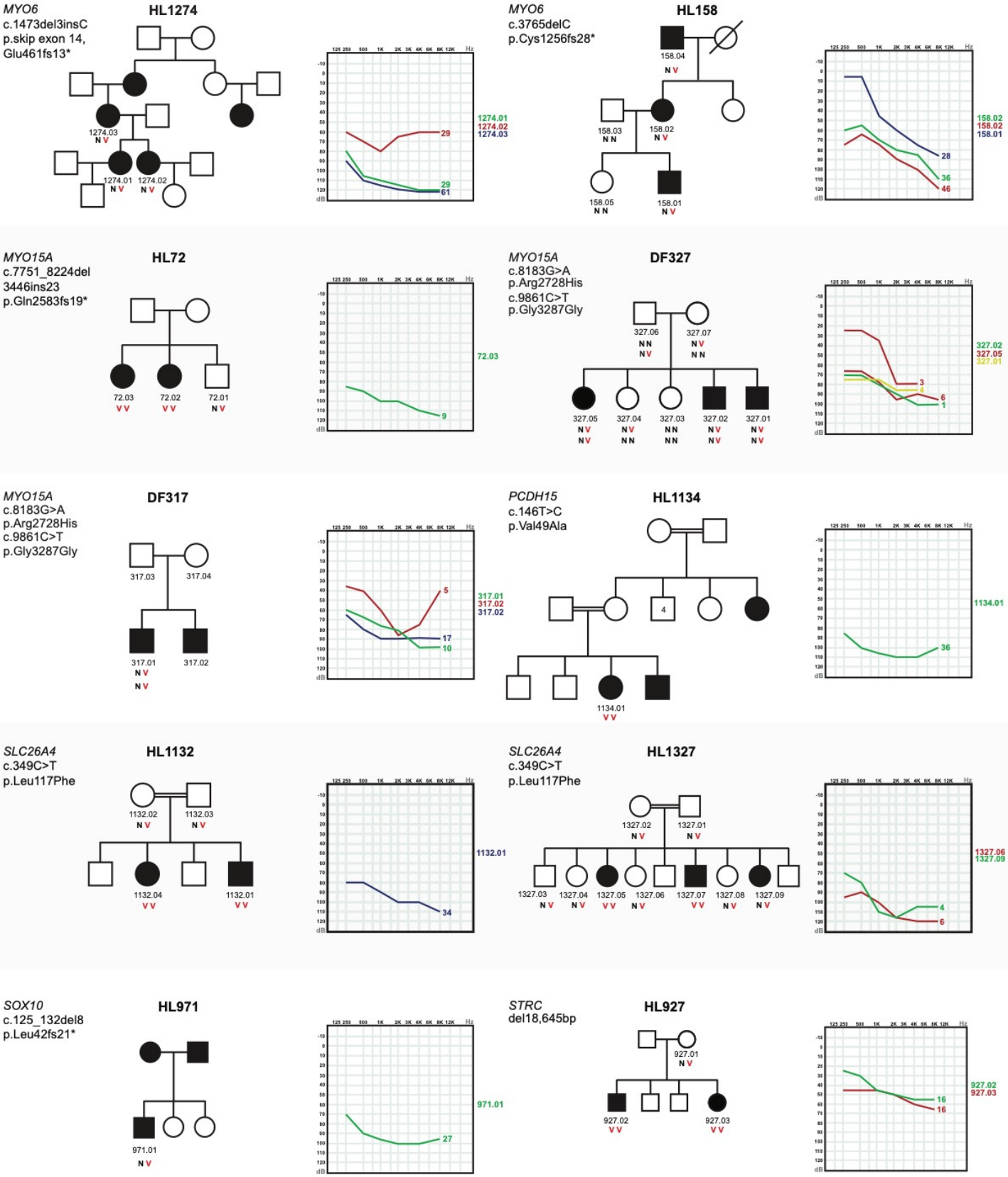
Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene *ATOH1*

Figure S1. Pedigrees, audiograms and genotypes of families with hearing loss solved by HEar-Seq. Age at the time of testing is noted on audiograms. N = wild type; V = variant.



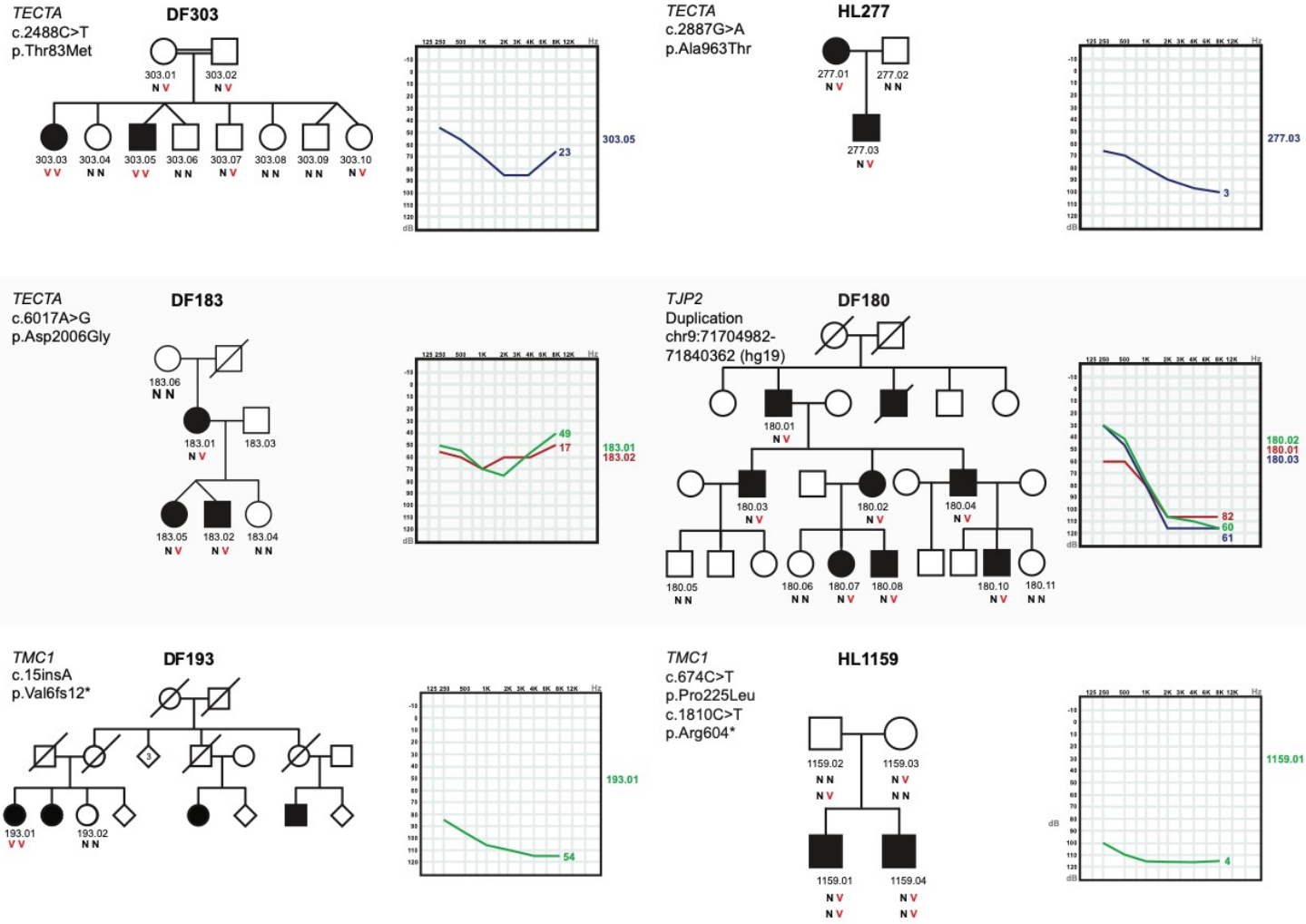
Supporting Information

Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene *ATOH1*



Supporting Information

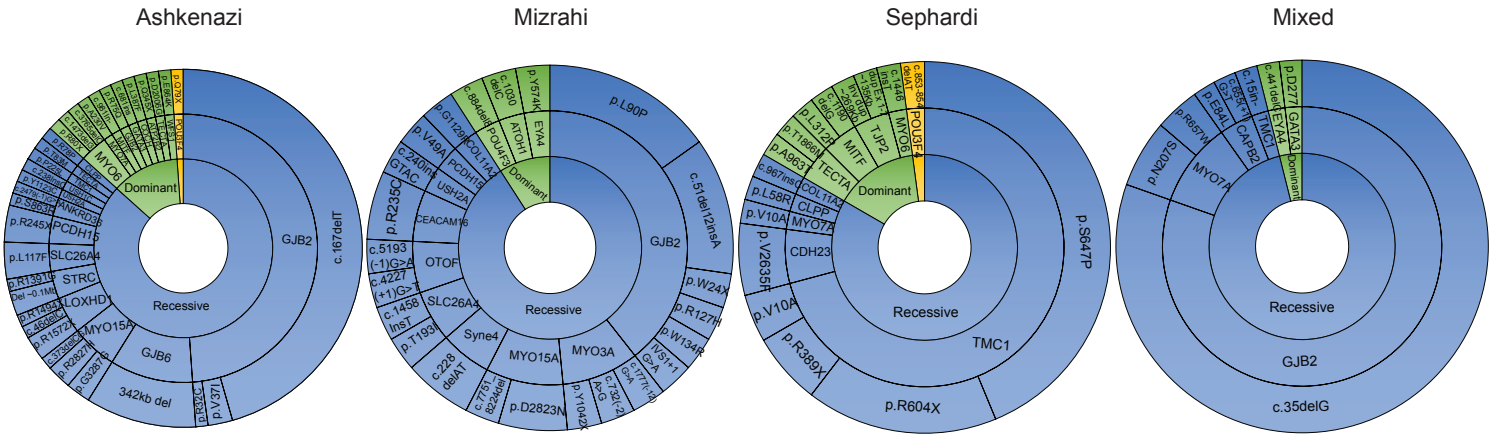
Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene *ATOH1*



Supporting Information

Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene *ATOH1*

Figure S2. Distribution of hearing loss variants in Jewish communities of different ancestries, including Jewish Ashkenazi, Mizrahi, Sephardi, and mixed.



Ashkenazi				Mizrahi				Sephardi				Mixed		
Profound	High-tone	Mild-moderate	Variable	Profound	High-tone	Mild-moderate	Variable	Profound	High-tone	Mild-moderate	Variable	Profound	Mild-moderate	Variable
SOX10	COCH	ATP2B2	POU3F4	USH1C	POU4F3	ATOH1	EYA4	MYO6	TJP2	COL11A2	POU3F4	EYA4	EYA4	GATA3
MITF		WFS1	GATA3	OTOF	MYO3A	USH2A	GJB2	MITF		MYO7A	TECTA	TMC1		MYO7A
GJB6		ANKRD36	GJB2	PCDH15	SYNE4	CEACAM16		CDH23			GJB2			GJB2
LOXHD1		STRC	TECTA	SLC26A4		COL11A2		CLPP			TECTA			MYO7A
PCDH15		USH2A	MYO6	MYO15A				TMC1						CAPB2
SLC26A4			GJB2											
USH1C			TECTA											
TMC1			MYO15A											
CLPP														
MYO7A														

Recessive Dominant X-linked

Supporting Information

Spectrum of genes for inherited hearing loss in the Israeli Jewish population, including the novel human deafness gene *ATOH1*

Figure S3. Transcriptional activity of the *MITF* c.1190delG, p.(Gly397fs15*) mutation. Western blot analysis showed a similar level of expression of the wild type MITF and mutant version.