PNAS RESEARCH ARTICLE | GENETICS OPEN ACCESS

Genetics of prelingual isolated deafness and Usher syndrome in the Maghreb and Jordan: Harnessing the potential of homozygosity

Zied Riahi[a,b], Sophie Boucher[c,d], Samia Abdi[e,f,g], Fabienne Wong Jun Tai[a], Amrit Singh-Estivalet[a], Asadollah Aghaie[a], Magali Niasme-Grare[a,h], Jean-Pierre Hardelin[i], Asma Behlouli[e,j], Malika Dahmani[k], Sonia Talbi[k], Yosra Bouyacoub[a,b], Rahma Mkaouar[b], Cherine Charfeddine[b,l], Ghita Amalou[m], Amina Bakhchane[m], Amale Bousfiha[n,o], Sara Salime[m], Soukaina Elrharchi[m], Malak Salame[p], Mouna Hadrami[p], Ely Boussaty[q], Hicham Charoute[r,s], Mustapha Detsouli[m], Khalid Snoussi[t,u,v], Hassan Rouba[m], Hala El Hachmi[p], Fatimetou Veten[p], Ghlana Meiloud[p], Jihene Marrakchi[b,w], Rim Zainine[b,x], Houda Chahed[x], Ghazi Besbes[x], Mediha Trabelsi[y,z], Ridha Mrad[y,z], Ichraf Kraoua[aa,bb], Sofiane Ouhab[cc], Djamel Djennaoui[dd], Farid Boudjenah[ee], Eliane Chouery[ff], Mirna Mustapha[gg], Ahmed Houmeida[p], Abdelhamid Barakat[m,r], Fatima Ammar Khodja[k], Mohamed Makrelouf[e], Akila Zenati[e], Najeh Beltaief[x], Sonia Abdelhak[b], Christine Petit[a,hh,1,2], and Crystel Bonnet[a,1,2]

Affiliations are included on p. 10.

Contributed by Christine Petit; received July 14, 2025; accepted October 22, 2025; reviewed by Karen B. Avraham and Mary-Claire King

The molecular genetic diagnosis of prelingual sensorineural hearing impairment (HI) is essential for genetic counseling and patient management. Effective diagnosis requires a knowledge of the genetic architecture of HI, which is often lacking. We established a cohort of 450 unrelated patients with familial (at least two affected relatives) severe-to-profound bilateral prelingual HI in five countries with high consanguinity rates: Tunisia, Jordan, Algeria, Morocco, and Mauritania (the TJAMM cohort). Recessive and dominant inheritance were observed in 92% and 8% of cases, respectively; 14% were syndromic. Genome analysis detected 211 different mutations (36% not reported before) in 49 deafness genes, and fully resolved 90% of cases of autosomal recessive isolated deafness (DFNB forms), 89% of the mutations being homozygous. The deafness genes involved were similar in different countries, but their mutations, except a few in *GJB2* and *LRTOMT*, differed considerably, suggesting an overrepresentation of private mutations. Biallelic missense mutations in *MYO7A*, *CDH23*, *PCDH15*, *USH1C* cause either DFNB forms or Usher syndrome type 1 (USH1) (*USH1/DFNB* genes). Such mutations were overrepresented (13% of patients), highlighting the importance of distinguishing between these two mutation classes. We hypothesized that current difficulties might stem from the misclassification of certain mutations. By studying the 65 *USH1/DFNB* missense mutations reported to cause DFNB in the homozygous state, we identified some that, when associated with a loss-of-function mutation, resulted in USH1, a characteristic pattern of some recessive hypomorphic mutations. This reappraised classification of *USH1/DFNB* mutations has the potential to improve molecular diagnosis and patient management significantly.

North Africa | homozygosity | hypomorphic mutations | USH1B/DFNB mutations | molecular diagnosis

Significance

Homozygosity for causal mutations in recessive deafness provides a unique opportunity to unravel the mutation/phenotype relationship. By studying familial congenital sensorineural deafness in highly consanguineous populations in North Africa, Mauritania, and Jordan, we deciphered the corresponding genetic architecture, highlighting the major contribution of highly heterogeneous private mutations. A crucial issue for molecular diagnosis is to predict the outcome of missense mutations of genes that can cause either Usher syndrome type 1 (USH1, a severe multisensory disorder) or isolated deafness (DFNB forms). We identified several missense mutations resulting in DFNB in homozygous patients and USH1 when associated with a loss-of-function mutation in heterozygous patients, leading to a reappraised classification of the *USH1/DFNB* mutations that should improve the quality of genetic counseling.

Hearing impairment (HI) is the most common congenital sensory disorder, with an incidence of about 1.3 per 1,000 newborns in developed countries (1). Gene defects are thought to account for more than half these HI cases. Isolated congenital/prelingual deafness (or nonsyndromic deafness) forms can display autosomal dominant (DFNA), autosomal recessive (DFNB), X chromosome linked (DFNX), Y-chromosome linked (DFNY), or mitochondrial transmission and are genetically highly heterogeneous, with 156 causal genes identified to date (Hereditary Hearing Loss Homepage: 2025). In addition, about 300 genes have been reported to cause syndromic HI (2), with an increasing number recognized as also responsible for isolated deafness. With few exceptions (3–5), DFNB forms are severe-to-profound and congenital or prelingual, whereas DFNA forms are often less severe, progressive, and with a later onset. The prevalence of DFNB forms is particularly high in countries with high consanguinity rates (6–8).

Identification of the causal mutations for prelingual HI is essential to improve the quality of genetic counseling for families, informing them of the risk of deafness in future children and the most likely HI outcome (based on increasingly documented genotype/phenotype correlations). It can also reveal disorders potentially associated with the HI and requiring clinical investigation, such as retinitis pigmentosa (9) or cardiac arrhythmia (10). Finally, it can help guide decisions about hearing rehabilitation.

[1]C.P. and C.B. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: christine.petit@pasteur.fr or crystel.bonnet@pasteur.fr.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2518445122/-/DCSupplemental.

Published December 8, 2025.

In recent years, next-generation sequencing techniques have greatly increased the efficiency of genomic analyses and decreased their cost (11–13). Some of these techniques (whole-exome or whole-genome sequencing) are still too expensive for routine use in molecular diagnosis in many developing countries (14). By contrast, targeted gene panel sequencing, focusing on particular genomic regions, is cheaper, with a faster turnaround time and results that are simpler to interpret. However, the development of dedicated targeted sequencing panels requires knowledge of the genetic architecture of the disorder to guide the selection of genes for analysis. This includes identification of the most prevalent mutations, which vary with ethnicity. Furthermore, the high frequency of consanguineous unions in some populations results in private mutations that must also be considered (15).

We investigated the genetic architecture of prelingual deafness in Tunisia, Algeria, Morocco, and Mauritania, home to some of the most understudied populations in Africa (16) as well as Jordan. We recruited a cohort of 450 unrelated cases from affected families and developed a targeted gene panel for sequencing analyses of 157 deafness genes identified in various populations worldwide.

About 13% of the patients carried mutations of *USH1/DFNB* genes that could underlie either Usher syndrome type 1 (USH1) or isolated deafness (DFNB forms). The association of retinitis pigmentosa with a prepubertal onset in patients with USH1 greatly impairs their overall sensory perception, making the search for molecular criteria for distinguishing between mutations causing USH1 and those causing DFNB a priority, particularly as visual impairment will occur long after parents have taken decisions about whether to use sign language or oral language (relying on cochlear implants) to communicate with their child. We addressed this issue by developing a strategy for mutation analysis based on homozygosity.

Results

HearPanel-IdA-1 Identifies Causal Variants for Prelingual HI in 90% of Patients in the TJAMM Cohort. We recruited a cohort of 450 unrelated index cases with prelingual HI from five countries: Tunisia, Jordan, Algeria, Morocco, and Mauritania, the TJAMM cohort (*SI Appendix,* Fig. S1). Pedigree analyses revealed recessive inheritance in 92% (414/450) and dominant inheritance in 8% (36/450); X-linked transmission could not be excluded in 1% (5/450). In 13.7% (62/450) of patients, syndromic deafness was clinically diagnosed before or during the study. Genomic DNA from patients and their relatives was analyzed with HearPanel-IdA-1 (*Patients and Methods* and *SI Appendix,* Table S1).

GJB2 mutations, causing DFNB1, are a highly prevalent cause of congenital deafness worldwide, except in black African populations (17). The prevalence of *GJB2* mutations was 36% in the TJAMM cohort, ranging from 16.6% in Mauritania to 46% in Algeria. The c.35delG;p.(Gly12Valfs*2) mutation accounted for more than 90% of the *GJB2* mutations in each country. Other mutations were occasionally observed (*SI Appendix,* Tables S2 and S3). Four unrelated patients were compound heterozygous for the c.35delG;p.(Gly12Valfs*2) mutation with either the c.139G>T;p.(Glu47*) or the intronic c.-23+1G>A mutation. A few additional missense mutations were observed (*SI Appendix,* Tables S2 and S3). All these mutations have already been reported.

We then screened the patients for *LRTOMT* mutations, causing DFNB63, the second most frequent cause of HI in Morocco (18). Three *LRTOMT* mutations have been reported, all in exon 7 (18–20) (*SI Appendix,* Tables S2 and S3). *LRTOMT* mutations were detected in 6% of the patients in the TJAMM cohort, all homozygous (Fig. 1 for country distribution). Together, *GJB2* and *LRTOMT* mutations accounted for 41.5% of all cases of deafness in this cohort.

We identified mutations responsible for HI in 87% (228/263) of the remaining 263 patients (99%, 95%, 83%, 75%, and 66% of the patients from Tunisia, Algeria, Jordan, Mauritania, and Morocco, respectively).

HearPanel-IdA-1 detected mutations in 92% of the patients with HI in the TJAMM cohort. Biallelic mutations were identified in 93.7% (388/414) of the 414 patients with DFNB or autosomal recessive syndromic deafness (ARSD), and monoallelic mutations, corresponding to partial resolution, were identified in 3% (13/414), with single mutations detected in 11 genes (*ADGRV1, BDP1, CDH23, ESRRB, MYO3A, OTOF, OTOG, SLC26A4, STRC, USH1C, and USH2A*). No apparent homozygosity suggestive of a possible deletion affecting the other allele was found. The last 3% (13/414) of cases remained fully unresolved. Monoallelic mutations were detected in only 39% (14/36) of the patients with familial segregation suggestive of DFNA, autosomal dominant syndromic deafness (ADSD) or DFNX forms. Overall, fully solved cases of recessive or dominant deafness accounted for 89% (402/450) of the entire cohort (*SI Appendix,* Table S2).

Mutation Analysis: Nature, Pathogenicity, and Recurrence. HearPanel-IdA-1 detected 211 different mutations in 49 deafness genes: 42 genes underlying DFNB forms and/or ARSD (predominantly USH), five underlying exclusively dominant forms (four ADSD genes and one DFNA gene), one (*GJB2*) both a DFNB and a DFNA form and the last one a DFNX form (*SI Appendix,* Tables S2 and S3).

About 50% of the 211 mutations (108/211) were loss-of-function (LOF) mutations [nonsense (23%), splice-site mutations (13.2%) all predicted to be pathogenic (*SI Appendix,* Table S4), frameshift deletions or insertions (12.8%), and large rearrangements (2%)]. The other half were missense mutations (99/211, 47%). In addition, four in-frame deletions, two of one amino acid, one of five amino acids, and one deletion/insertion mutation (4/211, 2%) were detected (*SI Appendix,* Fig. S2; see *SI Appendix,* Table S3 for all the mutations and *SI Appendix,* Table S4). Notably, 36% (76/211) of these mutations and four of the five large homozygous deletions had never been reported (*SI Appendix,* Figs. S2 and S3 and Table S3). Among them, all the 26 previously unknown missense mutations were predicted to be pathogenic by REVEL score but *MYO15A*:p.(Ser472Pro). Pathogenicity was further documented by studying the predicted effects on the known or predicted three-dimensional (3D) structure of the corresponding proteins (*SI Appendix, Methods*). Apart from *CDH23*:p.(Ile1265Thr) and the eight missense mutations of *MYO15A* that could not be analyzed due to the lack of a predicted 3D structure, eight of the remaining 17 mutations were predicted to modify the 3D structure of the corresponding protein due to α-helix breakages or steric clashes associated with the most frequent rotamer (as an illustration, see the p.(Cys254Arg) mutation in glutaredoxin and cysteine-rich domain-containing 1 (*GRXCR1*); *SI Appendix,* Fig. S4 and legend). For the other nine mutations, no structural modification was predicted, but changes in the chemical and physical properties of the amino acids may lead to protein instability (hydrophobic cluster modification, hydrogen bond polarization, etc.) (*SI Appendix,* Table S5).

Only a few recurrent mutations in genes other than *GJB2* and *LRTOMT* were found in several countries, with three of these mutations detected in at least four patients and two countries:

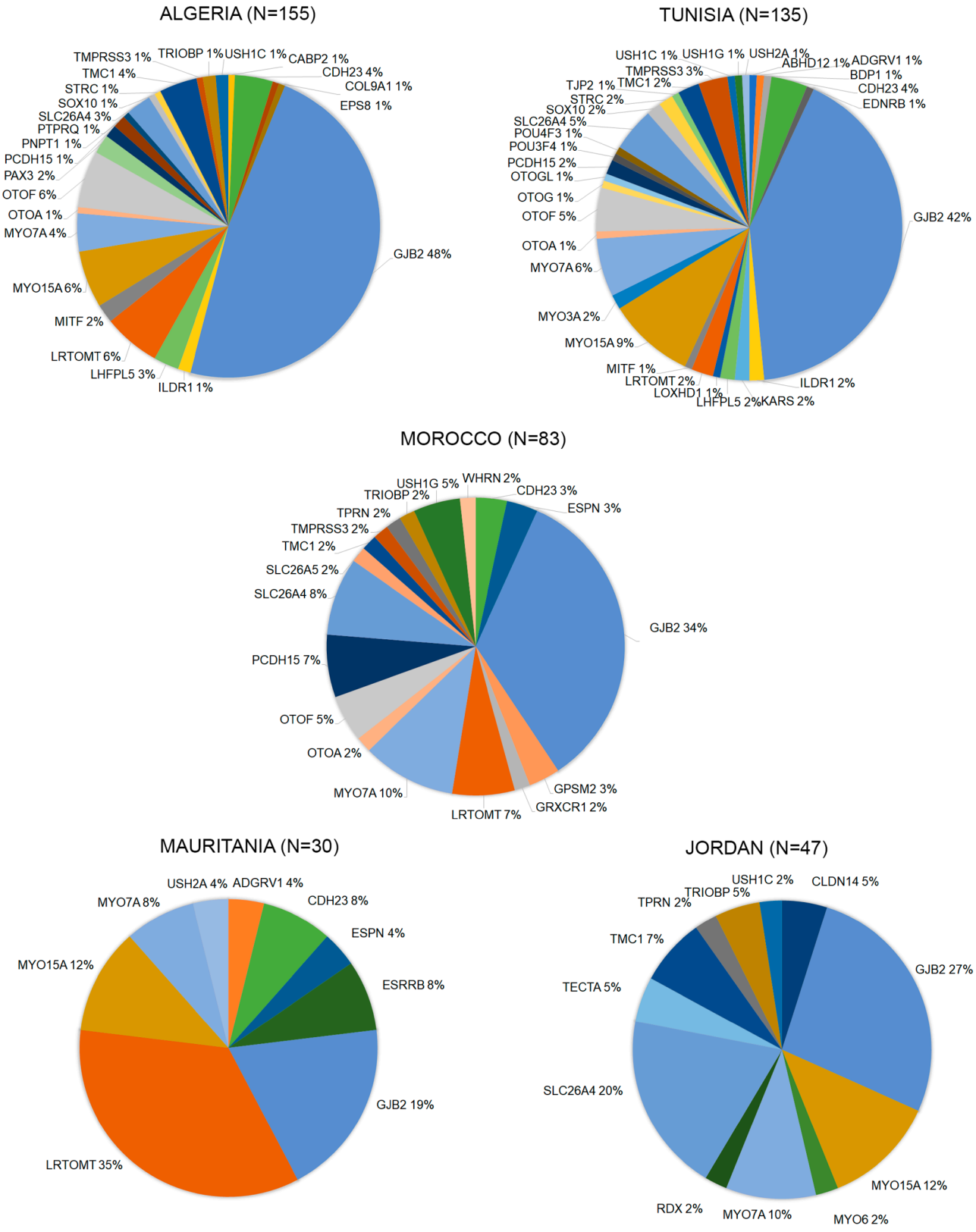

Fig. 1. Genes responsible for congenital/prelingual HI in the Tunisian, Jordanian, Algerian, Mauritanian, and Moroccan populations of the TJAMM cohort. Pie chart representations of the contribution of each deafness gene to HI cases in the TJAMM cohort.

MYO7A:c.2283-1G>T, *OTOF*:c.2239G>T;p.(Glu747*), and *TMC1*:c.100C>T;p.(Arg34*). A few others were present in only two to three patients from different countries (*SI Appendix*, Table S3).

Finally, mutations in syndromic deafness genes were found in 18.5% (77/415) of TJAMM cohort patients. Most (83%; 64/77) involved *USH* genes; 11 patients carried a monoallelic mutation in Waardenburg syndrome genes [*EDNRB* (1 patient), *MITF* (4 patients), *PAX3* (3 patients), and *SOX10* (3 patients)]. Two had rare syndromes: PHARC (*ABHD12*) and Stickler syndrome 4 (*COL9A1*) (*SI Appendix*, Tables S2 and S3).

High Genetic Heterogeneity of HI in the TJAMM Cohort. The distribution of the 211 different causal variants of 49 different deafness genes identified in the various countries of the TJAMM cohort is reported in *SI Appendix,* Table S6. The mutations found in this cohort increase the number of genes previously known to cause HI in these countries by about 2% in Jordan, 28% in Tunisia, 41% in Algeria, 50% in Morocco, and 200% in Mauritania (8, 18–31). The number of different individual mutations detected in each deafness gene is shown in *SI Appendix*, Fig. S5. Importantly, mutations of 10 of the 157 genes tested (Fig. 2) accounted for 81.1% of all deafness cases (41% in *GJB2*, 7.3% in *MYO15A*, 6.6% in *MYO7A*, 6.6% in *LRTOMT*, 5.1% in *SLC26A4*, 4.3% in *OTOF*, 3.6% in *CDH23*, 3.3% in *TMC1*, 2% in *PCDH15,* and 1.3% in *TRIOBP*). Mutations of these genes accounted for more than two thirds of the deafness cases in each country, with the contribution of individual genes varying substantially between countries (Figs. 1 and 2). Unsurprisingly, gene contributions were more similar for neighboring countries (e.g., Algeria and Tunisia or Tunisia/Algeria and Morocco) than for countries further apart [e.g., Mauritania and Jordan, this difference being partly accounted for by *LRTOMT* mutations, highly prevalent in Mauritania (35%) but absent from Jordan, and *SLC26A4* mutations, highly frequent in Jordan (20%)] (Fig. 1 and *SI Appendix,* Fig. S6).

Impact of Parental Consanguinity on the Molecular Diagnosis of HI. In the countries of origin of the TJAMM cohort patients, consanguinity rates are high, ranging from 28% in Morocco to 63% in Jordan (7). Self-reported parental consanguinity in the families with DFNB and ARSD was higher (372/436; 85%; from 80% in Moroccan patients to 98% in Jordanian patients). Homozygosity for mutations in causal deafness genes was observed in 92% (356/388) of patients (69% in Mauritanian patients, 83% in Moroccan patients, 89% in Jordanian patients, 96% in Tunisian patients, and 97% in Algerian patients). Several other manifestations of homozygosity were observed. Twelve patients with no known family ties between their parents turned out to be homozygous for causal mutations that were not recurrent. Two patients were homozygous for mutations in two different HI genes: one was homozygous for the *MYO15A*:c.6094C>T;p.(Arg2032*) and *CDH23*:c.5734C>T;p.(Arg1912Trp) mutations and the other for both a large *PCDH15*:c.92-?_594+? deletion and *OTOF*:c.2516C>T;p.(Ala839Val). Finally, we identified a patient with isolated deafness who was homozygous for a previously unknown nonsense (p.(Gln325*)) mutation in *POU4F3*. The *POU4F3* mutations described to date, including a nonsense mutation adjacent to the mutation described here (p.(Arg326*)), were all reported in patients with DFNA deafness (DFNA15) displaying mild-to-moderate HI. The affected homozygous child identified here was profoundly deaf, whereas the parents, who were heterozygous first cousins, had only mild-to-moderate HI. As a further illustration of the importance of analyzing all known HI genes in patients to prevent partial molecular diagnoses, which would impair genetic counseling, one patient compound heterozygous for two *GJB2* mutations (c.109G>A;p.(Val37Ile) and c.358_360del;p.(Glu120del) had an additional monoallelic *MYO7A* mutation [c.1121C>A;p.(Thr374Asn)].

Distinctive Features of Mutations in USH1/DFNB Genes Causing DFNB and USH1, Respectively in the TJAMM Cohort. There are three clinical subtypes of USH syndrome: USH1 to USH3. USH1 is the most severe, characterized by congenital/prelingual severe-to-profound deafness, balance defects due to vestibular impairment and early-onset retinitis pigmentosa (usually before puberty); USH1 and USH2 are the most frequent forms. USH3 is extremely rare (32). Mutations in all *USH1* genes except *USH1G* also cause DFNB forms (*USH1/DFNB* genes) (*SI Appendix,* Fig. S7).

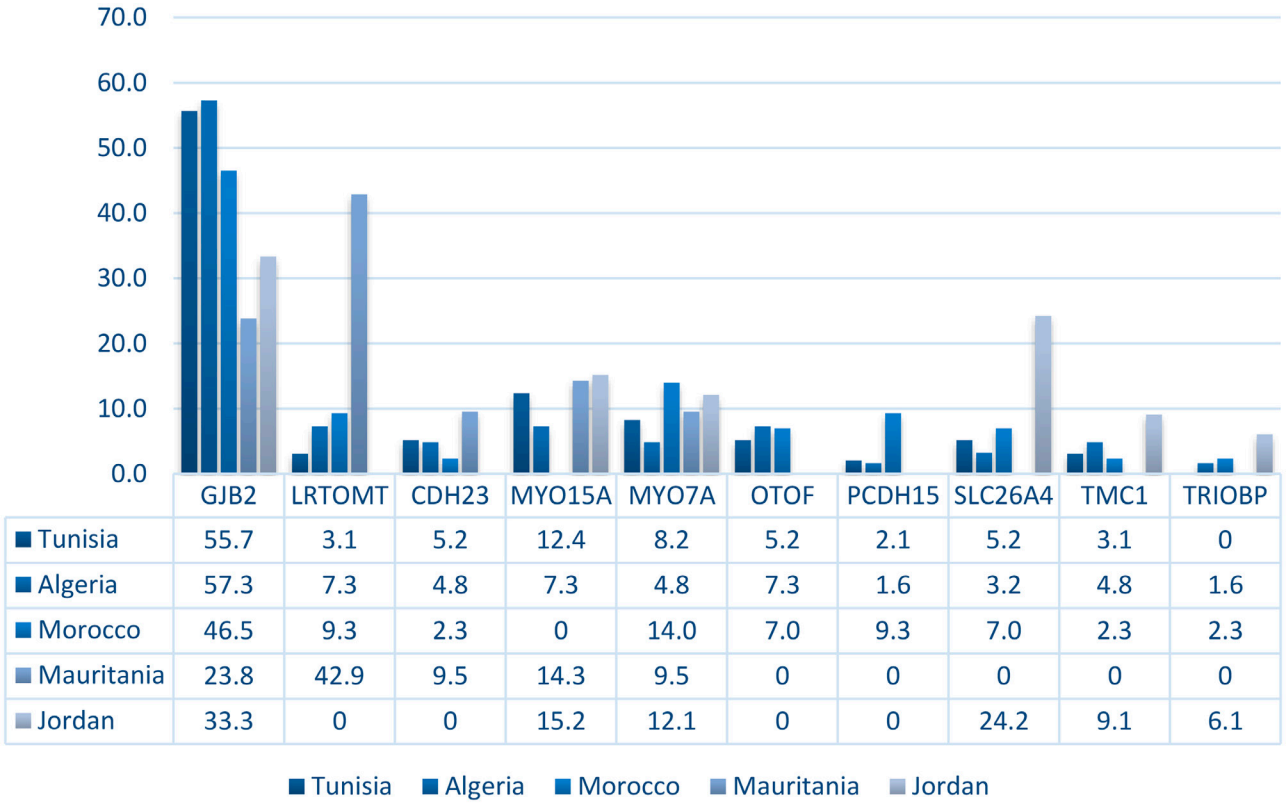

Fig. 2. Contributions of the 10 most prevalent deafness genes of the TJAMM cohort to congenital/prelingual HI cases in the different countries. The contribution of each of the 10 most common deafness genes in the TJAMM cohort in each country is expressed as a percentage (*y*-axis).

Of note, vestibular defects are only rarely reported in these DFNB forms. Only one of the three *USH2* genes, *WHRN*, also causes a DFNB form (DFNB31). One of the major issues in the molecular diagnosis of deafness is determining whether the mutations detected in *USH1/DFNB* genes will cause USH1 syndrome or DFNB.

Most *USH1/DFNB* mutations are reported in nonconsanguineous populations in which the patients are compound heterozygous. It is therefore almost impossible to correlate the various symptoms of the disease to the mutations. Homozygous mutations offer a unique opportunity to resolve this issue. In the TJAMM cohort, after ophthalmological examinations, 27 homozygous mutations were found to cause USH1 and eight DFNB. Sixteen of the 27 *USH1*-causing mutations were carried by *MYO7A*, six by *CDH23*, three by *PCDH15* and two by *USH1C*. Only one of the eight *DFNB*-causing mutations was carried by *MYO7A*, the other seven being carried by *CDH23* [four mutations including two new ones, p.(Asn772Ser) and p.(Ile1265Thr)], *PCDH15* (two mutations) and *USH1C* [p.(Phe237Leu), a new mutation] (*SI Appendix*, Table S7). For two of them, *CDH23*:p.(Asn772Ser) located in the canonical linker region between EC7 and EC8 and *USH1C*:p.(Phe237Leu) located in the PDZ2 domain, as mentioned above, modifications of the structure, the loss of a direct Ca^{2+} interaction to asparagine and introduction of steric clashes (in the most probable rotamer), respectively, were predicted (*SI Appendix*, Fig. S8 and Table S5).

We then investigated the nature of the mutations causing USH1 and DFNB. We found that 78% of the mutations causing USH1 (21/27) were LOF variants (12 *MYO7A*, 4 *CDH23*, 3 *PCDH15,* and 2 *USH1C* mutations), whereas the other six (6/27) were missense mutations (4 *MYO7A* and 2 *CDH23* mutations). Conversely, seven of the eight mutations causing DFNB were missense (1 *MYO7A*, 4 *CDH23*, 1 *PCDH15*, 1 *USH1C*); the single LOF mutation causing DFNB, *PCDH15*:(p.(Pro1515Thrfs*4)), was an atypical LOF mutation detected in two unrelated individuals from Tunisia and Morocco. This frameshift duplication mutation that we previously observed in two Algerian patients, affects only one of the three protocadherin-15 isoforms, CD2, which controls the gating of the auditory mechanotransduction channel (33).

Extended Analysis of the Distinctive Features of USH1/DFNB Mutations. We extended our investigations to other *USH1/DFNB* mutations reported in the homozygous state—304 *USH1/DFNB* mutations, including the 12 homozygous mutations reported here (*SI Appendix,* Table S8)—228 of which caused USH1, the others underlying DFNB (Table 1 and *SI Appendix,* Fig. S9 and Table S8).

The 304 homozygous mutations comprised 158 LOF mutations (52%), 136 missense mutations (45%), and 10 mutations that could not be classified (3.3%). These unclassified mutations included the aforementioned atypical LOF mutation *PCDH15* (p.(Pro1515Thrfs*4)) and five in-frame deletions of a single amino acid: three in *MYO7A*, one in *CDH23,* and another in *PCDH15* (*SI Appendix,* Table S8). The remaining four mutations affected noncanonical splice-sites: two in *MYO7A*, one in *CDH23,* and one in *USH1C* (*SI Appendix,* Table S9).

All but one of the LOF mutations led to USH1 (157/158) in the homozygous state. The single LOF mutation identified as underlying DFNB is a frameshift insertion (p.(Cys652Glyfs*11)) in the motor domain of myosin VIIa (34). These LOF mutations causing USH1 were distributed as follows between *USH1/DFNB* genes: 47% (74/157) were in *MYO7A*, 25.5% (40/157) in *CDH23*, 19% (30/157) in *PCDH15* and 8.3% (13/157) in *USH1C* (Table 1). Among the 136 *USH1/DFNB* homozygous missense mutations, 71 led to USH1 (52%) and 65 to DFNB (48%). The missense mutations causing USH1 were distributed as follows: 73% (52/71) were in *MYO7A* and 24% (17/71) in *CDH23*, with a single missense mutation in *PCDH15* and *USH1C*. By contrast, the homozygous missense mutations causing DFNB were distributed as follows: 66% (43/65) were in *CDH23*, 20% (13/65) in *MYO7A*, 10.7% (7/65) in *PCDH15* and 3% (2/65) in *USH1C* (Table 1). These results extended and confirmed the conclusions obtained in the TJAMM cohort.

Table 1. Number of independent homozygous genotypes leading to USH1 or to non-syndromic hearing loss (DFNB) phenotype

Gene	Homozygous allele	
Phenotype	LOF	Missense
MYO7A		
USH1B	74	52
DFNB2	1	13
CDH23		
USH1D	40	17
DFNB12	0	43
PCDH15		
USH1F	30	1
DFNB23	0	7
USH1C		
USH1C	13	1
DFNB18A	0	2
Proportion USH1	0.994	0.522

Distribution of 304 homozygous mutations (LOF and missense) between USH1 and DFNB phenotypes for each USH1/DFNB gene, MYO7A, CDH23, PCDH15, and USH1C. *MYO7A* defects cause USH1B and DFNB2; *CDH23* defects cause USH1D and DFNB12; *PCDH15* defects cause USH1F and DFNB23; *USH1C* defects cause USH1C and DFNB18B. LOF: loss-of-function.

In the clinical context, the information available is the identification of the causal gene and mutation(s). The critical question is whether a given missense mutation causes USH1 or DFNB. The distribution of homozygous missense mutations between USH1 and DFNB forms differs considerably between *USH1/DFNB* genes. Together missense mutations in *MYO7A* and *CDH23* accounted for 92% (125/136) of *USH1/DFNB* homozygous missense mutations. Among the homozygous missense mutations of *MYO7A,* 80% (52/65) cause USH1 syndrome. Conversely, 71.7% (43/60) of homozygous missense mutations in *CDH23*, are responsible for DFNB forms (*SI Appendix,* Fig. S7*)*. Seven of the eight *PCDH15* homozygous missense mutations and two of the three *USH1C* homozygous missense mutations were responsible for DFNB forms (*SI Appendix,* Fig. S7).

Revisiting the Classification of USH1/DFNB Gene Mutations. Biochemical and biophysical studies currently encounter difficulties when trying to correlate the changes in protein structure and stability caused by missense mutations of *USH1/DFNB* genes and the resulting phenotype, USH1 or DFNB. Such studies have characterized certain structural changes in great detail (35–37) but have been unable to predict whether a missense mutation will cause USH1 or DFNB. We hypothesized that these difficulties might stem from an incomplete classification or misclassification of certain mutations. We addressed the possible gene dosage effect of some of them, a characteristic of some recessive hypomorphic mutations, by taking advantage of *USH1/DFNB* homozygous missense mutations. Nine of the 65 *USH1/DFNB* missense mutations causing DFNB in the homozygous state have also been

reported in association with a loss-of-function (LOF; nonsense, frameshift, splice-site) mutation on the other allele. Five—two in *MYO7A* and three in *CDH23*—caused DFNB in association with a LOF mutation (Table 2) whereas the other four—three in *MYO7A* (p.(Ser617Pro), p.(Pro1243Leu), and p.(Pro1887Leu)) in the motor head, MyTH4 1 and MyTH4 2 domains, respectively, and one *CDH23* mutation (p.(Asp2148Asn)) affecting the extracellular cadherin 20 repeat (EC20) domain—resulted in USH1 (Table 2 and *Patients and Methods*), with documented retinal defects and vestibular dysfunctions (*Patients and Methods* and *SI Appendix*, Table S9). It follows that missense *USH1/DFNB* mutations causing DFNB in the homozygous state are of two types: genuine *DFNB*-causing mutations leading to DFNB regardless of the type of recessive mutation on the other allele (*DFNB* mutations) and mutations leading to USH1 when associated with a LOF mutation on the other allele (*USH1* hypomorphic-like mutations). Thus, any *USH1/DFNB* missense mutation resulting in a DFNB phenotype when associated with a LOF mutation can be directly classified as a *DFNB* mutation. By contrast, *USH1* hypomorphic-like mutations are not the only *USH1/DFNB* mutations leading to USH1 phenotype when associated with a LOF mutation: Any missense *USH1/DFNB* mutation causing USH1 in the homozygous state will also result in USH1 when associated with a LOF mutation. The phenotype of patients (DFNB or USH1) homozygous for these mutations must therefore be documented to distinguish between *USH1* hypomorphic-like and *USH1* mutations. We used these criteria to search in the Deafness Variation Database (DVD) and Leiden Open Variation Database (LOVD) for additional *DFNB* and *USH1* hypomorphic-like mutations. Based exclusively on their association with a LOF mutation, we identified 11 additional *DFNB* mutations: five in *MYO7A,* three in *CDH23*, and three in *PCDH15* (*SI Appendix*, Table S10). We could not extend the list of *USH1* hypomorphic-like mutations due to a lack of reported homozygous patients.

We searched for additional evidence supporting the distinction between *DFNB* and *USH1* hypomorphic-like mutations. *DFNB* mutations should yield a DFNB phenotype when associated with another recessive missense mutation, whether *DFNB*, *USH1* hypomorphic-like, or *USH1* mutation. Association with at least one other missense mutation (20 mutations in total) has been reported for seven of the 16 *DFNB* mutations mentioned above (Table 2 and *SI Appendix,* Table S10). As expected, all compound heterozygous patients had DFNB phenotype. Of

Table 2. Homozygous mutations in *MYO7A* and *CDH23* causing DFNB fall into two categories, *DFNB* and *USH1* hypomorphic-like mutations depending on the resulting phenotype when associated with a LOF mutation in heterozygous patients

	Gene	Homozygote		Compound het with a LOF			Compound het with a missense		
DFNB	MYO7A	p.G1159S	DFNB2	p.G1159S	c.849+1G>A	DFNB2	p.G1159S	p.R212H[†]	DFNB2
					p.Y477[*]	DFNB2		p.R1873W[†]	DFNB2
					p.C705[*]	DFNB2			
					p.W2107[*]	DFNB2			
		p.Y1780S	DFNB2	p.Y1780S	p.P2126Lfs*5	DFNB2	p.Y1780S	p.G329D	DFNB2
	CDH23	p.P240L	DFNB12	p.P240L	c.6712+1G>A	DFNB12	p.P240L	p.R301Q	DFNB12
					c.7055-1G>C	DFNB12		p.E956K	DFNB12
					p.D2858Efs*8	DFNB12		p.R1588W	DFNB12
								p.T1618K	DFNB12
								p.Q1716P	DFNB12
								p.N1845S	DFNB12
								p.N2287K	DFNB12
								p.T1368M	DFNB12
								p.N342S	DFNB12
								p.G539D	DFNB12
								p.E1595K	DFNB12
								p.R2029W[‡]	DFNB12
		p.D990N	DFNB12	p.D990N	c.6049+1G>A	DFNB12			
		p.F1888S	DFNB12	p.F1888S	p.D2962[*]	DFNB12			
USH1 hypomorphic-like	MYO7A	p.S617P	DFNB2	p.S617P	p.D1613Vfs*32	USH1B	p.S617P	p.G7V	DFNB2
		p.P1243L	DFNB2	p.P1243L	p.R669[*]	USH1B	p.P1243L	p.K527R	USH1B
								p.G163R	USH1B
								p.G1159V	USH1B
		p.P1887L	DFNB2	p.P1887L	p.Q1333Rfs*66	USH1B	p.P1887L	p.L1858P	USH1B
	CDH23	p.D2148N	DFNB12	p.D2148N	p.W2811[*]	USH1D	p.D2148N	p.R2608H	DFNB12

Identification of DFNB mutations: mutations leading to a DFNB phenotype 1) in the homozygous state and 2) when associated with a LOF mutation (compound heterozygous). Identification of USH1 hypomorphic-like mutations: mutations that result in DFNB in the homozygous state and USH1 when associated with a LOF mutation (compound heterozygous). On the left vertical column, missense mutations in the homozygous state are shown. On the central vertical column, the mutations of the left vertical column are each associated with one or several other LOF mutations in the same gene. On the right vertical column, mutations of the central vertical column are each associated with one or several other missense mutations in the same gene. MYO7A defects cause USH1B and DFNB2; CDH23 defects cause USH1D and DFNB12. Notes: 1-None of the MYO7A missense mutations included in this table have been reported to cause autosomal dominant DFNA11 (38). 2-The p.D2148N mutation has also been reported in association with p.R1746Q. However, homozygous patients for p.R1746Q showed phenotypic diversity with intra- and interfamilial heterogeneity including DFNB phenotype, USH1 phenotype, and even asymmetric hearing loss (39–41), making it impossible to predict the phenotype when the mutation is associated with a USH1 hypomorphic-like mutation.

[*]Translation termination (stop) codon.

[†]These mutations lead to USH1 when present in the homozygous state.

[‡]These mutations lead to DFNB when present in the homozygous state.

note, two of these patients carried a second missense mutation (p.Arg212His and p.Arg1873Trp) causing USH1 in the homozygous state (Table 2). Moreover, *USH1* hypomorphic-like mutations should result in DFNB when associated with a *DFNB* missense mutation and USH1 when associated with an USH1 missense mutation. As predicted, when associated with another missense mutation (six mutations in total), the four *USH1* hypomorphic-like mutations resulted in either DFNB or USH1 (Table 2 and *SI Appendix,* Table S9). The phenotype resulting from the association of an *USH1* hypomorphic-like missense mutation with another *USH1* hypomorphic-like missense mutation depends on the activity of the putative dimers (all proteins encoded by *USH1/DFNB* genes are dimeric and/or multimeric) formed by the proteins encoded by the two mutated alleles, which cannot currently be predicted.

We investigated the potential of protein modifications due to *USH1/DFNB* mutations for distinguishing between *DFNB* and *USH1* hypomorphic-like mutations. All 16 *DFNB* mutations analyzed had a REVEL score for pathogenicity >0.5 but two (Pro240Leu and Glu2438Lys) which however were predicted to be likely pathogenic or pathogenic by ClinVar. Twelve of the 16 *DFNB* mutations affected highly conserved residues, the other four affecting weakly to moderately conserved residues (Fig. 3 and *SI Appendix*, Fig. S10 and Table S11). The four *USH1* hypomorphic-like mutations had REVEL scores of 0.65 to 0.92 and were predicted to be likely pathogenic or pathogenic by ClinVar. All affected amino acid residues were highly conserved throughout evolution (*SI Appendix,* Fig. S10). The effect of *DFNB* and *USH1* hypomorphic-like mutations on the 3D structure of the corresponding proteins was investigated. The normal 3D

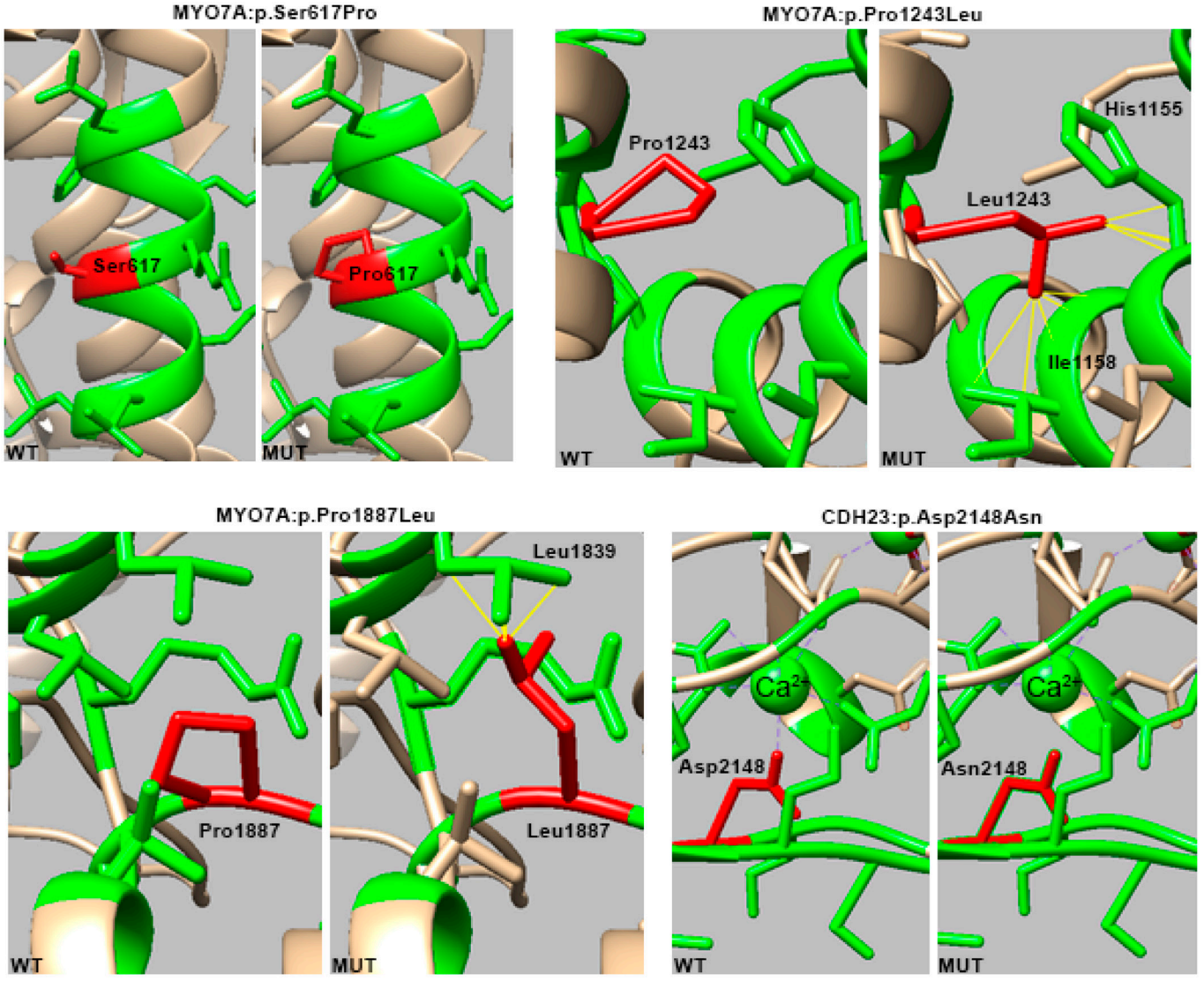

Fig. 3. Predicted structures of myosin VIIa and cadherin-23 carrying *USH1* hypomorphic-like mutations. 3D structure of wild-type (WT) and mutated (MUT) regions of myosin VIIa (MYO7A) and cadherin-23 (CDH23) carrying hypomorphic like mutations. (*Top Left*) *MYO7A*:p.(Ser617Pro); (*Top Right*) *MYO7A*:p.(Pro1243Leu); (*Bottom Left*) *MYO7A*:p.(Pro1887Leu); (*Bottom Right*) *CDH23*:p.(Asp2148Asn). In red, the amino acid affected by the mutation. For MYO7A, the most probable rotamer is represented for each modified amino acid. *MYO7A*:p.(Ser617Pro): the serine residue of p.(Ser617Pro) is located in an α-helix. The replacement of this serine residue by a proline residue is predicted to disrupt the α-helix (42). *MYO7A*:p.(Pro1243Leu) and *MYO7A*:p.(Pro1887Leu) based on the most probable rotamer, 90% for Pro1243Leu and 57% for Pro1887Leu: predicted structure of the human myosin VIIa (AlphaFold2: AF-Q13402-F1). Both p.(Pro1243Leu) and p.(Pro1887Leu) lead to the replacement of a proline by a leucine residue. The leucine residue of p.(Pro1243Leu) is predicted to interact with histidine 1155 and isoleucine 1158. The leucine residue of p.(Pro1887Leu) is predicted to interact with leucine 1839. These two modifications are predicted to cause steric clashes, leading to protein misfolding. *CDH23*:p.(Asp2148Asn): crystal structure of the normal human cadherin-23 EC19-20 domains (CDH23, 2.69 Å resolution, PDB: 5I8D). The aspartate residue in position 2148 located in the XDX motif of the EC20 domain interacts directly with the Ca^{2+} ion at site 3. Predicted crystal structure of the mutated form: the interaction of the asparagine residue with Ca^{2+} is lost. Adapted from Jaiganesh et al. (36).

structures of the regions carrying these mutations have been established by X-ray crystallography (12/20) or predicted by AlphaFold2 (7/20) (for local confidence of AlphaFold2, see *SI Appendix, Methods*). For the *USH1* hypomorphic-like mutations of *MYO7A*, the amino acid substitutions due to Pro1243Leu and Pro1887Leu were predicted to cause steric clashes and Ser617Pro was predicted to disrupt an α-helix (42) (Fig. 3). The Asp2148Asn, an *USH1* hypomorphic-like mutation in *CDH23* [Asp2125Asn in reference (36)] located in the XDX motif of EC20, causes the loss of interaction between aspartate and Ca^{2+}, reducing the affinity of the region for Ca^{2+}, which probably affects the mechanical rigidity of the region and, consequently, the transfer of sound forces to the mechanoelectrical transduction channel (Fig. 3 and *SI Appendix,* Table S11). By contrast, no conformational change, despite differences in the physicochemical properties of the amino acids concerned, was predicted for the proteins resulting from the 16 *DFNB* mutations (Gly158Glu, Glu314Lys, Glu513Gln, Gly1159Ser, Ala1224Asp, Tyr1780Ser, Trp2077Arg in *MYO7A*, Pro240Leu, Asp990Asn, Asp1626Ala, Phe1888Ser, Glu2438Lys, Pro2720His in *CDH23* and Cys273Tyr, Asp852Asn, Thr1378Ile in *PCDH15*) (*SI Appendix*, Table S11).

Discussion

In recent decades, several laboratories have used targeted genomic enrichment panels and massively parallel sequencing strategies to improve the molecular diagnosis of HI in newborns and children. This approach has identified causal mutations in 39 to 67% of patients in various populations (43–48). In the TJAMM cohort, full resolution of the genomic basis of HI was achieved in almost 90% of cases. The inclusion criteria for this cohort largely accounted for this high frequency: Only familial cases with bilateral severe-to-profound HI were included. Most cases of cytomegalovirus (CMV) infection, which is frequently associated with unilateral HI (49), were thereby excluded, together with most dominant forms of isolated deafness, DFNA, which are often moderate to moderately severe. The most relevant comparison between our results and those of other studies is with the results reported by Abu Rayyan et al. (8) for the familial deafness cases in their Palestinian cohort. In this subcohort, as in the TJAMM cohort, about 90% of the causal mutations were present in the homozygous state. The full genomic resolution rates for DFNB/ARSD patients were also similar: 87% in the Palestinian cohort and 93.7% in the TJAMM cohort. Only 3% of the TJAMM cohort patients with DFNB or ARSD had a partly solved genotype, i.e., a monoallelic mutation in a deafness gene. Given that the 11 genes involved (*MYO3A* being an exception) have been reported to underlie only DFNB forms and only six of the TJAMM cohort patients had mutations of two different deafness genes, the missing mutations are probably carried by the other allele. In the absence of detected deletions/duplications/inversions in this allele in patients and their relatives (eight relatives analyzed), the missing mutations probably lie in genomic regions not captured by targeted exome sequencing, such as the promoter and intronic regions (away from splice junctions), or distant regulatory regions (50). The genotypes of another 3% of patients with DFNB/ARSD remained completely unsolved, probably for similar reasons or due to the involvement of as yet unknown deafness genes.

The 10 genes most frequently implicated in deafness were similar from Mauritania to Jordan. However, their respective contributions to deafness varied between countries. Additional studies are required to improve the representativity of the data in each country, including, in particular, patients living in the regions underrepresented here. The same set of genes has also been implicated in Palestine (8) and Israel (51). By contrast, the spectrum of causal mutations differed considerably between the countries of the TJAMM cohort, with the exception of a few recurrent, probably ancestral mutations of *GJB2* and *LRTOMT*. The others are either private mutations or founder mutations at the regional scale (52). These findings are consistent with the general observation that very few recurrent mutations are detected for numerous inherited diseases in North Africa (52).

USH1 syndrome, which has a much more dramatic impact on the lives of sufferers than isolated prelingual deafness, was overrepresented in the TJAMM cohort (13% of cases). Being able to distinguish *USH1/DFNB* missense mutations causing USH1 from those causing DFNB is therefore crucial for an efficient molecular diagnosis of deafness. Studies of individuals homozygous for *USH1/DFNB* mutations provide a unique opportunity to establish direct phenotype/genotype correlations. We observed—first in the TJAMM cohort and then for the 304 *USH1/DFNB* mutations reported in the homozygous state to date—that all but one of the homozygous LOF mutations led to USH1. The exception [p.(Cys652Glyfs*11); 1 of 158 mutations] in *MYO7A*, was probably due to the fact that the patient had undergone a single ophthalmological electroretinogram (ERG) recording and at the age of 7 y, whereas conventional ERG testing is known to be potentially inconclusive at this age (53). These results, consistent with the conclusions of several previous studies (38), indicate that attention should focus on *USH1/DFNB* missense mutations. We observed that gene distribution of *USH1/DFNB* homozygous missense mutations responsible for USH1 and DFNB were highly contrasted, with 80% of missense mutations in *MYO7A* causing USH1 and 72% of missense mutations in *CDH23*, DFNB. Despite active research leading to in-depth characterizations of some recessive missense *USH1/DFNB* mutations (35), attempts to correlate phenotypes with genotypes, particularly for *MYO7A* and *CDH23* mutations (35, 38), have been only partially productive, raising the possibility of a misclassification or incomplete classification of *USH1*- and *DFNB*- causing missense mutations. By taking advantage of homozygosity, we identified a group of mutations, *USH1* hypomorphic-like mutations, providing information directly useful for the analysis of *USH1/DFNB* mutations. These mutations were identified as leading to DFNB in the homozygous state and as resulting in USH1 when associated with a LOF mutation in the other allele. This pattern is not uncommon for recessive hypomorphic mutations (54, 55) and is often reported in engineered mouse models for recessive human diseases, including syndromes (56). They are also referred to as mutations with a gene dosage effect (56). This led us to redefine *DFNB* mutations as mutations causing DFNB when associated with a LOF mutation. In support of the identification of *USH1* hypomorphic-like mutations, heterozygous patients for an *USH1* hypomorphic-like mutation and another recessive missense mutation were affected by either DFNB or USH1, whereas all patients heterozygous for a *DFNB* mutation, regardless of the type of missense mutation with which it was associated, had a DFNB phenotype. Moreover, among the four *USH1* hypomorphic-like mutations, three in *MYO7A* were predicted to modify the 3D structure of the corresponding proteins and one in *CDH23* to destabilize the protein, whereas, by contrast, none of the 16 *DFNB* mutations—seven in *MYO7A*, six in *CDH23* and three in *PCDH15*—were predicted to cause changes in the protein 3D structure. Studies of additional *USH1* hypomorphic-like mutations and resolutions of corresponding 3D structures are needed to evaluate structural changes

in proteins as possible criteria for distinguishing *DFNB* and *USH1* hypomorphic-like mutations. It is interesting to note that although *CDH23* mutations altering Ca^{2+}-binding sites at the EC linker regions cause hearing defects but no vestibular symptoms [probably due to the high Ca^{2+} concentration in the vestibular endolymph compared to cochlear endolymph (36)], the present analysis of the p.Asp2148Asn mutation associated with a LOF mutation on the other allele, suggests that they may nevertheless in such a context lead to vestibular dysfunction.

Recessive hypomorphic mutations affecting hearing have rarely been reported (57). In this clinical context, USH1 hypomorphism mainly refers to the effect of the mutations on the retinal phenotype. It should be noted that recessive hypomorphic mutations are particularly frequent in retinal disorders (58). Their prevalence in patients testing positive for biallelic mutations underlying *retinitis pigmentosa* has been estimated at about 18% (55). The prevalence of *USH1* hypomorphic-like mutations cannot be evaluated from the small number of mutations identified here. However, given that only five of the 13 missense mutations of *MYO7A* reported to cause DFNB in the homozygous state have also been reported in association with a LOF mutation, with two causing DFNB and the other three USH1 (Tables 1 and 2 and *SI Appendix,* Table S9), they may not be rare, especially among *MYO7A* missense mutations.

All USH1 proteins are structural proteins. The greater vulnerability of auditory hair cells than of photoreceptor cells to *USH1* hypomorphic-like mutations, which affect the 3D structures of myosin VIIa and cadherin-23, suggests that structural constraints on the functions of myosin VIIa and cadherin-23 are weaker in photoreceptors than in hair cells or differ between the two types of sensory cells. They also suggest that doubling the concentration of the modified protein (homozygous patients) might restore the tension forces generated by myosin VIIa and the resistance of cadherin-23 to tension forces to levels compatible with the performance of their functions in photoreceptor cells but not in auditory hair cells. Alternatively, the two types of sensory cells may differ in more general biological processes affecting these modified proteins, such as protein trafficking or stress responses. The characterization of hypomorphic mutations often also includes the study of animal models and in vitro assays. In the particular context of USH1 syndrome, explorations of the retinal phenotype caused by these mutations in mouse models are hampered by the absence of retinal degeneration in *Ush1* mouse mutants (59). As for other possible tests, their development requires a better understanding of the role of USH1 proteins in photoreceptor cells.

The crucial roles of USH1 proteins in the development and function of the stereocilia hair bundle—the sensory antenna of the auditory sensory cells, the hair cells—are well established, given that all are components of the auditory mechanoelectrical transduction machinery responsible for converting mechanical sound stimuli into electrical signals. Cadherin-23 and protocadherin-15 form the tip link and myosin VIIa and USH1C (harmonin) anchor the apical part of the tip link to the actin filaments of the stereocilia (*SI Appendix,* Fig. S11). The finding, in macaque photoreceptors, that all USH1 proteins are associated with densely packed cross-linked F-actin microvillus-like structures—the calyceal processes ensheathing the base of rod and cone outer segments (60, 61), which resemble the stereocilia forming the cochlear hair bundle—suggest that the activities of USH1 proteins in the two types of sensory cells may be related (*SI Appendix,* Fig. S11). Studies of mouse *Myo7A* mutants have suggested that this motor protein is involved in the translocation of proteins from the inner segment to the outer segment of the photoreceptors (62). A role for USH1 proteins in primate cone phototropism, in which the outer segments rapidly align with the oblique direction of incoming light, has recently been proposed (63). Finally, evidence has been obtained for a role of cadherin-23 and protocadherin-15 in the formation of rod disks and cone lamellae in *Xenopus tropicalis* (64) and a role of protocadherin-15b in photoreceptor disk growth in zebrafish (65), raising the attractive possibility that USH1 proteins are also involved in mechanosensing processes in photoreceptor cells.

We define here a subgroup of mutations of *USH1/DFNB* genes, *USH1* hypomorphic-like mutations, extending the spectrum of conditions associated with defects of *USH1/DFNB* genes. In parallel, we have redefined missense *DFNB*-causing mutations such that *DFNB* mutations result exclusively in a DFNB phenotype, regardless of the associated recessive mutation. These findings should help to extend the characterization of *USH1/DFNB* mutations to patients compound heterozygous for two missense mutations, by providing a framework for their analysis. In addition, the reclassification of certain mutations previously considered to be *DFNB* mutations as *USH1* hypomorphic-like mutations should contribute significantly to the search for molecular criteria for predicting the presence/absence of retinal abnormalities. In clinical management, hypomorphic mutations underlying other diseases have been reported to cause mild, late-onset phenotypes. The detection of *USH1* hypomorphic-like mutations in patients should therefore lead to thorough ophthalmological examinations throughout the patients' lives.

Altogether, this study highlights the critical added value of collecting detailed clinical and genetic information from various human populations around the world.

Materials and Methods

Patients and Methods. This study was approved by the local Ethics Committees (Biomedical Ethics Committee of the Pasteur Institute of Tunis in Tunisia, Rabat Biomedical Research Ethics Committee in Morocco, Ethics Committee of the University of Nouakchott in Mauritania, Ethics committee of the Bab El-Oued University Hospital in accordance with "Health Law No. 85-05 of February 16, 1985" applicable in Algeria, Ethics committee of St. Joseph university in Lebanon) and was performed in accordance with the ethical principles for medical research involving human subjects defined by the World Medical Association and laid down in the Declaration of Helsinki. Written informed consent was obtained from all patients or from the legal representatives of minors.

Patients. In total, 450 unrelated patients–each patient declaring having no known relationship to any of the others–were recruited between 2011 and 2016 at two centers in Algeria (Algiers and Blida; 155 patients), one center in Tunisia (Tunis; 135 patients), one center in Morocco (Casablanca; 83 patients), one center in Jordan (Amman; 47 patients), and one center in Mauritania (Nouakchott; 30 patients) (*SI Appendix,* Fig. S1). Most of the population in Algeria, Morocco, and Tunisia, and about one third of the population of Mauritania is of Arab-Berber origin. By contrast, about 95% of Jordanians are Arabs. These countries border the Mediterranean Sea and the Atlantic Ocean (*SI Appendix,* Fig. S1). For inclusion, the proband and at least one of his/her relatives had to have been diagnosed with congenital/prelingual, severe-to-profound bilateral HI in the absence of "environmental" factors for hearing loss, such as infections and ototoxic drugs. Audiological evaluation routinely included tympanometry, pure-tone audiometry, the recording of otoacoustic emissions and auditory evoked potentials, and computerized tomography or MRI of the inner ear if possible. Ocular fundus examination, electroretinogram (ERG), and cardiac and renal ultrasound scans were performed where possible. In the vast majority of cases, no other symptoms (visual, cardiac, or renal) indicative of a syndromic form were detected. The

patients were classified according to the most likely mode of HI inheritance based on pedigree analysis: DFNB, DFNA, or DFNX. Blood samples were obtained from all patients and, whenever possible, from their parents and siblings. Genomic DNA was extracted by standard procedures.

Patients carrying mutations in *USH1/DFNB* genes were analyzed in section *Revisiting the Classification of USH1/DFNB Gene Mutations*. The homozygous patients for the four mutations classified as USH1 hypomorphic-like mutations who displayed a DFNB phenotype, were all family cases; two of them belonging to large families with four and seven affected cases (34, 42). For the heterozygous patients carrying the *USH1* hypomorphic-like mutations associated with a LOF mutation who displayed an USH1 phenotype, information was only available for two cases who were family cases (66, 67).

A detailed description of the materials and methods (targeted exome sequencing, filtering strategy, and identification of mutations, qPCR analysis, protein 3D-structure modeling) is presented in *SI Appendix, Methods*.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. We thank the patients and their families for their participation in the study. We thank the *Association Marocaine Pour Enfants Sourds* from Morocco, Pr Yahia Rous from Université Saad Dahleb de Blida and the ENT department-CHU Blida, Pr Mokhtar Hasbelaoui from the CHU of Tizi Ouzou, Pr Djamel Selmane from CHU Bab Eloued, Pr Yahi Nadla from Hôpital Kouba, Algiers, and the school for deaf children of Béjaia, Tizi-Ouazou, Boumerdes, Ghardaia, Bouira, and Baraki from Algeria, Mr Mohamed Habib Allah Moussa, president of *Forum Mauritanien des Sourds* from Mauritania and the technical staff of the *Service de Biochimie et de Biologie Moléculaire* at Hôpital Armand Trousseau in Paris for their assistance. Financial support for this work was provided by the French national research agency under LabEx grant agreement LIFESENSES (ANR-10-LABX-65), (ANR-11-IDEX-0004-02), (ANR-11-BSV5-0011), (ANR-16-CE13-0015-02), RHU AUDINNOVE (ANR-18-RHUS-0007), the *Fondation pour l'Audition* (FPA-IDA05 to C.P. and FPA RD-2023-01 to S.B.), the LHW Stiftung foundation, the Raymonde & Guy Strittmatter and Mme Errera Hoechstetter Foundation, the Pasteur Institute of Morocco, the Tunisian Ministry of Public Health, the Ministry of Higher Education and Scientific Research (LR11IPT05), the Pasteur Institute of Tunisia and Mobidoc Fellowship under the *Programme d'appui au système de recherche et d'innovation* (PASRI-Europe Aid) (http://www.pasri.tn/). This work also benefited from a French government grant managed by the *Agence Nationale de la Recherche* under the France 2030 program, reference ANR-23-IAHU-0003.

Author affiliations: [a]Université Paris Cité, Institut Pasteur, Assistance Publique-Hôpitaux de Paris, INSERM, CNRS, Fondation Pour l'Audition, Institut de l'Audition, Institut Hospitalo-Universitaire reConnect, Auditory Therapies Innovation Laboratory, Paris F-75012, France; [b]Laboratory of Biomedical Genomics and Oncogenetics LR16IPT05, Pasteur Institute in Tunis, University of Tunis El Manar, Tunis 1002, Tunisia; [c]Department of ear, nose, throat, University Hospital Center in Angers, Angers 49933, France; [d]Univ Angers, MitoVasc, Equipe MitoLab, CNRS UMR6015, INSERM U1083, Angers 49100, France; [e]Laboratoire de Recherche Biochimie Génétique, Université des sciences de la santé, Algiers 16000, Algeria; [f]Central Laboratory of Clinical Biology, Frantz Fanon Hospital, University Hospital Center of Blida, Blida 9000, Algeria; [g]Faculty of Medicine, University Blida 1, Blida 9000, Algeria; [h]Service de Biochimie et Biologie Moléculaire, Hôpital d'Enfants Armand-Trousseau, Assistance Publique-Hôpitaux de Paris, Paris F-75012, France; [i]Brain Plasticity Unit, CNRS, École Supérieure de Physique et de Chimie Industrielles Paris, Paris Sciences et Lettres Research University, Sorbonne Université, Inserm, CNRS, Neuroscience Paris Seine-Institut de Biologie Paris Seine, Paris F-75005, France; [j]Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumédiène, Algiers 16000, Algeria; [k]Equipe de Génétique, Laboratoire de Biologie Cellulaire et Moléculaire, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumédiène, Bab-Ezzouar, Algiers 16000, Algeria; [l]Higher Institute of Biotechnology of Sidi-Thabet, University of Manouba, Ariana-Tunis, Tunis 2020, Tunisia; [m]Genomics and Human Genetics Laboratory, Institut Pasteur du Maroc, Casablanca 20360, Morocco; [n]Laboratory of Integrative Biology, Faculty of Sciences Ain Chock, Hassan II University, BP 2693, Maarif, Casablanca 20100, Morocco; [o]Department of Biology, Faculty of Sciences Ben M'Sick, Hassan II University, Casablanca 20670, Morocco; [p]Biomarker Research Unit for Mauritanian Populations, Nouakchott University, Nouakchott 2022, Mauritania; [q]Division of Otolaryngology, Department of Surgery, University of California, 9500 Gilman Drive, Mail Code 0666, La Jolla, San Diego, CA 92093; [r]Genomic Sequencing Laboratory, Institut Pasteur du Maroc, Casablanca 20360, Morocco; [s]Epidemiology Research Unit, Biostatistics and Bioinformatics, Institut Pasteur du Maroc, Casablanca 20360, Morocco; [t]Mohammed VI University of Sciences and Health (UM6SS), BP 82403 Casablanca, Morocco; [u]Mohammed VI Audition Center, Cheikh Khalifa International University Hospital, BP 82403 Casablanca, Morocco; [v]Mohammed VI University Hospital, Bouskoura 27182, Morocco; [w]Department of Otorhinolaryngology, District Hospital of Menzel Bourguiba, Bizerte 7050, Tunisia; [x]Department of Otorhinolaryngology and Maxilofacial Surgery, La Rabta Hospital in Tunis, Tunis 1007, Tunisia; [y]Department of Congenital and Hereditary Diseases, Charles Nicolle Hospital in Tunis, Tunis 1002, Tunisia; [z]LR99ES10 Laboratory of Human Genetics, Faculty of Medicine in Tunis, University of Tunis El Manar, Tunis 1006, Tunisia; [aa]Child and Adolescent Neurology Department of Neurology, National Institute of Neurology, Tunis 1007, Tunisia; [bb]LR18SP04 Department of Child Neurology, National Institute Mongi Ben Hmida of Neurology in Tunis. University of Tunis El Manar, Tunis 1007, Tunisia; [cc]Otorhinolaryngology Department, Etablissement Public Hospitalier Bachir Mentouri, Algiers 16000, Algeria; [dd]Otorhinolaryngology Department, Mustapha Pacha Hospital, Algiers 16000, Algeria; [ee]Otorhinolaryngology Department, Tizi Ouzou University Hospital Center, Algiers 16000, Algeriaa; [ff]Department of Human Genetics, Gilbert and Rose-Mary Chagoury School of Medicine, Lebanese American University, P.O. Box 36 Byblos, Lebanon; [gg]Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, United Kingdom; and [hh]Collège de France, Paris F-75005, France

Author contributions: S. Abdelhak, C.P., and C.B. designed research; Z.R., S.B., S. Abdi, A.H., A. Barakat, F.A.K., M.M., A.Z., N.B., C.P., and C.B. performed research; Z.R., S.B., S. Abdi., F.W., A.S.-E., A.A., M.N.-G., A. Behlouli, M. Dahmani, S.T., Y.B., R. Mkaouar, C.C., G.A., A. Bakhchane, A. Bousfiha, S.S., S.E., M.S., M.H., E.B., H. Charoute, M. Detsouli, K.S., H.R., H.E.H., F.V., G.M., E.C., M.M., C.P., and C.B. analyzed data; J.-P.H. and S. Abdelhak advised on paper framing and design; H.C., M.D., K.S., H.R., H.E.H., F.V., G.M., J.M., R.Z., H. Chahed, G.B., M.T., R. Mrad, I.K., S.O., D.D., F.B., and N.B performed clinical and audiological tests; and C.P., and C.B. wrote the paper.

Reviewers: K.B.A., Tel Aviv University; and M.-C.K., University of Washington.

The authors declare no competing interest.

1. A. M. L. Bussé *et al.*, Prevalence of permanent neonatal hearing impairment: Systematic review and Bayesian meta-analysis. *Int. J. Audiol.* **59**, 475–485 (2020).
2. A. E. Shearer, M. S. Hildebrand, A. M. Odell, R. J. Smith, "Genetic hearing loss overview" in *GeneReviews®*, M. P. Adam *et al.*, Eds. (University of Washington, Seattle, 1993).
3. E. Verpy *et al.*, Mutations in a new gene encoding a protein of the hair bundle cause non-syndromic deafness at the DFNB16 locus. *Nat. Genet.* **29**, 345–349 (2001).
4. T. Walsh *et al.*, From flies' eyes to our ears: Mutations in a human class III myosin cause progressive nonsyndromic hearing loss DFNB30. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 7518–7523 (2002).
5. L. J. Francey *et al.*, Genome-wide SNP genotyping identifies the Stereocilin (STRC) gene as a major contributor to pediatric bilateral sensorineural hearing impairment. *Am. J. Med. Genet. A.* **158A**, 298–308 (2012).
6. A. Bittles, Consanguinity and its relevance to clinical genetics. *Clin. Genet.* **60**, 89–98 (2001).
7. G. O. Tadmouri *et al.*, Consanguinity and reproductive health among Arabs. *Reprod. Health* **6**, 17 (2009).
8. A. Abu Rayyan *et al.*, Genomic analysis of inherited hearing loss in the Palestinian population. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 20070–20076 (2020).
9. R. K. Koenekoop, M. A. Arriaga, K. M. Trzupek, J. J. Lentz, "Usher Syndrome Type I" in *GeneReviews®*, M. P. Adam *et al.*, Eds. (University of Washington, Seattle, 1993).
10. J. R. Giudicessi, M. J. Ackerman, Prevalence and potential genetic determinants of sensorineural deafness in *KCNQ1* homozygosity and compound heterozygosity. *Circ. Cardiovasc. Genet.* **6**, 193–200 (2013).
11. Y. Yang *et al.*, Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N. Engl. J. Med.* **369**, 1502–1511 (2013).
12. E. L. van Dijk, H. Auger, Y. Jaszczyszyn, C. Thermes, Ten years of next-generation sequencing technology. *Trends Genet.* **30**, 418–426 (2014).
13. W. R. McCombie, J. D. McPherson, E. R. Mardis, Next-generation sequencing technologies. *Cold Spring Harb. Perspect. Med.* **9**, a036798 (2019).
14. I. Chakchouk *et al.*, NADf chip, a two-color microarray for simultaneous screening of multigene mutations associated with hearing impairment in North African Mediterranean countries. *J. Mol. Diagn.* **17**, 155–161 (2015).
15. E. M. Richard *et al.*, Global genetic insight contributed by consanguineous Pakistani families segregating hearing loss. *Hum. Mutat.* **40**, 53–72 (2019).
16. Y. Hamdi *et al.*, Genome Tunisia Project: Paving the way for precision medicine in North Africa. *Genome Med.* **16**, 104 (2024).
17. A. Wonkam *et al.*, No evidence for clinical utility in investigating the connexin genes *GJB2*, *GJB6* and *GJA1* in non-syndromic hearing loss in black Africans. *S. Afr. Med. J.* **105**, 23–26 (2015).
18. M. Charif *et al.*, The c.242G>A mutation in *LRTOMT* gene is responsible for a high prevalence of deafness in the Moroccan population. *Mol. Biol. Rep.* **39**, 11011–11016 (2012).
19. M. Salame *et al.*, Identification a novel pathogenic LRTOMT mutation in Mauritanian families with nonsyndromic deafness. *Eur. Arch. Otorhinolaryngol.* **280**, 4057–4063 (2023).
20. Z. Riahi *et al.*, Whole exome sequencing identifies new causative mutations in Tunisian families with non-syndromic deafness. *PLoS One* **9**, e99797 (2014).
21. A. Mahasneh, R. Battah, Prevalence of connexin 26 mutations in patients from Jordan with non syndromic hearing loss. *Int. J. Hum. Genet.* **6**, 119–124 (2006).
22. S. Abdi *et al.*, Diversity of the genes implicated in Algerian patients affected by Usher syndrome. *PLoS One* **11**, e0161893 (2016).
23. F. Ammar-Khodja *et al.*, Diversity of the causal genes in hearing impaired Algerian individuals identified by whole exome sequencing. *Mol. Genet. Genomic Med.* **3**, 189–196 (2015).
24. A. Bakhchane *et al.*, Recessive *TBC1D24* mutations are frequent in Moroccan non-syndromic hearing loss pedigrees. *PLoS One* **10**, e0138072 (2015).
25. A. Behlouli *et al.*, EPS8, encoding an actin-binding protein of cochlear hair cell stereocilia, is a new causal gene for autosomal recessive profound deafness. *Orphanet J. Rare Dis.* **9**, 55 (2014).
26. A. Behlouli *et al.*, A novel biallelic splice site mutation of *TECTA* causes moderate to severe hearing impairment in an Algerian family. *Int. J. Pediatr. Otorhinolaryngol.* **87**, 28–33 (2016).
27. M. Dahmani *et al.*, EPS8L2 is a new causal gene for childhood onset autosomal recessive progressive hearing loss. *Orphanet J. Rare Dis.* **10**, 96 (2015).
28. N. Hilgert *et al.*, Mutation analysis of TMC1 identifies four new mutations and suggests an additional deafness gene at loci DFNA36 and DFNB7/11. *Clin. Genet.* **74**, 223–232 (2008).
29. Z. Riahi *et al.*, Whole exome sequencing identifies mutations in Usher syndrome genes in profoundly deaf Tunisian patients. *PLoS One* **10**, e0120584 (2015).
30. R. L. P. Santos *et al.*, Novel sequence variants in the *TMIE* gene in families with autosomal recessive nonsyndromic hearing impairment. *J. Mol. Med. (Berl)* **84**, 226–231 (2006).

31. S. Delmaghani *et al.*, Mutations in *CDC14A*, encoding a protein phosphatase involved in hair cell ciliogenesis, cause autosomal-recessive severe to profound deafness. *Am. J. Hum. Genet.* **98**, 1266–1270 (2016).
32. V. M. Smirnov *et al.*, Retinal phenotype of patients with CLRN1-associated usher 3A syndrome in French Light4Deaf cohort. *Invest. Ophthalmol. Vis. Sci.* **63**, 25 (2022).
33. E. Pepermans *et al.*, The CD2 isoform of protocadherin-15 is an essential component of the tip-link complex in mature auditory hair cells. *EMBO Mol. Med.* **6**, 984–992 (2014).
34. S. Ben-Salem *et al.*, Analysis of two Arab families reveals additional support for a DFNB2 nonsyndromic phenotype of MYO7A. *Mol. Biol. Rep.* **41**, 193–200 (2014).
35. A. Jaiganesh, Y. Narui, R. Araya-Secchi, M. Sotomayor, Beyond cell-cell adhesion: Sensational cadherins for hearing and balance. *Cold Spring Harb. Perspect. Biol.* **10**, a029280 (2018).
36. A. Jaiganesh *et al.*, Zooming in on cadherin-23: Structural diversity and potential mechanisms of inherited deafness. *Structure* **26**, 1210–1225.e4 (2018).
37. D. Choudhary *et al.*, Structural determinants of protocadherin-15 mechanics and function in hearing and balance perception. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 24837–24848 (2020).
38. K. Watanabe, S.-Y. Nishio, S.-I. Usami, Deafness gene study consortium, The prevalence and clinical features of *MYO7A*-related hearing loss including DFNA11, DFNB2 and USH1B. *Sci. Rep.* **14**, 8326 (2024).
39. W. J. Kimberling *et al.*, Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. *Genet. Med.* **12**, 512–516 (2010).
40. L. M. Astuto *et al.*, CDH23 mutation and phenotype heterogeneity: A profile of 107 diverse families with Usher syndrome and nonsyndromic deafness. *Am. J. Hum. Genet.* **71**, 262–275 (2002).
41. H. Bolz *et al.*, Mutation of *CDH23*, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat. Genet.* **27**, 108–112 (2001).
42. R. I. Kabahuma *et al.*, Spectrum of *MYO7A* mutations in an indigenous South African population further elucidates the nonsyndromic autosomal recessive phenotype of DFNB2 to include both homozygous and compound heterozygous mutations. *Genes (Basel)* **12**, 274 (2021).
43. C. M. Sloan-Heggen *et al.*, Characterising the spectrum of autosomal recessive hereditary hearing loss in Iran. *J. Med. Genet.* **52**, 823–829 (2015).
44. C. M. Sloan-Heggen *et al.*, Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum. Genet.* **135**, 441–450 (2016).
45. A. E. Shearer *et al.*, Copy number variants are a common cause of non-syndromic hearing loss. *Genome Med.* **6**, 37 (2014).
46. J. Wu *et al.*, Molecular diagnose of a large hearing loss population from China by targeted genome sequencing. *J. Hum. Genet.* **67**, 643–649 (2022).
47. N. Yamamoto *et al.*, Comprehensive gene panel testing for hearing loss in children: Understanding factors influencing diagnostic yield. *J. Pediatr.* **262**, 113620 (2023).
48. J. Ma *et al.*, Genetic screening of a Chinese cohort of children with hearing loss using a next-generation sequencing panel. *Hum. Genomics* **17**, 1 (2023).
49. J. Goderis *et al.*, Hearing loss and congenital CMV infection: A systematic review. *Pediatrics* **134**, 972–982 (2014).
50. K. B. Avraham *et al.*, The noncoding genome and hearing loss. *Hum. Genet.* **141**, 323–333 (2022).
51. N. Idan, Z. Brownstein, S. Shivatzki, K. B. Avraham, Advances in genetic diagnostics for hereditary hearing loss. *J. Basic Clin. Physiol. Pharmacol.* **24**, 165–170 (2013).
52. L. Romdhane *et al.*, Consanguinity and inbreeding in health and disease in North African populations. *Annu. Rev. Genomics Hum. Genet.* **20**, 155–179 (2019).
53. W. M. Zein *et al.*, Cone responses in Usher syndrome types 1 and 2 by microvolt electroretinography. *Invest. Ophthalmol. Vis. Sci.* **56**, 107–114 (2014).
54. H. J. Muller, "Further studies on the nature and causes of gene mutations" in *Proceedings of the 6th International Congress of Genetics*, D. F. Jones Ed. (Brooklyn Botanic Garden, Menasha, WI, 1932), pp. 213–255.
55. T. B. T. Thuma, R. A. Procopio, H. J. Jimenez, K. B. Gunton, J. S. Pulido, Hypomorphic variants in inherited retinal and ocular diseases: A review of the literature with clinical cases. *Surv. Ophthalmol.* **69**, 337–348 (2024).
56. A. Horani *et al.*, The effect of *Dnaaf5* gene dosage on primary ciliary dyskinesia phenotypes. *JCI Insight* **8**, e168836 (2023).
57. W. R. Xie *et al.*, An Atoh1-S193A phospho-mutant allele causes hearing deficits and motor impairment. *J. Neurosci.* **37**, 8583–8594 (2017).
58. N. Schneider *et al.*, Inherited retinal diseases: Linking genes, disease-causing variants, and relevant therapeutic modalities. *Prog. Retin. Eye Res.* **89**, 101029 (2022).
59. A. El-Amraoui, C. Petit, The retinal phenotype of Usher syndrome: Pathophysiological insights from animal models. *C. R. Biol.* **337**, 167–177 (2014).
60. I. Sahly *et al.*, Localization of Usher 1 proteins to the photoreceptor calyceal processes, which are absent from mice. *J. Cell Biol.* **199**, 381–399 (2012).
61. M. Sharkova, E. Chow, T. Erickson, J. C. Hocking, The morphological and functional diversity of apical microvilli. *J. Anat.* **242**, 327–353 (2023).
62. S. G. Jacobson *et al.*, Usher syndromes due to MYO7A, PCDH15, USH2A or GPR98 mutations share retinal disease mechanism. *Hum. Mol. Genet.* **17**, 2405–2415 (2008).
63. A. Verschueren *et al.*, Planar polarity in primate cone photoreceptors: A potential role in Stiles Crawford effect phototropism. *Commun. Biol.* **5**, 89 (2022).
64. C. Schietroma *et al.*, Usher syndrome type 1-associated cadherins shape the photoreceptor outer segment. *J. Cell Biol.* **216**, 1849–1864 (2017).
65. A. Miles, C. Blair, A. Emili, V. Tropepe, Usher syndrome type 1-associated gene, pcdh15b, is required for photoreceptor structural integrity in zebrafish. *Dis. Model. Mech.* **14**, dmm048965 (2021).
66. K. M. Bujakowska *et al.*, Targeted exon sequencing in Usher syndrome type I. *Invest. Ophthalmol. Vis. Sci.* **55**, 8488–8496 (2014).
67. C. Bonnet *et al.*, An innovative strategy for the molecular diagnosis of Usher syndrome identifies causal biallelic mutations in 93% of European patients. *Eur. J. Hum. Genet.* **24**, 1730–1738 (2016).