# Supplementary Materials:

**Table S1.** List of SNPs used for haplotype construction.

| RS id | Position in chromosome 13 (Build GRCh37) |
|---|---|
| rs7984141 | 19,660,641 |
| rs9578792 | 19,662,154 |
| rs4769387 | 19,666,481 |
| rs7320243 | 19,714,717 |
| rs518637 | 19,732,341 |
| rs11617480 | 20,211,434 |
| rs7988277 | 20,411,701 |
| rs6490494 | 20,493,020 |
| rs7327207 | 20,494,558 |
| rs9506408 | 20,507,957 |
| rs1407961 | 20,572,751 |
| rs4624004 | 20,684,742 |
| rs2031283 | 20,687,836 |
| rs7982556 | 20,688,590 |
| rs1011038 | 20,695,554 |
| rs1886176 | 20,715,801 |
| rs9509057 | 20,723,127 |
| rs7981756 | 20,790,742 |
| rs7992230 | 20,848,317 |
| rs2050500 | 20,854,763 |
| rs1953516 | 20,881,146 |
| rs1572371 | 20,887,860 |
| rs4409941 | 20,951,425 |
| rs4331204 | 20,962,029 |
| rs4770024 | 20,995,731 |

**Table S2.** Summary of clinical features of cases.

| Clinical features | |
|---|---|
| **Age at onset** | |
| Mostly prelingual | 18.2 months [0 – 72 m] |
| **Sex** | |
| Males | 69 |
| Females | 64 |
| **Family history** | |
| Multiplexcases | 63 |
| Simplex cases | 70 |
| **Level hearing loss on left ear** | |
| Moderate | 2.9% |
| Severe | 14.7% |
| Profound | 82.4% |
| **Level hearing loss on right ear** | |
| Moderate | 1% |

| | |
|---|---|
| Severe | 13.7% |
| Profound | 85.3 |

*Complete audiometry information was available for 102 cases. Grades of hearing loss according to WHO: Mild (26 – 40 dB), Moderate (41 – 60 dB), Severe (61 – 80 dB), Profound (Over 81 dB).

**TableS3.** Characteristics of identified *GJB2* variants.

| cDNA | Protein | SIFT | Mutation Taster | GERP[1] | PhastCons[1] | gnomAD | Reference | ClinVar | ACMG |
|---|---|---|---|---|---|---|---|---|---|
| c.19C>T | p.(Gln7Ter) | NA | DC | 5.36 | 0.023 | 0.00000802 | Pandya et al. 2003 | Pathogenic/Likely pathogenic | Pathogenic |
| c.29T>C | p.(Leu10Pro) | DM | DC | 5.36 | 0.335 | - | Dalamón et al. 2013 | NA | Likely pathogenic |
| c.35G>T | p.(Gly12Val) | 0.001/DM | DC | 5.36 | 0.591 | 0.00009221 | Rabionet, R. et al. 2000 | Pathogenic/Likely pathogenic | Pathogenic |
| c.35delG | p.(Gly12fs) | NA | NA | 5.36 | NA | 0.006188 | Zelanmte, L. et al. 1997 | Pathogenic | Pathogenic |
| c.59T>C | p.(Ile20Thr) | 0.02/DM | DC | 5.21 | 0.961 | - | Loffler, J. et al. 2001 | Likely pathogenic | Likely pathogenic |
| c.94C>A | p.(Arg32Ser) | 0/DM | DC | 5.21 | 0.8939 | 0.000007984 | Hayashi C. et al. 2011 | Likely pathogenic | Likely pathogenic |
| c.109G>A | p.(Val37Ile) | T | DC | 5.21 | 0.5149 | 0.007556 | Kelley, P., et al. 1998 | Conflicting interpretations of pathogenicity | Likely pathogenic |
| c.167delT | p.(Leu56fs) | NA | NA | 4.1199 | NA | 0.0008036 | Zelante et al. 1997 | Pathogenic | Pathogenic |
| c.232dupG | p.(Ala78fs) | NA | NA | 5.3299 | NA | NA | Putcha et al 2007 | Likely pathogenic | Likely pathogenic |
| c.235delC | p.(Leu79fs) | NA | NA | 5.3299 | - | 0.0004738 | Kudo T et al. 2000 | Pathogenic | Pathogenic |
| c.283G>A | p.(Val95Met) | 0.001/DM | DC | 4.2399 | 0.9959 | 0.00004779 | Kelley, P., et al. 1998 | Pathogenic/Likely pathogenic | Pathogenic |
| c.427C>T | p.(Arg143Trp) | 0/DM | DC | 4.56 | 0.92 | 0.0001311 | Brobby, G.; Muller-Myhsok, B. 1998 | Pathogenic | Pathogenic |
| c.617A>G | p.(Asn206Ser) | T | DC | 4.449 | 0.448 | 0.0001027 | Marlin et al. 2001 | Pathogenic | Pathogenic |
| c.645delT | p.(Arg216fs) | NA | NA | -2.96 | NA | NA | Angeli 2009 | Likely pathogenic | Likely pathogenic |
| c.-15C>T | NA | NA | NA | -5.0799 | NA | 0.004994 | Gasmelseed NM et al. 2004 | Benign/Likely benign | Benign |
| c.79G>A | p.(Val27Ile) | T | POL | 5.21 | 0.898 | 0.05038 | Kelley, P., et al. 1998 | Benign | Benign |
| c.*84T>C | NA | NA | NA | 5.82 | NA | 0.7486 | Wilch et al. 2006 | Benign | Benign |
| c.*1C>T | NA | NA | NA | -3.96 | NA | 0.0004986 | Dalamón et al. 2010 | Conflicting interpretations of pathogenicity | Uncertain significance |
| c.-22-6T>C | NA | NA | NA | 1.74 | NA | 0.0004889 | Tang et al. 2006 | Uncertain significance | Uncertain significance |
| c.154G>T | p.(Val52Phe) | DM | POL | -7.3499 | 0.05099 | NA | NA | NA | Uncertain significance |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| c.503A>G | p.(Lys168Arg) | T | DC | 5.4699 | 0.8399 | 0.0000531 | Samanich J et al. 2007 | Conflicting interpretations of pathogenicity | Uncertain significance |

**DM**: Damaging, **T**: Tolerated, **DC**: Disease Causing, **NA**: Not Available, **ACMG**: American College of Medical Genetics Guideline (Richards et al., 2015).
[1]PhastCons and GERP scores are obtained from SeattleSeq Annotation 138 (http://snp.gs.washington.edu/SeattleSeqAnnotation138/).

**Table S4.** List of *GJB2* genotypes.

| COMPOUND HETEROZYGOUS GENOTYPES | | PROBANDS |
|---|---|---|
| c.[427C>T(;)94C>A] | p.[Arg143Trp(;)Arg32Ser] | 5 |
| c.[427C>T(;)59T>C] | p.[Arg143Trp(;)Ile20Thr] | 4 |
| c.[427C>T(;)35delG] | p.[Arg143Trp(;)Gly12fs] | 3 |
| c.[427C>T(;)645delT] | p.[Arg143Trp(;)Arg216fs] | 3 |
| c.[427C>T(;)35G>T] | p.[Arg143Trp(;)Gly12Val] | 1 |
| c.[427C>T(;)167delT] | p.[Arg143Trp(;)Leu56fs] | 1 |
| c.[427C>T(;)109G>A] | p.[Arg143Trp(;)Val37Ile] | 1 |
| c.[427C>T(;)427C>T] | p.[Arg143Trp(;)Arg143Trp] | 1 |
| c.[427C>T(;)19C>T] | p.[Arg143Trp(;)Gln7Ter] | 1 |
| c.[35delG(;)35delG] | p.[Gly12fs(;)Gly12fs] | 3 |
| c.[35delG(;)94C>A] | p.[Gly12fs(;)Arg32Ser] | 2 |
| c.[35delG(;)19C>T] | p.[Gly12fs(;)Gln7Ter] | 2 |
| c.[35delG(;)283G>A] | p.[Gly12fs(;)Val95Met] | 1 |
| c.[35G>T(;)35G>T] | p.[Gly12Val(;)Gly12Val] | 3 |
| c.[35G>T(;)235delC] | p.[Gly12Val(;)Leu79fs] | 1 |
| c.[59T>C(;)283G>A] | p.[Ile20Thr(;)Val95Met] | 2 |
| c.[59T>C(;)59T>C] | p.[Ile20Thr(;)Ile20Thr] | 1 |
| c.[645delT(;)167delT] | p.[Arg216fs(;)Leu56fs] | 1 |
| c.[645delT(;)283G>A] | p.[Arg216fs(;)Val95Met] | 1 |
| c.[645delT(;)59T>C] | p.[Arg216fs(;)Ile20Thr] | 1 |
| c.[94C>A(;)19C>T] | p.[Arg32Ser(;)Gln7Ter] | 1 |
| c.[94C>A(;)94C>A] | p.[Arg32Ser(;)Arg32Ser] | 1 |
| c.[109G>A(;)617A>G] | p.[Val37Ile(;)Asn206Ser] | 1 |
| c.[167delT(;)232dupG] | p.[Leu56fs(;)Ala78fs] | 1 |
| c.[19C>T(;)283G>A] | p.[Gln7Ter(;)Val95Met] | 1 |
| HETEROZYGOUS GENOTYPES | | PROBANDS |
| c.[283G>A];[=] | p.[Val95Met];[=] | 1 |
| c.[29T>C];[=] | p.[Leu10Pro];[=] | 1 |
| c.[35delG];[=] | p.[Gly12fs];[=] | 2 |
| c.[35G>T];[=] | p.[Gly12Val];[=] | 1 |
| c.[59T>C];[=] | p.[Ile20Thr];[=] | 1 |
| c.[94C>A];[=] | p.[Arg32Ser];[=] | 1 |
| WILDE TYPE | | PROBANDS |
| WT | WT | 83 |

Genotypes were annotated according to HGVS (http://www.hgvs.org/).

**TableS5.** Causative genetic variants identified via WGS. Transcript NM_016239.3 was used for the *MYO15A*.

| Family ID | Gene | OMIM# | Associated Phenotype | OMIM# | Zygosity | cDNA | Protein | Reference | gnomAD | CADD | GERP RS | Mutation Taster | SIFT | ClinVar | ACMG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2641 | *MYO15A* | 602666 | Deafness, autosomal recessive 3 | 600316 | HM | c.3757-2A>G | - | This study | NA | 28.9 | 4.76 | DC | NA | NA | LP |
| 2726 | *MYO15A* | 602666 | Deafness, autosomal recessive 3 | 600316 | HT | c.843C>A | p.(Tyr281*) | This study | NA | 34 | 2.5899 | DC | NA | NA | LP |
| | | 602666 | Deafness, autosomal recessive 3 | 600316 | HT | c.8798delT | p.(Phe2933Serfs*101) | This study | NA | 35 | 3.65 | NA | NA | NA | LP |

**HM:** Homozygous, **HT**: Heterozygous, **DC**: Disease Causing, **NA**: Not Available, **LP**: Likely Pathogenic, **VUS**: Variant of Uncertain Significance, **ACMG**: American College of Medical Genetics Guideline (Richards et al., 2015).