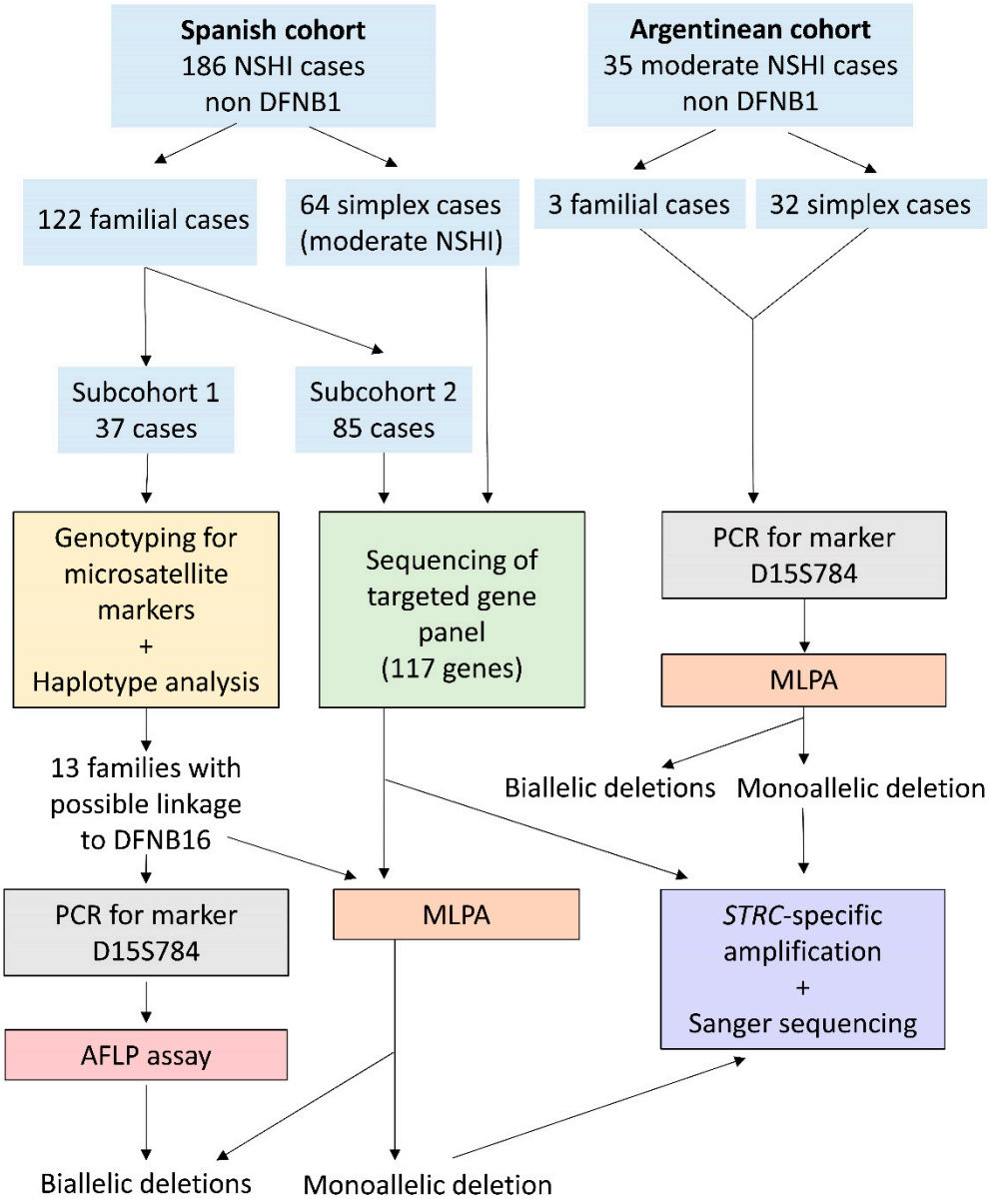

Figure S1. Diagram of the study setup.

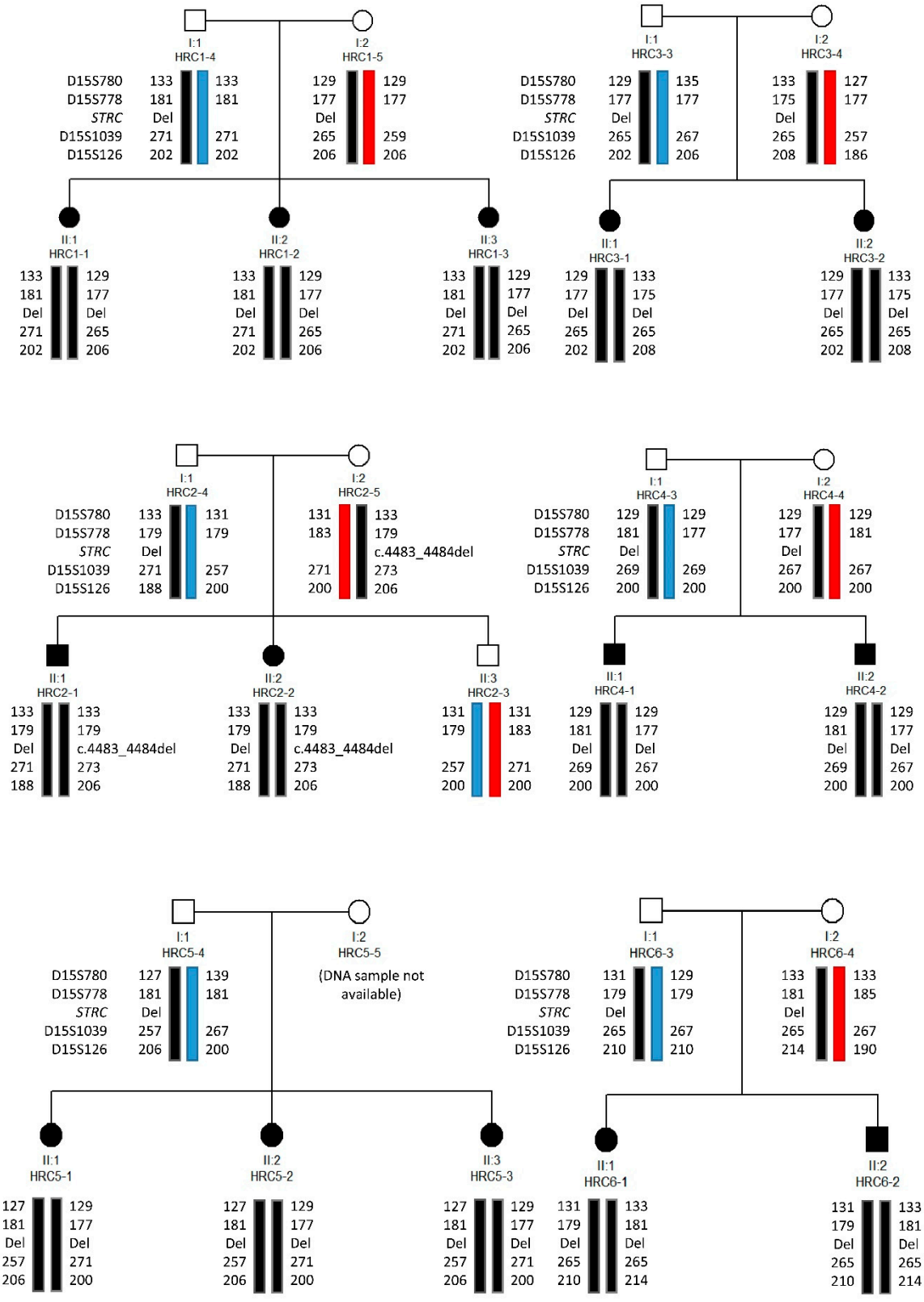

Figure S2. Pedigrees of the six families with biallelic pathogenic variants in the *STRC* gene for which haplotype analysis was performed. Haplotypes are indicated by vertical bars. In black, the haplotype associated with the disease. The four microsatellite markers that were genotyped and the position of the *STRC* gene are indicated on the left of the first subject in each family. Numbers indicate allele size, using as reference the genotype of CEPH subject 134702 (D15S780: 127/133; D15S778: 179/181; D15S1039: 267/267; D15S126: 188/202) [10, 11].

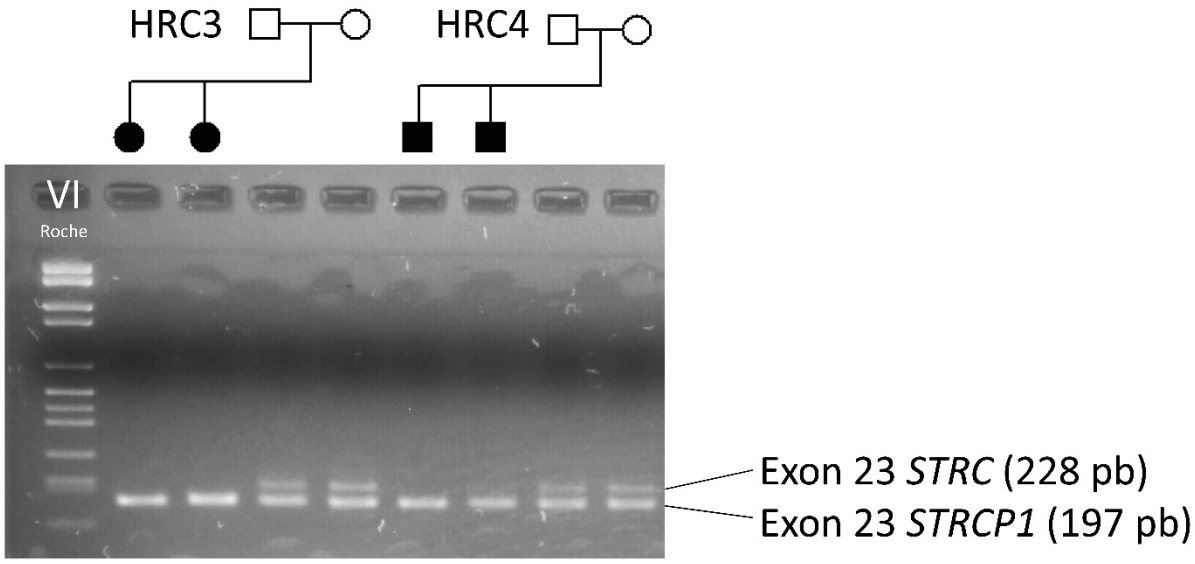

Figure S3. Example of AFLP test for the 3′ end of intron 22 in *STRC* and *STRCP1*. PCR products were resolved on a 2.5% agarose gel. Affected subjects of families HRC3 and HRC4 show only the band corresponding to *STRCP1*, whereas their parents show the two bands, corresponding to *STRC* and *STRCP1*, respectively.

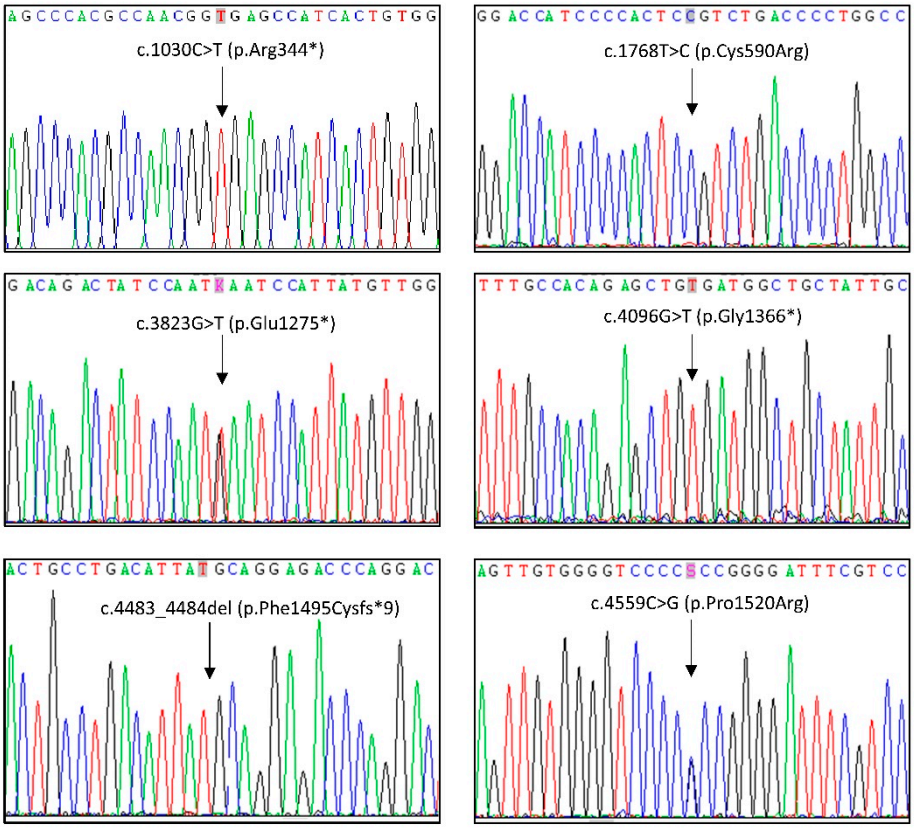

Figure S4. Electropherograms of the pathogenic SNVs in *STRC* that were found in this study.

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                                                Cys590Arg
H.sapiens     ISRGGNDTCFLEGLLGPLLPSLPPLGPSPLCLTPGPFLLGMLSQLPRCQSSVPALAHPTR
B.taurus      ISRGGNDTCFLEGLLGPLLPSLPPLGPSPLCLAPGPFLLGMLSQLPRCQSSVPALAHPTR
M.musculus    ISRGGNDTCFLEGMLGPLLPSLPPLGPSPLCLAPGPFLLGMLSQLPRCQSSVPALAHPTR
M.domestica   VSRGGNDTCFLEGVLGPLLPPLPPLGPSPLCLAPGPFLLGMLSQLPRCQAAVPALSHPTR
               ************ ****** ********** ****************  **** ****
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Figure S5. Alignment of estereocilin orthologous sequences from human and three other mammals in the stretch containing the residue that is affected by the p.(Cys590Arg) variant. Asterisks indicate identical residues across all sequences. Sequence accession numbers: *Homo sapiens* (NP_714544.1), *Bos taurus* (XP_024838009.1), *Mus musculus* (XP_006498797.1), *Monodelphis domestica* (XP_056671975.1). No orthologues were found in the databases for *Gallus gallus* (chicken), *Xenopus tropicalis* (clawed frog), *Danio rerio* (zebrafish), *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (nematode).

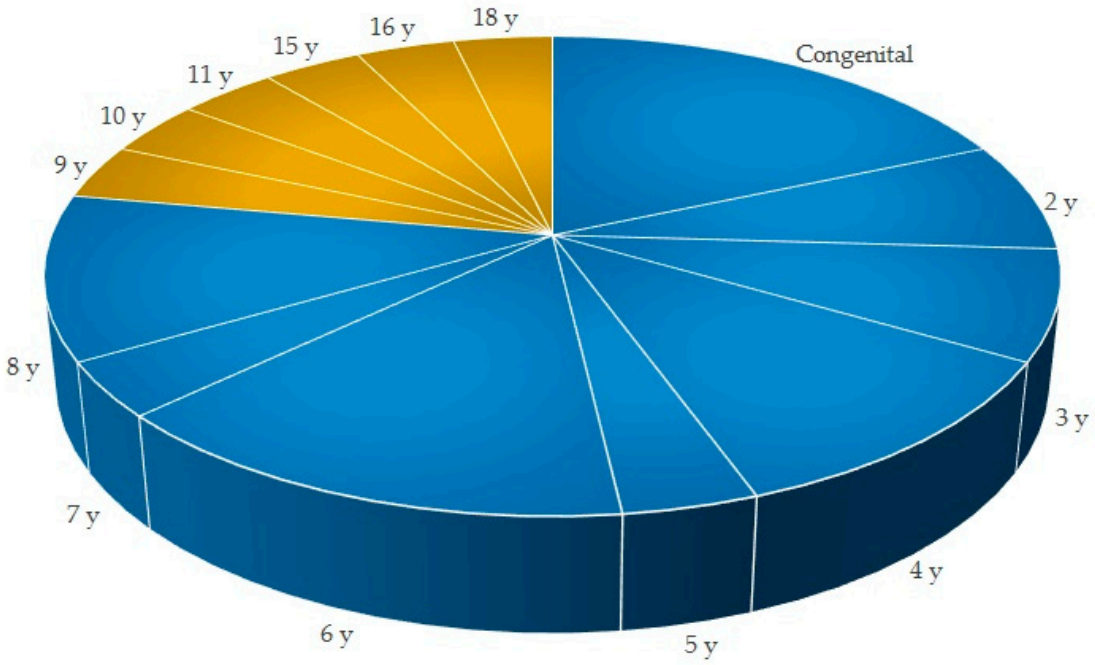

Figure S6. Age of onset of the hearing loss in 27 subjects with biallelic pathogenic variants in *STRC*. In blue, onset in early childhood (ages 0-8 years). In brown, apparently delayed onset (ages 9-18 years).

Table S1. Primer sequences for Sanger sequencing of the *STRC* gene.

Amplicon	Upper primer (5′→3′)	Lower primer (5′→3′)
STRC_EX1	TCCCCTATCATTAGTCCCTCTCACA	CACAAGTCTCTGCCTCTCTCCTTTG
STRC_EX2F1	GCTCAGGGGCAGGGTTGAG	GTCAGCAGCCAGGACACAGTCA
STRC_EX2F2_EX3	TGGACTTGTGGAGGTGCTGCT	CAGCTCTTCACCTCCAGATTTTCC
STRC_EX4F1	GCTTGCCATGCCCCTTGAG	GTCGCTCAGCCCACAGAGTCTC
STRC_EX4F2	CTGCTCCCACCCCCTACCA	GCGTGTGGGGTGAGCAAGA
STRC_EX4F3	TCTGACCCCTGGCCCCTTC	GCTTTCCCTCTGGCAGAGCAC
STRC_EX5_EX6	TGCAGGAGAGACAGAGAAGACAGCT	GAGATGCTCAGTATCCCTGCCATT
STRC_EX7	GCTTGCTTTAGTGGGAGGAGGAA	ACCGTGACAGGAGCAGGAGGA
STRC_EX8_EX9	AGGGCTACAGAGGGTCAGAGGAA	TGACAGCTGTGCCTTCCTCTCTC
STRC_EX10	GTGCCCCTGTGTAGTGGTGTAGTGT	AACAGGTAAGAATGGCCATGGAGT
STRC_EX11	TGGGAAATTCAGATGTGGGATTAGA	GCGTCCTCCTGCAGACAGAGTT
STRC_EX12_EX13	TTGCCTTAGGAACCCACTTAGGAC	GATGCCTTCCTCCCAACCAGA
STRC_EX14	GTGGCAAGGAGGATGAGATAATCAG	ACCAGAACTCTCAGGGCCACTG
STRC_EX15	CCCCATGGTTCCTTTTCTCTGA	GGCTAAGGGATAGGTAAAGAAGGTAGACTA
STRC_EX16	CACAGAGTGAGGAGGCAGGACTG	CAAAGGGAGGTGCTAGGAGAACG
STRC_EX17_EX18	ACAGGCTGTTGTATGTGTTGAATGAG	GGGAACCAGTCAATACATAGAGCAACT
STRC_EX19	CACTAAATTTGGTCTGGGGTCACAT	AGAGATTTCTCGGACTCCAAGAGGTAT
STRC_EX20	AGGGGGCTTGGTGTCTTGATC	GGAACAGGCTGCCACTGATG
STRC_EX21	GCATGTCCTCTTTTCCCCTTCC	GCTCACCAGTCCCCTCTATGCA
STRC_EX22	TGCAAAACAGAGACAGGACTTTGG	TTTGATACTCCCTGTACATCCTGCTG
STRC_EX23	TATTTCTCACAGTGCTCTATCCTCCAA	ATCTCTTGCTTCCCCCACCTCT
STRC_EX24	AATAAGCGTCTCTGTGTCTGATGCA	GATGATCTATAGGGCTGGGTCTGTG
STRC_EX25	TCCTCAGCCTTCCCCATGACT	AAATGAGTTCCCTTCCTCCATGG
STRC_EX26	TCTCATGACCACAGCCCCTTTC	CTAGCCCCTTATTTAAACACCCTCAG
STRC_EX27_EX28	AGGGCACTTTGGGAGTAGTTAGAGAA	GAGCCAGACAGCACCAAATTAGG
STRC_EX29	ACAGGCAGAGCGCTAATTTCCA	CGCCAGCTGATGACTCAAGATTC