## Supplemental Data

# Noncoding Mutations of *HGF* Are Associated with Nonsyndromic Hearing Loss, DFNB39

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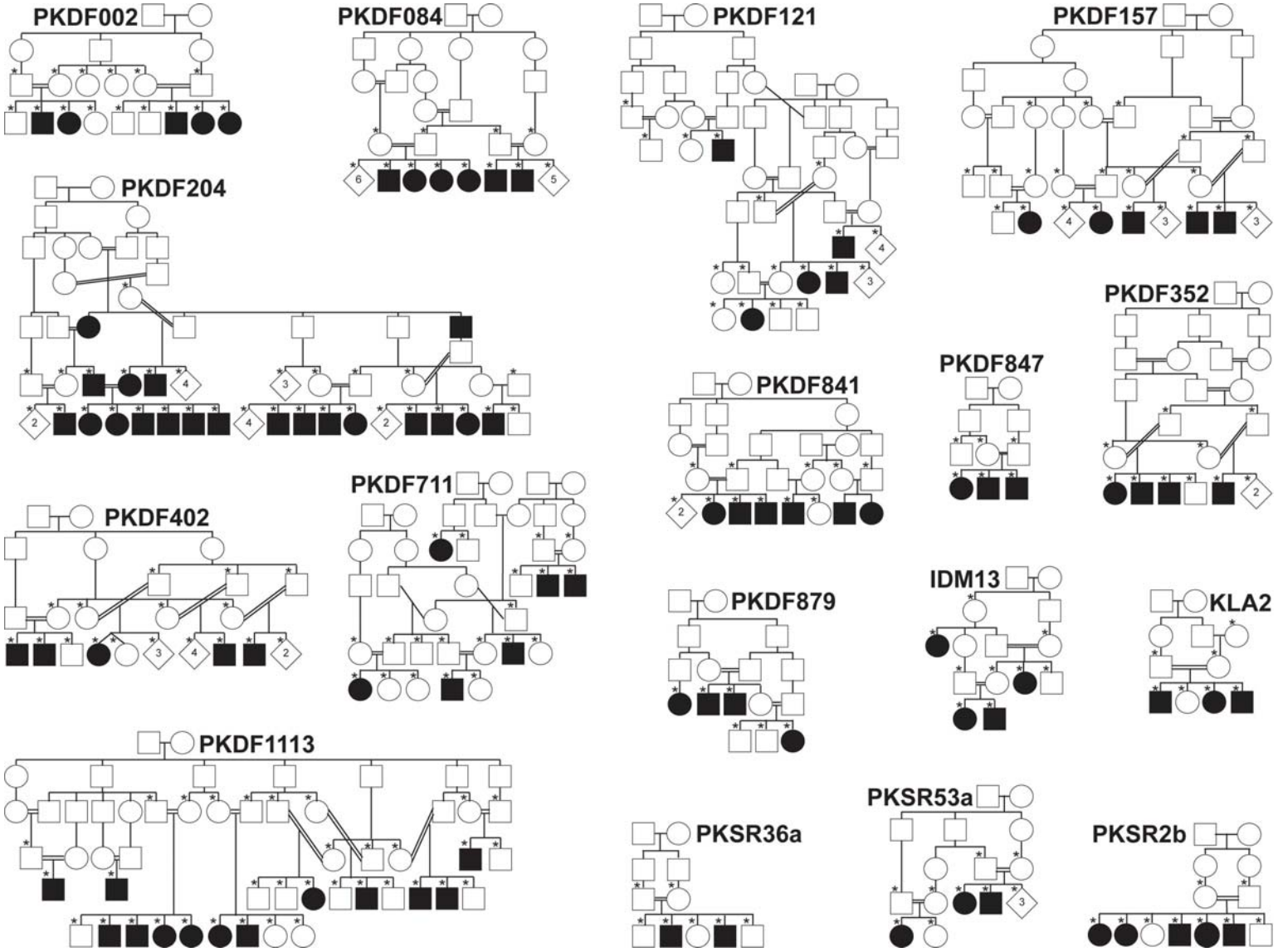
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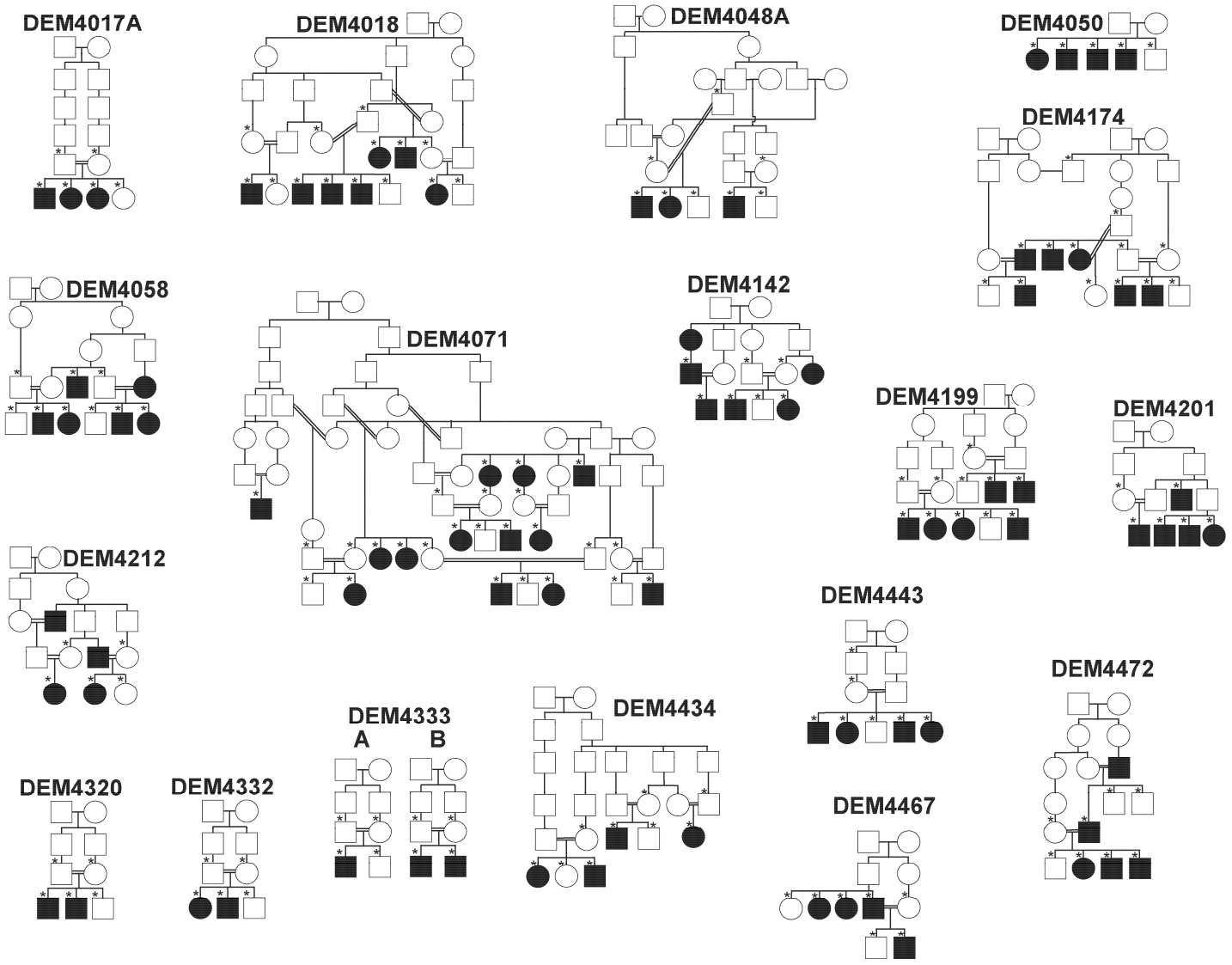
PKDF121
PKDF157
PKDF204
PKDF352
PKDF847
PKDF841
PKDF711
PKDF402
PKDF879
IDM13
KLA2
PKDF1113
PKSR53a
PKSR36a
PKSR2b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DEM4174
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DEM4071
DEM4142
DEM4199
DEM4201
DEM4212
DEM4443
DEM4472
DEM4333
A
B
DEM4434
DEM4320
DEM4332
DEM4467

**Figure S1. Pedigrees of 36 Families Cosegregating Profound Deafness and c.482+1986_1988delTGA within Intron 4 of *HGF***

Filled symbols represent individuals with prelingual, severe to profound, hearing loss. Asterisks indicate subjects enrolled in the protocol who contributed DNA samples. The pedigree for family DEM4011 was reported.[16] The exact relationship between families DEM4333A and DEM4333B was not determined at the time of family history interview.

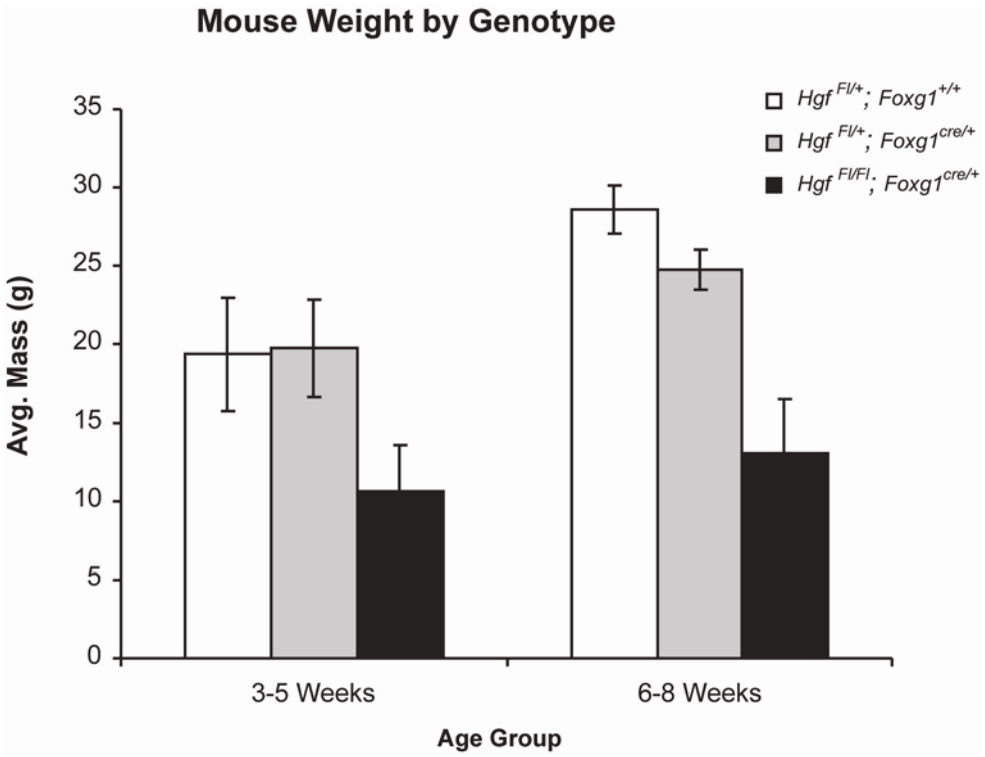


**Figure S2. Differences in Weights of *Hgf* Conditional Knockout Mice and Littermate Controls Weighed before ABR Testing**

Conditional knockout mice were significantly smaller than littermate controls. Vertical bars indicate standard deviations.

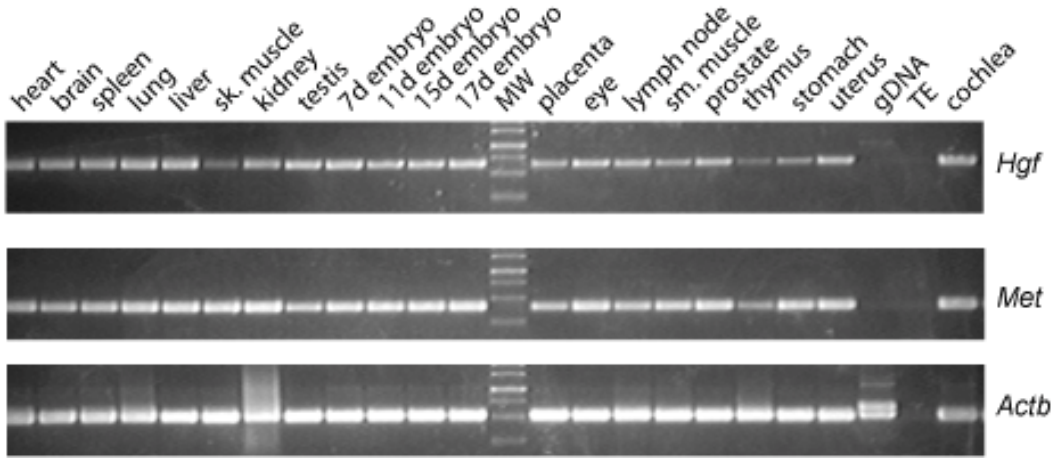


**Figure S3. RT-PCR of *Hgf* and *Met* from Mouse cDNAs**

Mouse MTC panels I and III (Clontech) and cDNA from mouse cochlea were amplified with PCR primers for *Hgf* (forward 5'-cgacaagggctttgatgataa-3', reverse 5'-agggcaataatcccaaggaa-3'), *Met* (forward 5'-tgtcaaggttgctgatttcg-3', reverse 5'-ggacgtagtgttccccaatg-3') and *Actb* (forward 5'-agtgtgacgttgacatccgta-3', reverse 5'-gtttgctccaaccaactgct-3'). Mouse genomic DNA and TE are included as controls. *Hgf* and *Met* are ubiquitously expressed in all mesenchymal and epithelial tissues, respectively.

**Table S1. Primer Sequences Used to Amplify and Sequence *HGF* Genomic DNA**

| Exon | Forward Primer | Reverse Primer | Product (bp) [a] |
|---|---|---|---|
| 1 | gttgcagggatctgtttggt | gaggggagttgagggaaagt | 582 |
| 2 | gctcctaacccctggaaatc | ggtagcagtttttgcgctct | 508 |
| 3 | tgcatttgtttctatattgtcca | gagggaggaagggaatcaag | 461 |
| 4 | acagcgactgctctctgga | aaaccttgccgtaatacaattca | 502 |
| IVS4 | gatgtttatggccgagagga | ggctttaagagagacaagtgagg | 500 |
| 5 | tcagcaaattcacaggctca | tagttgcatttgcacgaaca | 517 |
| 6 | gcgtcgtggccctatagtaa | ccctgctgatgattttgtgtt | 669 |
| 7 | actcgatttggaacctcagc | tccaaaaatgcaaaagattgg | 555 |
| A (7b) | tgtgcagcatcacaaagtca | gccctgtattcaaagaaatgaaa | 471 |
| 8 | tgaatgaaaggaaaattggaatg | tttgaggaccaaatcccaga | 558 |
| 9 | aggccaatgttttgaaatgg | caaaaccagtgcagcaagaa | 580 |
| 10 | tctcgatctcctgacctcgt | cttggaggttggaaccaaaa | 600 |
| 11 | atctttgccatctgcttgct | ttgggaataaatgccagacc | 583 |
| 12 | aagtagctgggtgtggtggt | gcattgtgccccaaattaaa | 622 |
| 13 | tgagggtggtgagggattag | gggtacaaccttcaggacca | 514 |
| 14 | gtgtgttcgggatggctatt | gcaaaattttccccaactga | 598 |
| 15 | tgctttacctgagcatttttca | atcagactgttggcccaatg | 654 |
| 16 | catttggacattcccacctt | acctcacatggtcctgatcc | 518 |
| 17 | tggatgcacaattcctgaaa | ggagttccggctctacacac | 571 |
| 18 | cagttgcagttattctctttttctg | ccaacatcagaaagcagcttag | 640 |

[a] All PCR products were amplified with 1.5 mM $MgCl_2$ and 57°C annealing temperature, except exons 5 and 8, where 2.5 mM $MgCl_2$ was used.

**Table S2. Control Chromosomes Screened for Mutations of *HGF***

| Mutation [a] | Pakistani | Indian | Coriell Human Diversity Panel | Coriell Caucasian Panel | Total |
|---|---|---|---|---|---|
| Δ3 | 2/858 | 0/262 | 0/168 | 0/400 | 2/1688 |
| Δ10 | 0/858 | 0/262 | 0/168 | 0/400 | 0/1688 |
| c.495G>A (p.S165S) | 0/474 | | 0/172 | 0/394 | 0/1040 |

[a] Δ3 = c.482+1986_1988delTGA; Δ10 = c.482+1991_2000delGATGATGAAA

**Table S3. SNP Genotypes in 221,150 bp Surrounding *HGF* Mutations**

| | rs4732399 | rs10231299 | rs5745752 | rs2286194 | B39 [a] | rs1558001 | rs969705 | rs12155338 |
|---|---|---|---|---|---|---|---|---|
| **~Mb [b]** | **81.12** | **81.12** | **81.17** | **81.19** | **81.22** | **81.25** | **81.26** | **81.34** |
| PKDF002 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF084 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF121 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF157 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF204 | TT | CC | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF239 [c] | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF351 [c] | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF352 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF402 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | TT | AA |
| PKDF711 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF841 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | AA |
| PKDF847 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | AA |
| PKDF879 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF1113 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKSR36a | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKSR53a | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKSR2b | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| IDM13 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| Kla2 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4011 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4017A | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4018 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | AA |
| DEM4048 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4050 | **CC** | CC | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4058 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4071 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4142 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4174 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4199 | **CC** | CC | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4201 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4212 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4320 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4332 | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| DEM4333 (A,B) | **CC** | **TT** | **GG** | **AA** | **Δ3** | **GG** | **CC** | **TT** |
| PKDF601 | CC | TT | AA | AA | Δ10 | AA | TT | TT |
| PKDF210 | CC | CC | GG | TT | G>A | GG | CC | AA |

[a] *HGF* mutations: Δ3=c.482+1986_1988delTGA; Δ10=c.482+1991_2000delGATGATGAAA; G>A=c.495G>A (p.S165S)

[b] Nucleotide location in megabases according to human genome reference sequence (NCBI build 36.1)

[c] Pedigrees that define the proximal and distal breakpoints for the minimal *DFNB39* interval