Supplementary Figures:

Figure S1 Audiograms of affected familiar members showing bilateral conductive or mixed hearing loss (Symbols ◇, ×: air conduction pure-tone thresholds at different frequencies in the right and left ear; Symbols △, □: bone conduction pure-tone thresholds at different frequencies right and left ear; dB, decibels; Hz, Hertz). PTA showed that they exhibited bilateral, mild to severe, conductive or mixed hearing loss. Three of them presented bilaterally asymmetric hearing loss. No other family members, including two presumed obligate carrier mothers (III-4, IV-11), had detectable hearing impairment.

Figure S2 The distribution of per-base sequencing depth in target regions for each sample. Y-axis indicated the percentage of total target region under a given sequencing depth.

Figure S3 Cumulative depth distribution in target regions for each sample. X-axis denotes sequencing depth, and Y-axis indicated the fraction of bases that achieves at or above a given sequencing depth.

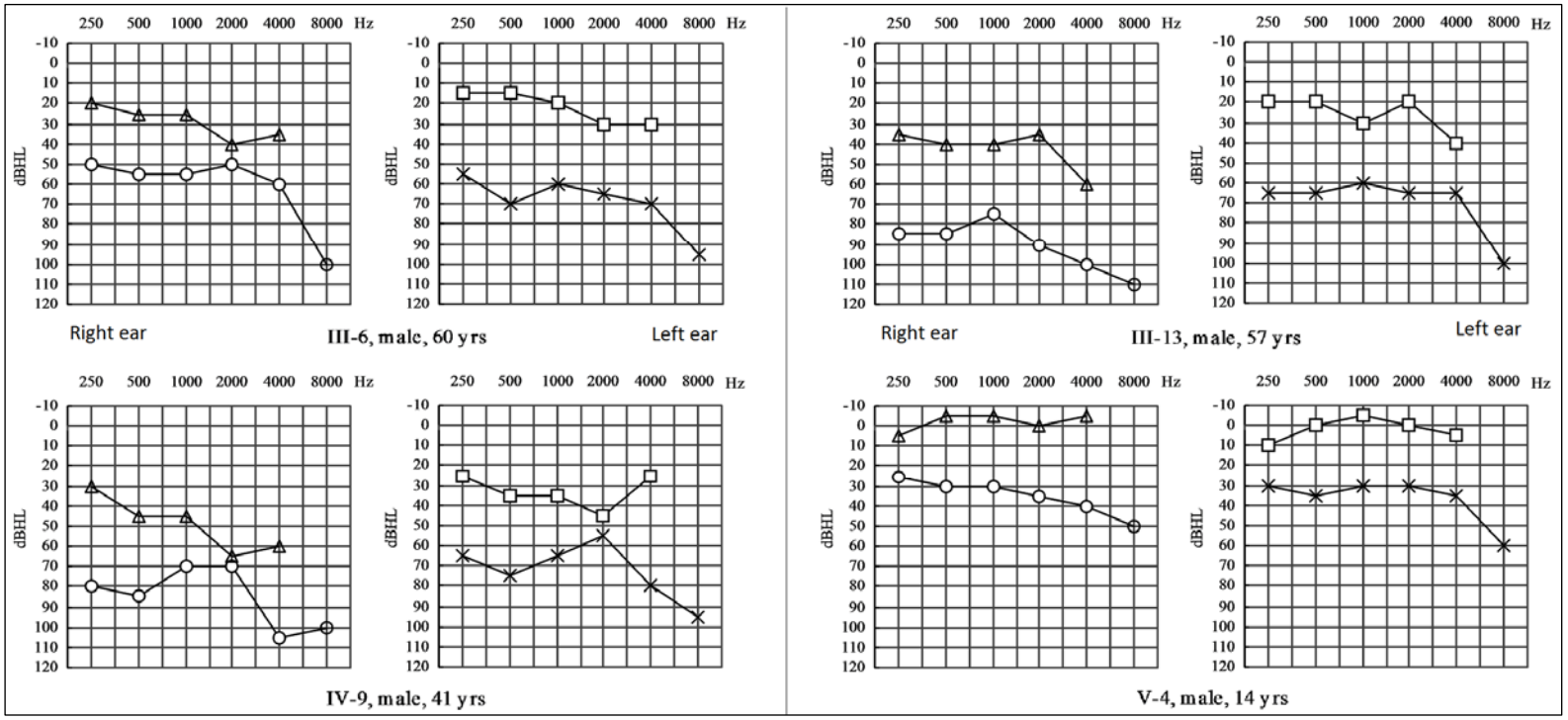
250 500 1000 2000 4000 8000 Hz
-10 0 10 20 30 40 50 60 70 80 90 100 110 120
dBHL
Right ear
III-6, male, 60 yrs
Left ear
III-13, male, 57 yrs
IV-9, male, 41 yrs
V-4, male, 14 yrs

Figure S1

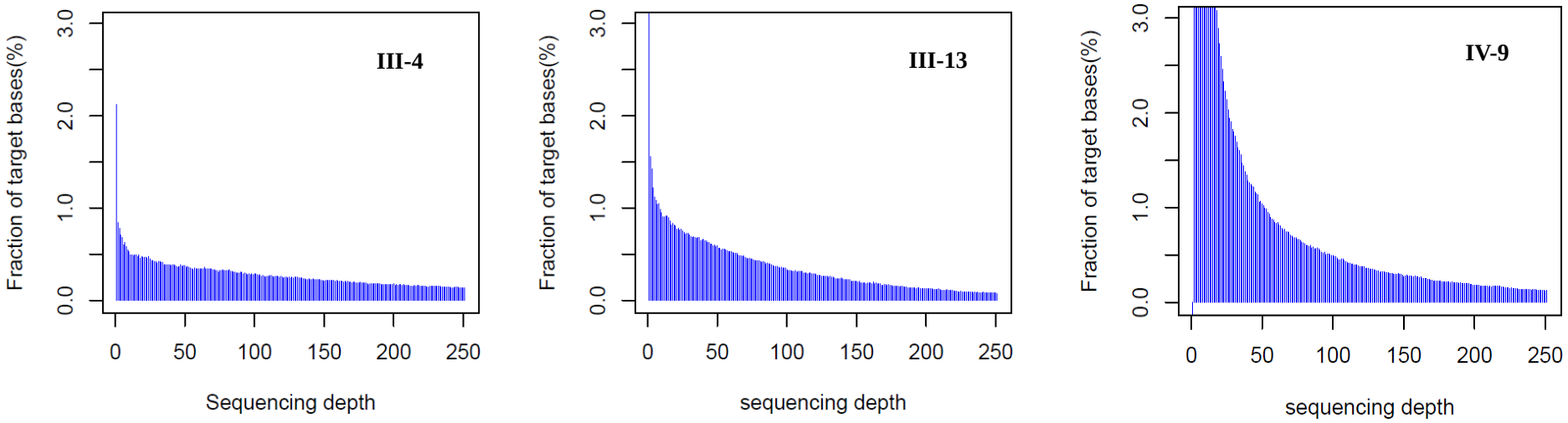
III-4
III-13
IV-9
Fraction of target bases(%)
0.0
1.0
2.0
3.0
0
50
100
150
200
250
Sequencing depth
sequencing depth

Figure S2

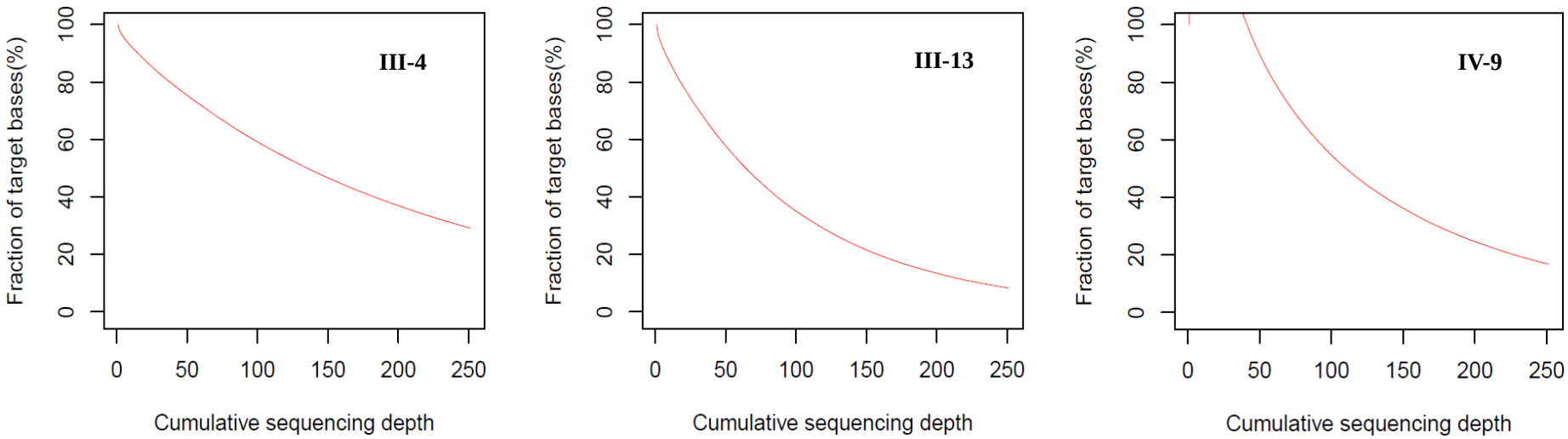
III-4
III-13
IV-9
Fraction of target bases(%)
0
20
40
60
80
100
0
50
100
150
200
250
Cumulative sequencing depth

Figure S3

Supplementary Tables:

Table S1 Summary of clinical features in affected family members

Table S2 Primers for sequencing analysis of *GPRASP2*

Table S3 Primers for gene expression by RT-PCR

Table S4 Summary of XES data for each sample

Table S5 Summary of coding SNVs and indels for each sample

Table S6 Screening and identification of the causal genes by XES (SNPs*)

Table S7 Screening and identification of the causal genes by XES (indels*)

Table S8 Screening and identification of the causal genes by XES (Functional SNPs*)

Table S9 Screening and identification of the causal genes by XES (Functional indels*)

Table S10 Candidate variants shared by three included individuals in XES

Table S1 Summary of clinical features in affected family members*

Phenotype	Patients (age)			
	III-6 (60y)	**III-13 (57y)**	**IV-9 (41y)**	**V-4 (14y)**
Hearing loss	Congenital, mixed	Congenital, mixed	Congenital, mixed	Congenital, conductive
Ear anomalies				
Canal atresia/stenosis	Atresia in the left, severe stenosis in the right	Atresia in the right, severe stenosis in the left	Bilateral atresia, corrected for right ear	Bilateral atresia
Abnormal auricular shape	No	Bat ear	Posteriorly rotated small ear, incomplete antihelix and antitragus formation	Incomplete antitragus and antihelix formation
Inner ear malformation detected by CT scan	Bulbous dilatated IAC; Incomplete separation of the cochlea basal turn from the fundus of IAC	Bulbous dilatated IAC; Incomplete separation of the cochlea basal turn from the fundus of IAC	Dilatation of IAC (mild)	No
Facial dysmorphism				
Ptosis	No	Bilateral ptosis with asymmetric face	Bilateral severe ptosis, Corrected	Bilateral ptosis
Heavy eyebrows	Yes	No	Yes	Yes
Microphthalmic eye	No	Yes (right)	No	No
Telecanthus	Yes	Yes	Yes	Yes
Broad nasal root	Yes	No	Yes	Yes
Developmental delay	No	No	No	No
Intellectual disability	No	No	No	No

* Of 4 affected family members, the congenital hearing loss was reported to be progressive in three subjects (III-6, III-13, IV-9) and non-progressive in one case (V-4). No treatment was ever made to correct the hearing loss for all of them except IV-9, who underwent a canaloplasty of the right external auditory meatus in 1990. This operation, however, unfortunately deteriorated his hearing and induced an occurrence of persistent, high-pitched tinnitus in the right ear. None of the patients complained of vestibular symptoms.

Table S2 Primers for sequencing analysis of *GPRASP2* coding region*

Exons (Coding region)	Forward primer (5' to 3')	Reverse primer (5' to 3')
Exon 1	ATCACGGGGCGGTTTGGTATC	GGCACTGCGCACCTCTCTCAA
Exon 2	CGCGTTTGCTACTCCGGGTCC	TGGGGGAGACAGGTGGGGATG
Exon 3+Exon 4	TGCTCTTCTGCCACCACTCAT	GTGGGAAAGTGTGTCGGCAAT
Exon 5 (1-744)	TCCCACCTAGCATTCAACATC	GGGCCATGATCTGACACTTGT
Exon 5 (689-1879)	CCTCTACAGCGTCTTCTTTCT	AAGCACTTCTCATACCCATTG
Exon 5 (1857-2517)	TTGCAATGGGTATGAGAAGTG	CCCACAATAATCTCTCCCTTC

* The primers flanking the candidate loci, including all coding exons and exon-intron boundaries, were designed using Primer Premier 5.0 software (Premier, Polo Alto, CA, USA) and synthesized by BGI-Beijing, Shenzhen, China).

Table S3 Primers for gene expression by RT-qPCR

Gene name	Gene ID	Primer sequences (5' to 3')	Product length
Gprasp2	NM_001163015.1	Forward: ATGAAACAGTCGAACAAGAGTCC	90 bp
		Reverse: CAGCCTCCACATTAGTCTCCT	
β-actin	NM_007393.3	Forward: AAAGACCTGTACGCCAACAC	219 bp
		Reverse: GTCATACTCCTGCTTGCTGAT	

Table S4 Summary of XES data for each sample

Exome Capture Statistics	**III-4**	**III-13**	**IV-9**
Target region (bp)	1855306	1855306	1855306
Raw data yield (Mb)	715.78	387.76	390.46
Data mapped to target region (Mb)	462.05	215.45	237.09
Average sequencing depth of target region(X)	249.04	116.13	127.79
Coverage of target region (%)	98.30%	97.00%	97.30%
Fraction of target covered >=4X (%)	96.10%	93.20%	94.00%
Fraction of target covered >=10X (%)	92.90%	87.40%	88.70%
Fraction of target covered >=20X (%)	88.50%	79.50%	81.10%
Duplication rate (%)	4.6641	5.5005	5.4762

Table S5 Summary of coding SNVs and indels for each sample

Number of SNVs*/indels	**III-4**	**III-13**	**IV-9**
Synonymous coding	197	158	161
Missense variant (MV)	219	176	189
Nonsense variant(NV)	6	5	5
Splice site SNPs (SS)**	1	1	3
Unknow	1	1	1
Frame shift indels	4	3	2
Nonframeshift indels	3	3	2
Splice site indels (SI) ***	1	0	0
Unknown	1	1	1
Total variants (MV, NV, SS, and indel)	234	188	201

*SNVs (Single nucleotide variants)
**Splice site SNPs (SS) is intronic SNPs within 10 bp of exon/intron boundary
** Splice site indels (SI) is intronic indels within 10 bp of exon/intron boundary

Table S6 Screening and identification of the causal genes by XES (SNP*)

Feture SNP	**III-4**	**III-13**	**IV-9**
Total SNPs	424	341	359
Filtered_DBsnp	16	22	18
Filtered_DBsnp_1000gene	16	22	18
Share all cases		6	

(*Function: missense|readthrough|nonsense|splicesite|synonymous-coding|5-UTR|3-UTR|intron|intergenic)

Table S7 Screening and identification of the causal genes by XES (indels*)

Feture indels	**III-4**	**III-13**	**IV-9**
Total indels	9	7	5
Filtered_DBsnp	3	4	2
Filtered_DBsnp_1000gene	3	4	2
Share all cases		2	

(*Function: frameshift|cds-indel|splicesite|5-UTR|3-UTR|intron|promoter|intergenic)

Table S8 Screening and identification of the causal genes by XES (Functional SNPs*)

Feture SNP	**III-4**	**III-13**	**IV-9**
Functional_SNPs	226	182	197
Filtered_DBsnp	8	10	8
Filtered_DBsnp_1000gene	8	10	8
Share all cases		3	

(*Function: missense|readthrough|nonsense|splicesite)

Table S9 Screening and identification of the causal genes by XES (Functional indels*)

Feture indels	**III-4**	**III-13**	**IV-9**
Functional indels	8	6	4
Filtered_DBsnp	3	4	2
Filtered_DBsnp_1000gene	3	4	2
Share all cases		2	

(*Function: frameshift|cds-indel|splicesite)

Table S10 Candidate variants shared by three included individuals in XES

Chromosome	Position	Reference	Change	Gene	Variant	Substitution	MutRatio%[1] (III-4/III-13/ IV-9)	Genotype[2] (III-4/III-13/ IV-9)
chrX	114425181	-	GAGGCCGCTCGCCC AACGCCCACAGCG	*RBMXL3*	c.1177_1178insGAGGCCGCT CGCCCAACGCCCACAGCG	p.R393delinsR GRSPNAHSG	100.0/100.0/100.0	Homo/hemi/hemi
chrX	122336600	-	G	*GRIA3*	c.381_382insG	p.G127fs	100.0/100.0/100.0	Homo/hemi/hemi
chrX	**101971514**	**G**	**A**	***GPRASP2***	**c.1717G>A**	**p.A573N**	44.5/100.0/100.0	Hetero/hemi/
chrX	**101971515**	**C**	**A**	***GPRASP2***	**c.1718C>A**		hemi 44.4/100.0/100.0	Hetero/
chrX	140994062	C	G	*MAGEC1*	c.872C>G	p.T291S	9.4/ 14.7/ 14.3	hemi/hemi N /N /N

[1] MutRatio: mutation ratio (%)
[2] Genotype: homo – homozygote (MutRatio = 100%), hemi – hemizygote (MutRatio = 100%), hetero – heterozygote (MutRatio ≈ 50%), N – not certain (MutRatio ≤ 30%)