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Table S1. Case report form of infants who showed deafness-causing genotypes in *GJB2* and *SLC26A4*

Serial number:
Sample number:
Mother's name:
Baby's name:
Gender:
Birth date:

Items	Please tick at □	Remark
1. Were guardians contacted?	1. No□ 2. Yes□	If no, jump to 20
2. Results of hearing screening results before follow-up	1. Referred in left□ 2. Referred in right □ 3. Referred bilateral □ 4. Pass bilateral□	Found from original data
3. Was hearing diagnosis performed?	1. No□ 2. Yes□	If no, jump to 18. 19
4. Is there any hearing loss?	1. No□ 2. Yes□	If no, jump to 18. 19
5. Is there any family history of deafness?	1. No□ 2. Yes□	
6. Sides of hearing loss?	1. Left ear□ 2. Right ear□ 3. Bilateral □	
7. Types of hearing loss?	1. Sensorineural hearing loss□ 2. Conductive hearing loss□ 3. Mixed Hearing Loss□	
8. Hearing threshold in 0.5kHz, 1 kHz, 2 kHz, and 4 kHz.	1. Left ear 2. Right ear	
9. Degree of hearing loss?	1. Mild□ 2. Moderate□ 3. Severe□ 4. Profound□	
10. Was intervention given	1. No□ 2. Yes□	If no, jump to 18. 19
11. Hearing aids (HA)	1. No□ 2. Yes□	If no, jump to 13
12. Age in month when given HA	□□Age in month	
13. Cochlear implantation (CI)	1. No□ 2. Yes□	If no, jump to 15
14. Age in month when given CI	□□Age in month	
15. Exercise at home	1. No□ 2. Yes□	
16. Habilitation training	1. No□ 2. Yes□	
17. Parents' satisfaction	1. Satisfied □ 2. Dissatisfied □	
18. Hearing status from self-observation	1. Normal □ 2. Basically normal □ 3. Abnormal □	
19. Results of hearing screening in community	1. Referred in left□ 2. Referred in right□ 3. Referred bilateral □ 4. Pass bilateral□ 5. Uncertain□ 6. No□	
20. Loss of follow-up	1. No□ 2. Yes□	

Recorder name:
Recording time:

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Table S2. Schematic diagram of the detection sites and quality control settings of the chip

Row \ Column	1-3	4-6	7-9
1	QC	BC	PC
2	35W	538W	1975W
3	35M	538M	1975M
4	176W	2168W	2027W
5	176M	2168M	2027M
6	MC	IC	MC
7	235W	IVS7-2W	1226W
8	235M	IVS7-2M	1226M
9	299W	1174W	1229W
10	299M	1174M	1229M
11	1494W	IVS15+5W	1555W
12	1494M	IVS15+5M	1555M
13	PC	NC	QC

QC: Surface chemical quality control probes; IC: Gene amplification internal control probes MC: Magnetic bead quality control probes; PC: Microarray quality control probes; BC: Blank control probes; NC: Negative control probes.

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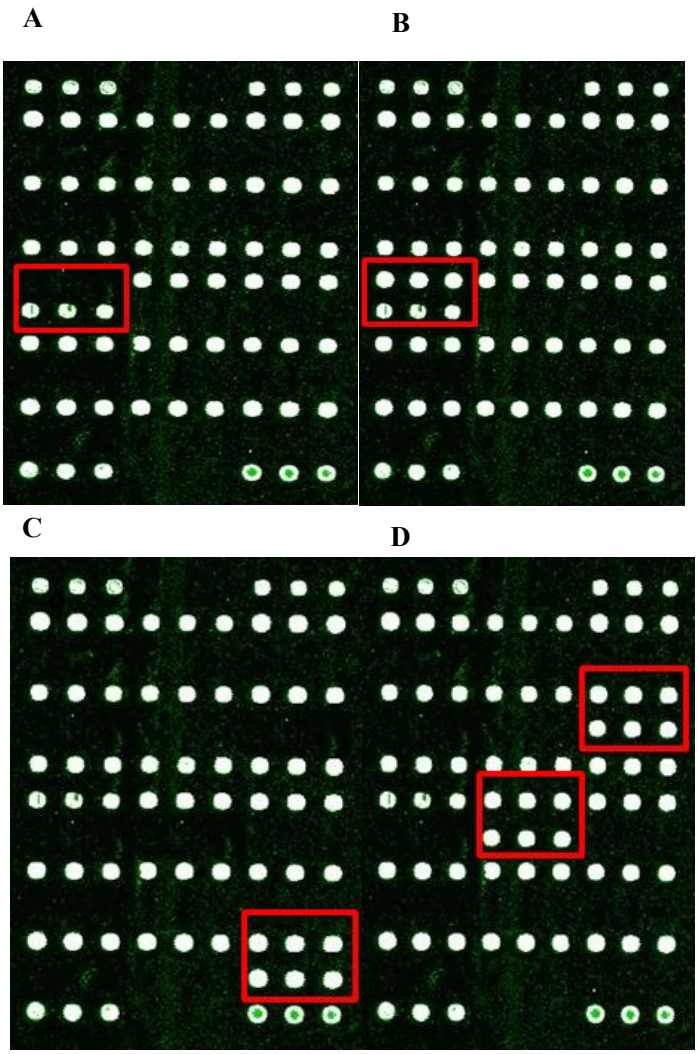

Figure S1. Mutation detection examples

(A) *GJB2* gene c.235delC homozygous mutation; (B) *GJB2* gene c.235delC heterozygous mutation; (C) MtDNA 12SrRNA m.1555A>G homogeneous mutation; (D) *SLC26A4* gene c.2027T>A/ c.919-2A>G compound heterozygous mutation.

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Table S3. Beijing Newborn Deafness Genetic Screening Report Card

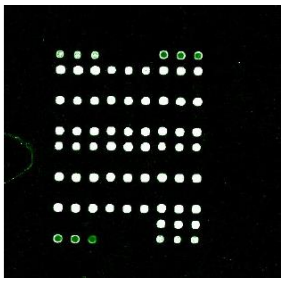
北京市新生儿耳聋基因筛查报告单

【基本信息】

筛查编号		采血单位		采血日期	
母亲姓名		户籍		体重	
性别		出生日期		手机	
样品编号		筛查单位		样本类型	干血斑

检测项目：十五项遗传性耳聋相关基因检测
检测方法：微阵列芯片法
仪器型号：LuxScan-10K/B 微阵列芯片扫描仪

【检测结果】（以线粒体 12SrRNA m.1555A>G 异质突变为例）

序号	基因名称	突变位点	检测结果	结果说明	检测结果图
1	*GJB2* (NM_004004.5)	c.35delG	正常	正常	
2	*GJB2* (NM_004004.5)	c.176_191del16	正常	正常	
3	*GJB2* (NM_004004.5)	c.235delC	正常	正常	
4	*GJB2* (NM_004004.5)	c.299_300delAT	正常	正常	
5	*GJB3*(NM_024009.2)	c.538C>T	正常	正常	
6	*SLC26A4*(NM_000441.1)	c.2168A>G	正常	正常	
7	*SLC26A4*(NM_000441.1)	c.919-2A>G (同:c.IVS7-2A>G)	正常	正常	
8	*SLC26A4*(NM_000441.1)	c.1174A>T	正常	正常	
9	*SLC26A4*(NM_000441.1)	c.1226G>A	正常	正常	
10	*SLC26A4*(NM_000441.1)	c.1229C>T	正常	正常	
11	*SLC26A4*(NM_000441.1)	c.1975G>C	正常	正常	
12	*SLC26A4*(NM_000441.1)	c.2027T>A	正常	正常	
13	*SLC26A4*(NM_000441.1)	c.1707+5G>A (同:c.IVS15+5G>A)	正常	正常	
14	*MT-RNR1*(12SrRNA)	m.1494C>T	正常	正常	
15	*MT-RNR1*(12SrRNA)	m.1555A>G	异质突变型	异常	

【筛查结果】
☐通过 ☑未通过

【用药指南】
您本人及母系家族成员应绝对终生禁用或慎用氨基糖甙类抗生素：链霉素. 卡那霉素. 妥布霉素（抗普霉素）. 大观霉素. 新霉素. 庆大霉素. 威地霉素. 西索米星（紫苏霉素. 西索霉素）. 小诺霉素. 阿司米星. 阿米卡星（丁胺卡那霉素）. 奈替米星（奈特. 力确兴. 诺达）. 核糖霉素. 爱大（硫酸依替米星）. 依克沙（硫酸异帕米星）. 小儿利宝（硫酸庆大霉素）等。

【建议】
☐通过本次相关耳聋基因筛查，并请关注听力筛查结果。
■未通过本次相关耳聋基因筛查，此结果异常并非最终诊断，需要进一步的听力学随访和耳聋基因诊断，请于工作时间致电下方医院预约就诊时间。

检验员：_____ 报告签发者：_____ 筛查日期： 年 月 日

【说明】
此结果仅对本份检测标本负责！由于儿童耳聋发病原因的复杂性，本次耳聋基因筛查仅对中国常见的 4 个耳聋相关基因（*GJB2*. *GJB3*. *SLC26A4* 及线粒体 *12SrRNA*）的 15 个突变位点进行筛查，无法涵盖与遗传性耳聋相关的所有突变位点。当检测结果为“正常”时，并不能排除受检者携带其它与遗传性耳聋相关的突变基因及其他原因造成的耳聋。当受检者在待测位点附近出现罕见或未知突变时，可能导致该位点无检测信

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号。因此，即便是筛查结果为通过，也并不能完全排除听力障碍的可能，如在发现孩子听力异常时应及时就医。

【遗传咨询医院】

医院名称	医院地址	咨询电话	就诊须知
首都医科大学附属北京同仁医院	北京市东城区东交民巷 1 号（崇文门）	01058265831	门诊须知：关注“京医通”公众号，点击“就诊服务”-挂号-北京同仁医院-崇文门院区（东西区），有两个时间段： 1.周一下午：点击“耳鼻咽喉头颈外科”-耳研所门诊（西区）-周一下午-儿童听力诊疗专台黄丽辉主任。 2.周三上午：点击“耳鼻咽喉头颈外科会诊中心”-耳研所会诊中心（东区）-周三上午-儿童听力诊疗专台黄丽辉主任。 程晓华大夫（儿童听力言语主治医师 01），周二和周五上午，周四下午。就诊地点：北京同仁医院东西区（崇文门）。提示：就诊时，请持听力筛查和基因筛查报告单。

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Table S3. Beijing Newborn Deafness Genetic Screening Report Card

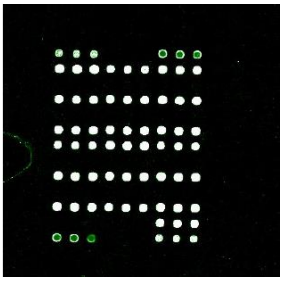
Beijing Newborn Deafness Genetic Screening Report Card

【Basic Information】

Screening Number		Blood Collection Institution		Blood Collection Date	
Mother's name		Domicile Address		Birth Weight	
Gender		Birth Date		Mobile Phone	
Sample Number		Screening Institution		Sample Type	Dried Blood Spots

Test item：Fifteen deafness genetic mutations test
Detection Method：Microarray chip method
Instrument model：LuxScan-10K/B Microarray chip scanner

【Test Result】（Take mtDNA *12SrRNA* m.1555A>G heteroplasmy as an example）

No.	Gene	Mutation	Detection Result	Result Description	Test result figure
1	*GJB2* (NM_004004.5)	c.35delG	Wild Type	Normal	
2	*GJB2* (NM_004004.5)	c.176_191del16	Wild Type	Normal	
3	*GJB2* (NM_004004.5)	c.235delC	Wild Type	Normal	
4	*GJB2* (NM_004004.5)	c.299_300delAT	Wild Type	Normal	
5	*GJB3*(NM_024009.2)	c.538C>T	Wild Type	Normal	
6	*SLC26A4*(NM_000441.1)	c.2168A>G	Wild Type	Normal	
7	*SLC26A4*(NM_000441.1)	c.919-2A>G (c.IVS7-2A>G)	Wild Type	Normal	
8	*SLC26A4*(NM_000441.1)	c.1174A>T	Wild Type	Normal	
9	*SLC26A4*(NM_000441.1)	c.1226G>A	Wild Type	Normal	
10	*SLC26A4*(NM_000441.1)	c.1229C>T	Wild Type	Normal	
11	*SLC26A4*(NM_000441.1)	c.1975G>C	Wild Type	Normal	
12	*SLC26A4*(NM_000441.1)	c.2027T>A	Wild Type	Normal	
13	*SLC26A4*(NM_000441.1)	c.1707+5G>A (c.IVS15+5G>A)	Wild Type	Normal	
14	*MT-RNR1*(12SrRNA)	m.1494C>T	Wild Type	Normal	
15	*MT-RNR1*(12SrRNA)	m.1555A>G	Heteroplasmy	Abnormal	

【Screening Result】
☐Pass ☑Refer

【Medication Guide】
You and the members of your maternal family should absolutely prohibit or carefully use aminoglycoside antibiotics for the whole life: streptomycin, kanamycin, tobramycin (antipromycin), spectinomycin, neomycin, gentamycin, widamycin, sisomicin (perilla mycin), micronomicin, astromicin, amikacin, netilmicin, ribomycin, etimicin sulfate, isopalmicin sulfate, gentamycin sulfate, etc.

【Suggestion】
☐Pass the deafness genetic screening, and pay attention to the hearing screening results.
■Fail the deafness genetic screening, and the abnormal result is not the final diagnosis. Further audiological follow-up and deafness gene diagnosis are required. Please call the following hospital during working hours to make an appointment.
Inspector：_____ Issuer of report：_____Screening date：_____

【Explanation】
This result is only responsible for this test sample! Due to the complexity of deafness pathogenesis in children, this deafness gene screening only screened 15 mutations of four common deafness genes in China (*GJB2*, *GJB3*, *SLC26A4* and mtDNA *12SrRNA*), and could not cover all mutations related to hereditary deafness. When the test

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result is "Normal", it cannot be ruled out that the subject carries other deafness genes and other deafness pathogenesis. When the subject has a rare or unknown mutation near the mutation to be tested, it may lead to no detection signal at the mutation. Therefore, even if pass the genetic screening, the possibility of hearing impairment cannot be completely excluded. If children's hearing abnormalities are found, they should seek medical advice in time.

【Genetic Counseling Hospital】

Hospital Name	Address	Consulting telephone	Patients Need-to-Know
Beijing Tongren Hospital, Capital Medical University	No.1 Dong-Jiao-Min Lane, Dong-Cheng District, Beijing (Chong-Wen-Men)	01058265831	Notice for outpatient service: subscribe the We-Chat official account "Jing-Yi-Tong", click "medical service" - “registration” - “Beijing Tongren Hospital” - “Chong-Wen-Men Hospital Area (Eastern and Western District)”. There are two time periods for doctor Huang Li-Hui: 1. Monday afternoon: click "ENT Head and Neck Surgery" - “outpatient service of Beijing Institute of Otolaryngology (Western District) - “Monday afternoon” - “doctor Huang Li-Hui of the Children's Hearing Diagnosis and Treatment”. 2. Wednesday morning: click "ENT Head and Neck Surgery Consultation Center" - “Beijing Institute of Otolaryngology (Eastern District)” - “Wednesday morning”- “Doctor Huang Li-Hui of Children's Hearing Diagnosis and Treatment”. There are three time periods for doctor Cheng Xiao-Hua: Doctor Cheng Xiao-Hua (doctor-in-charge of children's hearing and speech 01), Tuesday and Friday morning, Thursday afternoon. Location: Eastern and Western District of Beijing Tongren Hospital (Chong-Wen-Men). Tip: Please take your hearing screening and genetic screening report cards at the time of your outpatient visit.

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Table S4. Profile of cases withdrew from the study

	Year group	
	2019	**2020**
Cases included in cohort (N)	54,359	39,106
Cases withdrew (N)	12,669	4,336
Cases included in analysis (N)	41,690	34,770
Dropout rate (%)	23.31	11.09

N, number of cases. In general, cases were withdrawn for several reasons: a) Absence of hearing screening results, b) Incomplete information of parent's name, c) Invalid results of hearing screening, d) Infant with non-Chinese nationality, e) Unmatched information due to twins or multiples, f) Lost to follow-up in second hearing screening, g) Unmatched information of unknown reason.

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Table S5. Clinical and genetic characteristics of 11 newborns with deafness-causing phenotypes in *GJB2* and *SLC26A4*

No.	Sex	Year	Hearing screening	Genotypes	Hearing follow-up diagnosis (L/R) *	Hearing follow-up diagnosis (L/R) **	Hearing management(L/R)
1	M	2019	Refer	*GJB2* c.235delC/ c.235delC	Profound/Severe	Profound/Severe	CI/HA
2	M	2019	Refer	*GJB2* c.235delC/ c.235delC	Moderate/ Moderate	Moderately Severe /Moderately severe	HA/HA
3	M	2019	Refer	*GJB2* c.235delC/ c.235delC	Lost to follow-up	Lost to follow-up	NA/NA
4	F	2019	Pass	*SLC26A4* c.2168 A > G/ c.1975 G > C	Moderate /Normal	Moderately severe /Normal	N/N
5	M	2019	Refer	*SLC26A4* c.2168 A > G/ c.2027 T > A	Profound/ Profound	Profound/ Profound	HA/HA
6	M	2019	Refer	*SLC26A4* c.2168 A > G/ c.2027 T > A	Moderate/Profound	Moderately Severe/Profound	HA/CI
7	M	2020	Refer	*GJB2* c.235delC/ c.235delC	Severe/ Severe	Severe/ Severe	HA/HA
8	F	2020	Refer	*SLC26A4* c.919-2 A > G/ c.919-2 A > G	Severe/Severe	Severe/ Severe	HA/HA
9	F	2020	Refer	*SLC26A4* c.919-2 A > G/ c.919-2 A > G	Moderate /Moderate	Moderately Severe /Moderately severe	HA/HA
10	F	2020	Refer	*SLC26A4* c.919-2 A > G/ c.1229 C > T	Mild/Moderate	Mild/Moderate	N/N
11	M	2020	Pass	*SLC26A4* c.2027 T > A/ c.2027 T > A	Normal/Normal	Normal/Normal	N/N

M=Male, F=Female, N = No, NA = not available, HA = hearing aid, CI = cochlear implant.

"Hearing threshold" refers to the minimum sound intensity that an ear can detect as an average of values at 500, 1000, 2000, 4000 Hz.

***** The hearing degree was diagnosed according to the grades of hearing loss and related hearing experience in the World Hearing Report conducted in 1997.

******The hearing degree was diagnosed according to the grades of hearing loss and related hearing experience in the World Hearing Report conducted in 2021.