

# A novel variant in MITF in a child from Yunnan-Guizhou Plateau with autosomal dominant inheritance of nonsyndromic hearing loss: A case report

ZHEN ZHANG<sup>1,2\*</sup>, QUAN-DONG CHEN<sup>3\*</sup>, LI-PING ZHAO<sup>3</sup>, JING MA<sup>3</sup>, TIE-SONG ZHANG<sup>3</sup>, JING-XUE PANG<sup>4</sup>, YANG-FANG LI<sup>4</sup>, MEI-FEN WANG<sup>4</sup>, AI-PING WANG<sup>4</sup>, LI TANG<sup>4</sup>, LI-JUN LI<sup>4</sup>, WEN-JI HE<sup>4</sup> and HUAIYU GU<sup>1</sup>

<sup>1</sup>Department of Human Anatomy, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, Guangdong 510080;

<sup>2</sup>Yunnan Pediatric Institute; <sup>3</sup>Department of Otolaryngology-Head Neck Surgery; <sup>4</sup>Department of Ultrasonic Cardiogram, Kunming Children's Hospital, Kunming Medical University, Kunming, Yunnan 650228, P.R. China

Received June 29, 2017; Accepted January 2, 2018

DOI: 10.3892/mmr.2018.8627

**Abstract.** Deafness and hearing loss may have functional, economic, social and emotional impacts on humans, including the ability of an individual to communicate with others, feelings of isolation and frustration, and health sector costs. The World Health Organization reported that there are 32 million children worldwide with hearing loss. In order to investigate genetic mutations in children of 26 nationalities with hearing loss in Yunnan, Sanger sequencing was employed to screen for mutations in four of the most common pathological genes, including gap junction protein  $\beta 2$  and 3, solute carrier family 26 member 4 and mitochondrial DNA. Whole exome sequencing was used to detect the mutation in the proband of a family in which these four genes were normal. Subsequently, the mutation was identified by Sanger sequencing. The present study reports a novel mutation, c.718C>G; p. (Arg240Gly) in the melanogenesis associated transcription factor gene, in Han people with hearing loss. The results of the present study may provide parents and children an accurate diagnosis, which may allow physicians to how to rehabilitate children's hearing.

## Introduction

Hearing loss (HL), which is a common sensory disorder, may affect humans of all ages, from newborns to the elderly. According to the World Health Organization, >5% of the world's population, 360 million people, have disabling HL (328 million adults and 32 million children) (1). In China, the office of the Second Sampling Survey on Disabled People reported that there were 27,800,000 people with this disorder in 2006; this disorder is a birth defect that affects ~1 in 1,000 children in China (2).

HL may be caused by genetic or environmental factors, or a combination of the two, and >50% of cases are caused by genetic factors (3). With respect to the mode of inheritance, this disorder may be classified as autosomal recessive (77%), autosomal dominant (22%), or X- or Y-linked and mitochondrial inheritance (1%) (4). There are two clinical types: Syndromic (S)HL and nonsyndromic (NS)HL. SHL is usually distinguished via systemic medical examination (5); however, NSHL is frequently classified as SHL by doctors, as NSHL does not display other associated clinical features (6). NSHL accounts for ~70% of hereditary HL (6). At present, >80 genes and >100 genetic loci have been implicated in NSHL (7). In China, the most frequent causative genes, in order of frequency, are gap junction protein  $\beta$  (GJB)2, GJB3, solute carrier family 26 member 4 (SLC26A4) and mitochondrial (mt)DNA for HL (3), and these four genes are screened by doctors in persons with HL. However, certain patients with NSHL do not have mutations in these four genes; certain patients, therefore, remain genetically unexplained. Traditionally, studies into a family pedigree were performed by linkage analysis and candidate gene Sanger sequencing. When the pedigree is very large or very small, it is difficult to identify causative mutations via regular Sanger sequencing for numerous cases of NSHL (8). Whole exome sequencing (WES), a technique for sequencing all of the expressed genes in a genome, has become an effective alternative strategy (9).

In the present study, to investigate the genetic causes of deafness in children in the Yunnan province, which has been inhabited by 26 nationalities throughout history, mutations in

*Correspondence to:* Dr Huaiyu Gu, Department of Human Anatomy, Zhongshan School of Medicine, Sun Yat-sen University, 74 Zhongshan Road, Guangzhou, Guangdong 510080, P.R. China  
E-mail: 952259505@qq.com

Dr Wen-Ji He, Department of Ultrasonic Cardiogram, Kunming Children's Hospital, Kunming Medical University, 288 Qianxing Road, Kunming, Yunnan 650228, P.R. China  
E-mail: hewenj@etiy.cn

\*Contributed equally

**Key words:** novel variant, melanogenesis associated transcription factor gene, hearing loss, children, Yunnan-Guizhou Plateau

two deaf families were investigated. A novel mutation in the melanogenesis associated transcription factor (MITF) gene was identified in one family from Yunnan-Guizhou Plateau with two siblings affected by congenital sensorineural HL and heterozygous GJB2 c.235delC del/del variant was detected in another family from Yunnan-Guizhou Plateau with two siblings affected by congenital sensorineural HL.

## Case report

**Subjects.** A total of two consanguineous Chinese Han families from Yunnan-Guizhou Plateau were recruited by the Children's Hospital, Kunming Medical University (Kunming, China), for genetic diagnosis. Within these two families, two siblings suffered from bilateral prelingual deafness, although the hearing of the parents was normal. The present study was approved by the ethics committee of the Children's Hospital, Kunming Medical University; written informed consent was obtained from participants or their guardians.

**Clinical evaluations.** All clinical and audiometric assessments were performed in the Children's Hospital and First Affiliated Hospital of Kunming Medical University. Pure tone audiometry, auditory brainstem response and distortion product otoacoustic emission were performed. To examine the inner malformation, computed tomography or magnetic resonance imaging (MRI) were employed in the present study. The 250, 500, 1,000, 2,000, 4,000 and 8,000 Hz settings of pure tone audiometry were used to assess the hearing level as follows: Normal [ $<20$  decibels hearing level (dBHL)], mild (20–40 dBHL), moderate (41–70 dBHL), severe (71–95 dBHL), and profound ( $>95$  dBHL) deafness (10).

**WES and variant analysis.** Peripheral blood samples (2 ml) in tubes containing 0.2 M EDTA were collected from the siblings and their parents. The peripheral blood samples for each subject were extracted using a blood DNA extraction kit, QIAamp DNA (Qiagen GmbH, Hilden, Germany). Following extraction, DNA was randomly fragmented with DNA enzymes (Tagment DNA Enzyme 1, TruSight One Sequencing Panel Series, Illumina Inc., San Diego, CA, USA) and purified with magnetic beads as described (11). DNA fragments (80.4 ng/ $\mu$ l) were amplified by ligation-mediated polymerase chain reaction (PCR) as described (11). Sequences of 'TAAGGCGA' and 'CTCTCTAT' were added using a 10-cycle PCR program. The thermocycling conditions were: An initial denaturation of 98°C for 30 sec, 10 cycles of denaturation at 98°C for 10 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec and a final extension of 72°C for 5 min (11). The DNA fragments were captured and purified twice using a TruSight One Sequencing panel according to the manufacturer's protocols (Illumina China, Shanghai, China).

Size-selected DNA fragments were amplified by PCR as described (11). A total of 5  $\mu$ l PCR Primer Cocktail was added to each well, and 20  $\mu$ l Enrichment Amp Mix was added. The total volume per well was 50  $\mu$ l. Subsequently, the mixture was centrifuged at 1,200  $\times$  g for 1 min at 4°C and then at 280  $\times$  g for 1 min at 4°C. The thermocycling conditions were described as aforementioned. Samples were purified to obtain a qualified captured library.

Each resulting qualified captured library was loaded on MiSeq Next 500 sequencing platforms (Illumina Inc.), and high-throughput sequencing for each captured library was performed to ensure that each sample met the desired average sequencing coverage. As an autosomal recessive pattern of inheritance was suspected, the candidates were two affected siblings and their parents who were expected to be heterozygous for the variant.

**Mutation analysis.** The debased reads or clean reads without adapters were mapped to the human reference genome (UCSC hg19; genome.ucsc.edu) by Burrows-Wheeler Alignment. All variants were screened with the single nucleotide polymorphism database version 142 (www.ncbi.nlm.nih.gov/projects/SNP/snp\_summary.cgi?build\_id=142), the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php), ClinVar (www.ncbi.nlm.nih.gov/clinvar), the 1,000 Genomes Project (www.internationalgenome.org), UniProt (www.uniprot.org/uniprot) and the National Heart, Lung and Blood Institute Exome Sequencing Project 6500 (https://esp.gs.washington.edu/drupal/) Functional prediction was performed via Sorting Intolerant from Tolerant (http://sift.jcvi.org/), a likelihood ratio test (12), Mutation Taster (www.mutationtaster.org), Mutation Assessor (mutationassessor.org/r3), Functional Analysis through Hidden Markov Models (fathmm.biocompute.org.uk), Genomic Evolutionary Rate Profiling (mendel.stanford.edu/SidowLab/downloads/gerp), PhyloP-2 (http://genetics.bwh.harvard.edu/pph2/), SiPhy (portals.broadinstitute.org/genome\_bio/siphy/index.html) and Polymorphism Phenotyping-2 (genetics.bwh.harvard.edu/pph2). Candidate variants were annotated using Annotate Variation software (http://annovar.openbioinformatics.org/en/latest/) (13).

**Mutation validation.** Sanger sequencing was performed with an ABI3500 sequencer (Applied Biosystems, Thermo Fisher Scientific Inc., Waltham, MA, USA) and PCR; thermocycling conditions: An initial denaturation of 94°C for 4 min, 32 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec and a final extension of 72°C for 7 min (14). The sequences of forward and reverse primers are presented in Table I and were used to confirm potential causative variants in this family.

## Clinical findings

**Clinical presentation of family I.** The family included two affected siblings and two unaffected parents. Audiogram analysis of this family revealed that the siblings had bilateral HL. The proband was a 12-year-old boy (II1; Fig. 1A) with deafness, although MRI analysis demonstrated that the inner ear was normal. When the proband was 6 years old, pure tone audiometry revealed profound bilateral sensorineural HL. The results of the acoustic immittance measurement demonstrated a type A tympanometric curve. The auditory brainstem response at 95 dB and distortion product otoacoustic emission were absent in both ears of the proband. The younger affected sibling, a 2-year-old girl (II2; Fig. 1A) with severe sensorineural HL, had a normal inner ear and a type A tympanometric curve. The younger sibling had bilaterally absent distortion product otoacoustic emission and a 90-dB auditory brainstem response. The father

Table I. PCR primers for amplification.

Gene	Exon	Forward primers	Reverse primers
MITF	8	TACACGGCTTGGGTGGTG	GCACATGTCCAAGAATGACTG
CHD7	29	AATCTTAATGAGTCATCCTGTTTG	ACTTGGGGGAGAATTCAAGGG

CHD7, chromodomain DNA helicase binding protein 7; MITF, melanogenesis associated transcription factor.

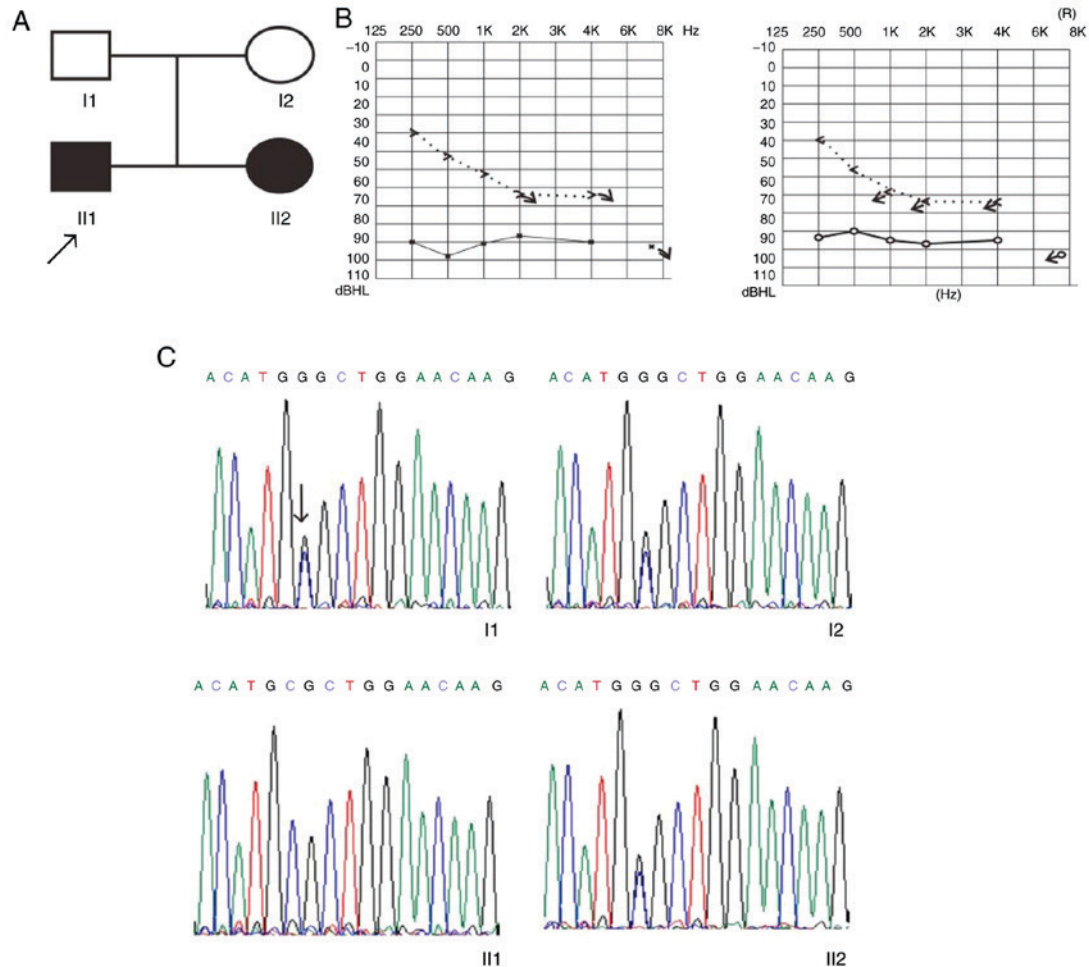


Figure 1. (A) Pedigree of family 1 with nonsyndromic hearing loss. Affected subjects were denoted as blank. The arrow indicates the proband. (B) Audiograms of the proband. 'o' indicates air conduction for the right ear, while 'X' indicates air conduction for the left ear; '<' indicates bone conduction for the right ear, while '>' indicates bone conduction for the left ear. '∟' and '∟' represent no response in the right and left ear, respectively. (C) Heterozygous melanogenesis associated transcription factor c.718C>G variant of the proband. Arrows denote the mutations. I1, the unaffected father; I2, the unaffected mother; II1, the proband; II2, the affected younger sister. dBHL, decibels hearing level.

and mother (45 and 35 years old, respectively) had no HL (I1 and I2; Fig. 1A). The clinical information for this family is summarized in Table II.

The genes GJB2, GJB3, SLC26A4 and mtDNA were sequenced in this family to exclude mutations in the four genes known to be associated with hereditary HL; however, no mutations were detected in these four genes (Table III).

**Clinical presentation of family 2.** This family included two affected siblings (a 3-month-old and a 3-year-old, both girls) and two unaffected parents. Sanger sequencing demonstrated

a homozygous and heterozygous GJB2 c.235delC del/del variant in the two siblings and unaffected parents, respectively (Table III).

**WES.** WES was used to sequence the proband of family 1, and heterozygous MITF c.718C>G and chromodomain DNA helicase binding protein 7 (CHD7) c.5770T>C variants were detected in this proband.

**Identification of pathogenic mutation.** In family 1, the heterozygous MITF c.718C>G and CHD7 c.5770T>C variants were confirmed by Sanger sequencing. The heterozygous MITF

Table II. Phenotypes and genotypes of family 1.

Subject	Age, years	Hearing loss	ABR	DPOAE	CT	MRI	Genotype
Father	45	N/A	Present	Present	N/A	Normal	Wild-type
Mother	35	N/A	Present	Present	N/A	Normal	Mutation
Boy	12	L (profound), R (severe)	Absent	Absent	Normal	Normal	Mutation
Girl	2	Bil (severe)	Absent	Absent	Normal	Normal	Mutation

DPOAE, distortion product otoacoustic emission; ABR, auditory brainstem response; L, left; R, right; MRI, magnetic resonance imaging; CT, computed tomography; Bil, bilateral.

Table III. Gene mutations of family 1 and 2.

Gene or mitochondria	Family 1				Family 2			
	Proband	Younger sister	Father	Mother	Proband	Younger sister	Father	Mother
GJB2	Normal	Normal	Normal	Normal	c.235delC (homozygous mutation)	c.235delC (homozygous mutation)	c.235delC (heterozygous mutation)	c.235delC (heterozygous mutation)
GJB3	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
SLC26A4	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
mtDNA	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

GJB3, gap junction protein  $\beta$ ; mt, mitochondria; SLC26A4, solute carrier family 25 member 4.

c.718C>G variant was identified in the affected sibling and unaffected mother (Fig. 1C).

## Discussion

In the present study, GJB2, GJB3, SLC26A4 and mtDNA were screened in two families with NSHL, and a novel c.718C>G variation in the MITF gene in family 1 was detected by WES. The MITF gene was named after mice with a mutation in this particular gene (15), and Hemesath *et al* (16) demonstrated that MITF functions in melanocyte survival; pigmentation is mediated by Mitf family interactions and transcriptional activities. In 1994, a mutation in the MITF gene was detected in humans with HL (17). According to the study of Yang *et al* (14) proposed that a MITF mutation (Mitf) database should be created for those with Waardenburg syndrome in the Chinese population. The locus of human and mouse MITF covers 229 kb and starts at ~214 kb from the beginning of exon 1A to the end of exon 9 (including the extended 3' untranslated region), respectively. The MITF/Mitf locus is over 200 kb in length, with strong but imperfect exon conservation between human and mouse (18). A total of  $\geq 9$  isoforms of MITF/Mitf have been identified, and the expression profile of each isoform varies (19,20). MITF/Mitf functions are associated with numerous inherited disorders of humans and mice (21). Elimination of the MITF-M isoform alone is sufficient to cause deafness and depigmentation (22).

According to genome databases (grenada.lumc.nl/LOVD2/ for humans, and www.informatics.jax.org for mice), ~89 MITF mutations in human and mouse alleles have been reported. A total of >40 MITF mutations have been identified in a number of people with Waardenburg's syndrome type 2 and in Tietz syndrome families; 9 mutations of the MITF gene were identified in Chinese patients with Waardenburg's syndrome type 2 (14).

At present, severe or profound HL is frequently treated with a cochlear implant (23). In particular, when those with HL secondary to GJB2 (or Cx26) (OMIM: 121011) mutations were fitted with cochlear implants, excellent speech and language performance was observed (24). However, the cochlear implant was only used for analysis study in a large mammal Rongchang pigs with mutations in the MITF gene (25).

In the present study, the heterozygous c.718C>G variant in the MITF gene was identified in two affected siblings, although it was absent in one unaffected parent. This variant of MITF may be the deafness disease-causing mutation in this particular family.

Congenital causes led to HL in the second child of the family again, although the affected parents were told that the first child has hearing loss. These might be that the parents did not initially receive a genetic diagnosis for the first child, and were thus unaware of the possible inherited nature of this HL. Therefore, the present study aimed to characterize the severity of HL and advise on the use of hearing aids, early identification and prenatal genetic diagnosis to parents in Yunnan.



## Acknowledgements

The authors would like to thank Mrs. Yu Wang (Beijing Myogenostics Co., Ltd., Beijing, China) for advice regarding data analysis. We would also like to thank the Miss Yali Guo of the Department of Otolaryngology-Head Neck Surgery, Kunming Children's Hospital, Kunming Medical University (Kunming, China) for the clinical data collection.

## Funding

The present study was supported by funds from the Health and Family Planning Commission of Kunming and Yunnan, respectively [grants nos. 2015-1-S-00967, 2016-SW (Sheng)-30, 2017-SW (Yang)-02, 2016NS125 and D-201254].

## Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding authors upon reasonable request.

## Authors' contributions

Conceptualization of the present study was performed by ZZ, HG, WJH and QC, ZZ, JM, YFL and MFW conducted data curation. Acquisition of funding was carried out by ZZ and QDC. ZZ, TSZ, JXP, YFL, MFW and LPZ conducted the investigations. Methodologies were suggested by ZZ, QDC, LPZ, APW, LT and LJL. Project administration was performed by HG and WJH. Software were suggested by APW, LT and ZZ. Supervision was performed by WJH and HG. ZZ prepared the manuscript, ZZ and HG reviewed and edited the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The present study was approved by the ethics committee of the Children's Hospital, Kunming Medical University; written informed consent was obtained from participants or their guardians.

## Consent for publication

Written informed consent for publication of their clinical details and data was obtained from participants or their guardians.

## Competing interests

The authors declare that they have no competing interests.

## References

- Deafness and hearing loss fact sheet No. 300. WHO; <http://www.who.int/mediacentre/factsheets/fs300/en/>, 2015.
- Li L, Lu JG, Liu Y and Xiao YL: Common deafness genes detection and hot mutation spots analysis in patients with congenital non-syndromic hearing loss. *Chinese Journal of otology* 10: 246-251, 2012.
- Jiang L, Ling Y, Cai G and Wang R: Research on the clinical application of DNA microarray on deafness gene mutations. *J Mol Diag Ther* 3: 170-172, 2011.
- Smith RJ: Clinical application of genetic testing for deafness. *Am J Med Genet A* 130A: 8-12, 2004.
- Petersen MB and Willems PJ: Non-syndromic, autosomal-recessive deafness. *Clin Genet* 69: 371-392, 2006.
- Chen PH and Yang T: Whole exome sequencing helps diagnose Muckle-Wells syndrome in a Chinese family with autosomal dominant hearing loss. *J Shanghai Jiao Tong Univ (Med Sci)* 36: 1135-1139, 2016.
- Li S, Peng Q, Liao S, Li W, Ma Q and Lu X: A reverse dot blot assay for the screening of twenty mutations in four genes associated with NSHL in a Chinese population. *PLoS One* 12: e0177196, 2017.
- Shearer AE, DeLuca AP, Hildebrand MS, Taylor KR, Gurrola J II, Scherer S, Scheetz TE and Smith RJ: Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. *Proc Natl Acad Sci USA* 107: 21104-21109, 2010.
- Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloglu A, Ozen S, Sanjad S, *et al*: Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci USA* 106: 19096-19101, 2009.
- Bae SH, Baek JI, Lee JD, Song MH, Kwon TJ, Oh SK, Jeong JY, Choi JY, Lee KY and Kim UK: Genetic analysis of auditory neuropathy spectrum disorder in the Korean population. *Gene* 522: 65-69, 2013.
- TruSight One Sequencing Panel Series Reference Guide. <https://support.microsoft.com/en-us/help/17621/internet-explorer--downloads>. Accessed August, 2017.
- Chun S and Fay JC: Identification of deleterious mutations within three human genomes. *Genome Res* 19: 1553-1561, 2009.
- Wang K, Li M and Hakonarson H: ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 38: e164, 2010.
- Yang SZ, Dai P, Liu X, Kang D, Zhang X, Yang W, Zhou C, Yang S and Yuan H: Genetic and phenotypic heterogeneity in Chinese patients with Waardenburg syndrome type II. *PLoS One* 8: e77149, 2013.
- Hertwig P: Neue mutationen und Kopplungsgruppen bei der Hausmaus. *Z Induct Abstammungs-Vererbungs* 80: 220-246, 1942.
- Hemesath TJ, Steingrímsson E, McGill G, Hansen MJ, Vaught J, Hodgkinson CA, Amheiter H, Copeland NG, Jenkins NA and Fische D E: Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. *Genes Dev* 8: 2770-2780, 1994.
- Tassabehji M, Newton VE and Read AP: Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. *Nat Genet* 8: 251-255, 1994.
- Hershey CL and Fisher DE: Genomic analysis of the Microphthalmia locus and identification of the MITF-J/Mitf-J isoform. *Gene* 347: 73-82, 2005.
- Steingrímsson E, Moore KJ, Lamoreux ML, Ferré-D'Amaré AR, Burley SK, Zimring DC, Skow LC, Hodgkinson CA, Arnheiter H, Copeland NG, *et al*: Molecular basis of mouse microphthalmia (mi) mutations helps explain their developmental and phenotypic consequences. *Nat Genet* 8: 256-263, 1994.
- Steingrímsson E, Copeland NG and Jenkins NA: Melanocytes and the microphthalmia transcription factor network. *Annu Rev Genet* 38: 365-411, 2004.
- Kawakami A and Fisher DE: The master role of microphthalmia-associated transcription factor in melanocyte and melanoma biology. *Lab Invest* 97: 649-656, 2017.
- Chen L, Guo W, Ren L, Yang M, Zhao Y, Guo Z, Yi H, Li M, Hu Y, Long X, *et al*: A de novo silencer causes elimination of MITF-M expression and profound hearing loss in pigs. *BMC Biol* 14: 52, 2016.
- Wu CC, Lee YC, Chen PJ and Hsu CJ: Predominance of genetic diagnosis and imaging results as predictors in determining the speech perception performance outcome after cochlear implantation in children. *Arch Pediatr Adolesc Med* 162: 269-276, 2008.
- Sinnathuray AR, Toner JG, Clarke-Lyttle J, Geddis A, Patterson CC and Hughes AE: Connexin 26 (GJB2) gene-related deafness and speech intelligibility after cochlear implantation. *Otol Neurotol* 25: 935-942, 2004.
- Chen W, Yi H, Zhang L, Ji F, Yuan S, Zhang Y, Ren L, Li J, Chen L, Guo W and Yang S: Establishing the standard method of cochlear implant in Rongchang pig. *Acta Otolaryngol* 137: 503-510, 2017.