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p.Y556C is a Recurrent Mutation in Pendred Syndrome causing Gene *SLC26A4* in Punjabi Population

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ABSTRACT

Previous studies have reported that Pendred Syndrome (PDS) contributes approximately 4.7-7.2% of recessive hereditary deafness in Pakistani population. More than 367 mutations of *SLC26A4* have been reported so far with PDS. Identification of recurrent mutations reveals widespread lineage diversity and mutational specificity of a population. This study reports p.Y556C mutation as a recurring mutation in Punjabi population of Pakistan. A detailed genetic evaluation of four unrelated but ethnically similar families from Punjab province was performed. Linkage to *SLC26A4* locus was confirmed through linkage analysis using Short Tandem Repeat Markers (D7S2420, D7S2459 and D7S2456). p.Y556C was identified as causative mutation by Sanger Sequencing. A known missense mutation p.Y556C in exon 15 of *SLC26A4* gene was identified in all the four families. It may be concluded that p.Y556C mutation is a recurrent mutation in Punjab province of Pakistan.

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Ion exchange, Pendred syndrome, p.Y556C, Founder mutation, Recurrent mutation.

INTRODUCTION

Pendred syndrome (PDS) (OMIM#274600) is reported worldwide as the second most common cause of syndromic deafness. It is a genetic disorder that shows clinical variability for sensorineural hearing loss and enlarged vestibular aqueduct (EVA) accomplished with manifestation of goiter due to thyroid dysfunction, whereas sensorineural deafness along with EVA characterizes DFNB4 (OMIM#600791) (Li *et al*., 1998). PDS accounts for up to 7.5% cases of congenital hearing loss (http://www.orpha.net). DFNB4/PDS is the second most common locus in Pakistani population (Jamal *et al*., 2012). More than 367 mutations of *SLC26A4* have been reported to be associated with DFNB4 locus (OMIM # 600791) and PDS (http://www.hgmd.cf.ac.uk). *SLC26A4* mutations outline a broad phenotypic spectrum between PDS and DFNB4 along with EVA (Tsukada *et al*., 2015), thus *SLC26A4* mutational screening is a valued test as these two conditions are difficult to diagnose clinically (Prasad *et al*., 2004).

SLC26A4 belongs to a group of genes called Solute Carriers (SLC) that have a vital role in maintaining ion balance in cochlea. After *GJB2, SLC26A4* is the second most common gene reported worldwide (Zhanga *et al*., 2012). Cytogenetic position of *SLC26A4* is at chromosome 7 long arm at position 31 (7q31). It comprises of 21 exons and encodes pendrin protein. This transmembrane protein exchanges Cl^-, I^-, HCO_3^- and other anions across cell membrane (Rehman *et al*., 2016). This anion transport executed by pendrin is critical for keeping the appropriate ionic balance within inner ear fluid, which has a crucial role in the hearing process (Everett *et al*., 1999).

SLC26A4 mutations account for the second most common cause of inherited hearing loss worldwide (Tsukada *et al*., 2015). Approximately 5% of *SLC26A4* mutations account for autosomal recessive hereditary deafness in Southern Asian population (Park *et al*., 2003). In Pakistani population, *SLC26A4* mutations account for 4.7-7.2% deafness (Yan *et al*., 2015). The present study was carried out on four Pendred families defining p.Y556C mutation recurrent and to be included in mutation spectrum of Pakistani population as founder mutation.

MATERIALS AND METHODS

For all subjects in study an approval from Institutional Review board at the National Centre of Excellence in Molecular Biology, Lahore, Pakistan. Informed written consents were obtained from all individuals enrolled in study. A comprehensive clinical and medical history was obtained from individual families. All participating members voluntarily provided 5-10 ml of a blood sample approximately that was kept in 50 ml Sterilin® falcon tubes having 400 ul of 0.5 M EDTA. Blood samples were kept at -20°C for long-term storage.

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Genomic DNA extraction

A described non-organic modified procedure was used to extract the genomic DNA from white blood cells (Grimberg *et al*., 1989). SmartSpec plus Bio-Rad Spectrophotometer (Bio-Rad, Hercules, CA) was used to estimate genomic concentration of the extracted DNA.

Exclusion analysis

For most prevalent loci (DFNB1, DFNB2, DFNB3, DFNB4, DFNB12 and DFNB39) in Pakistani population exclusion analysis was performed. Highly polymorphic fluorescent STR markers (D7S2420, D7S2459 and D7S2456) were used to perform linkage analysis for DFNB4 locus. A cocktail having HD-400 size standards and PCR products was prepared (Applied Biosystems) and run in Applied Bio systems 3100 DNA Analyzer. Gene Mapper software from Applied Bio systems was used to align genotypes.

Linkage analysis

SUPERLINK version of Easy linkage Program Package was used to perform linkage analysis on alleles obtained after exclusion analysis. MLINK of the FASTLINK computer Program Package was used to calculate maximum LOD scores, having allele frequency of disease at 0.001 while disease was considered fully penetrant. Equal meiotic recombination frequencies was set for males and females.

Mutation screening

Sequencing and PCR amplification were done, for 21 exons primer pairs, as describe earlier (Everett *et al*., 1997). PCR reactions for 10 ul volume containing 25 ng of genomic DNA was done. The reaction has a denaturation step at 94°C for 3 min followed by denaturation at 94°C for 45 seconds, followed by annealing for primer set specific at 57°C for 45 seconds for 30 cycles and elongation at 72°C

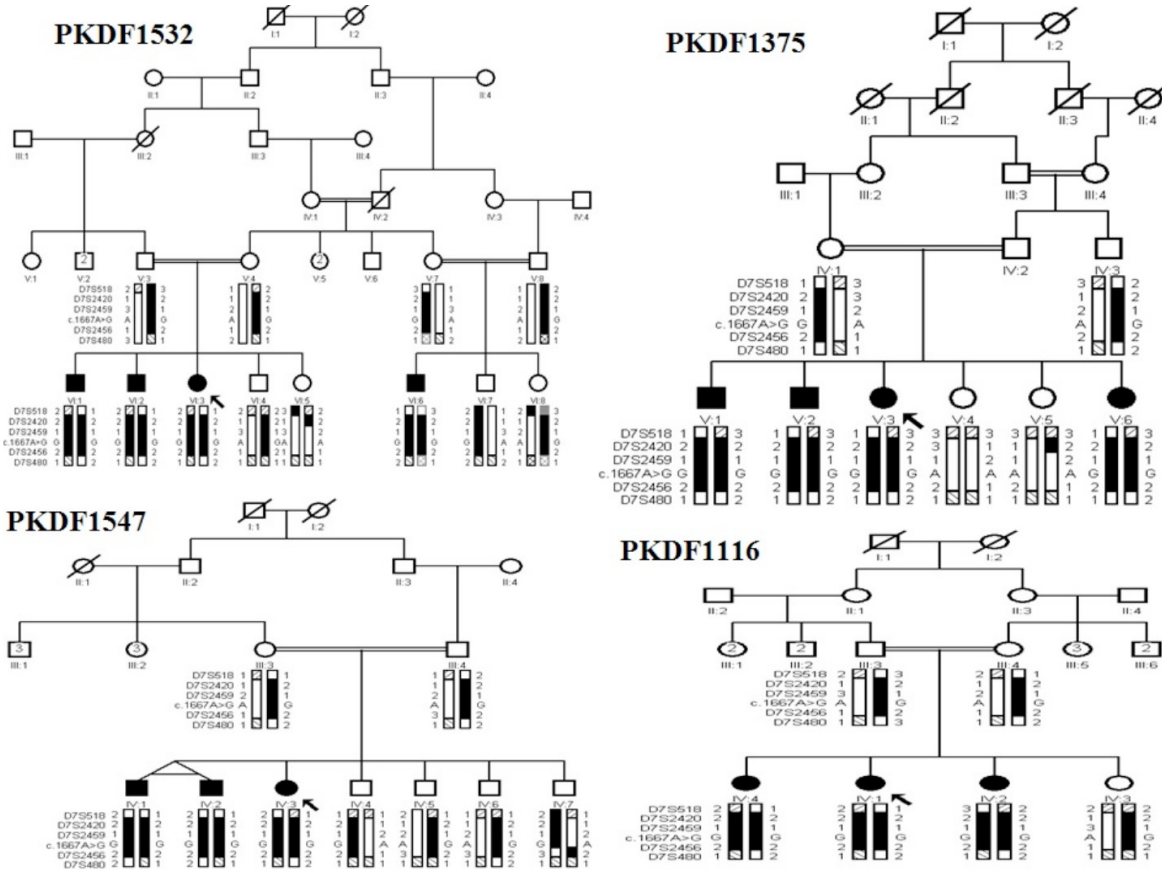

Fig. 1. Pedigrees showing haplotypes of chromosome 7q microsatellite markers. The alleles forming the risk haplotype shaded black, and alleles not segregating with Pendred syndrome shown in white. The vertical lines shows recombination crosses at proximal and distal sides of linkage region. Squares: males; Circles: females; Filled symbols: affected individuals; Double line between individuals: consanguineous marriages and a diagonal line through the symbol: deceased person. Carriers of c.1667A>G share a common haplotype (black color) not observed in non-carrier members.

for 45 seconds, followed by a final elongation 72°C for 10 min. For bidirectional sequencing PCR primers for each exon were used in BigDye Terminator Ready reaction mix, according to manufacturer instructions. The ABI PRISM 3100 DNA analyzer (Applied Biosystems) was used to analyze sequencing products and results were evaluated with Applied Biosystems SeqScape software.

Evolutionary conservation and in silico *analysis*

By aligning the protein sequence of *SLC26A4* orthologs, evolutionary conservation of amino acid was investigated. SIFT (http://sift.jcvi.org) and PolyPhen2 (http://genetics.bwh.harvard.edu) were referred for the evolutionary conservation and imaginable effect of amino acid substitution on the structure of *SLC26A4* protein.

RESULTS

In this study four consanguineous families: PKDF1375, PKDF1532, PKDF1547, and PKDF1116 with three or more individuals affected with hearing impairment were enlisted from Punjab province of Pakistan.

Exclusion studies resulted in linkage to DFNB4 locus. In family PKDF1532 a maximum LOD score of 2.37 at θ = 0 was observed with marker D7S2459 while in PKDF 1375, PKDF1547 and PKDF1116 a maximum LOD scores of 1.4, 0.65 and 1.53 at θ = 0 was observed. In addition the linkage is supported to this region at distal side (D7S2420) and at proximal side (D7S2456) with LOD scores 2.53 at θ = 0 and 2.53 at θ = 0 in PKDF1532 while in PKDF1375 2.3 at θ = 0 and 0.92 at θ = 0 and PKDF1547 0.65 at θ = 0 and 0.76 at θ = 0 moreover in PKDF1116 1.41 at θ = 0 and 1.41 at θ = 0.The recombination cross was obtained with D7S518 proximally and 0.00 at θ = 0 distally for marker D7S480 in PKDF 1532 with LOD score of 0.00 at θ = 0 (Table I). The constructed haplotype using the alleles of the STR markers for all the families showed same pattern (Fig. 1). Linkage analysis supports the haplotype and PDS locus was localized at 13.63cM (20.1Mb) interval between markers D7S518 and D7S480.

Table I.- Two point LOD score yielded by chromosome7q markers for Pendred families.

STR	PKDF1375	PKDF1532	PKDF1547	PKDF1116
D7S518	0.0000	0.0000	0.0000	0.0000
D7S2420	2.3100	2.5353	0.6589	1.4191
D7S2459	1.4000	2.3748	0.6589	1.5317
D7S2456	0.9275	2.5353	0.7609	1.4191
D7S480	0.1851	0.0000	0.2993	0.0356

STR, short tandem repeats markers. LOD max theta at zero at chromosome 7.

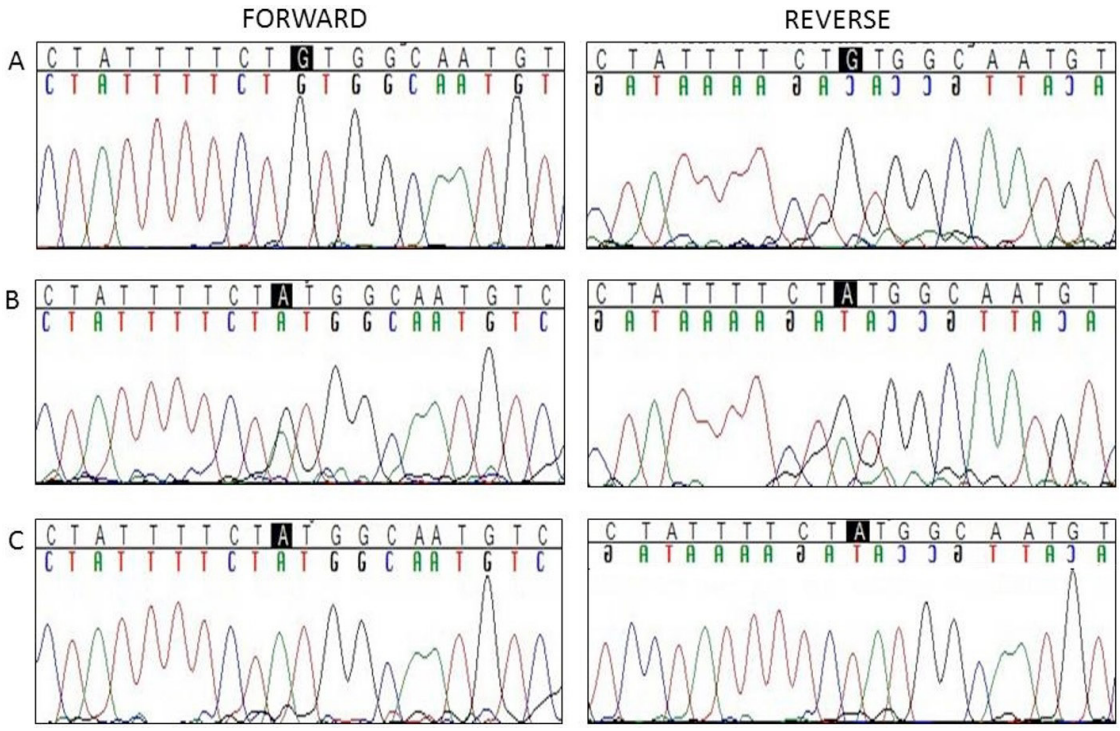

Fig. 2. The forward and reverse sequence chromatograms of the c.11167A>G variation. The arrow indicates the mutation site (A) the affected individual (B) the heterozygous carrier (C) the wild type.

Bidirectional Sanger sequencing of 21 exons of *SLC26A4* in all families revealed a missense variation c.1667A>G; p.Y556C that penetrated with disease phenotype in all families (Fig. 2). In 192 control chromosomes this variant was not found in ethnically and geographically matched participants with normal hearing. It is to be noted that in pendrin orthologues Tyrosine amino acid at position 556 is highly conserved (Fig. 3) whereas SIFT and Polyphen 2 analysis showed that this pathogenic variant is probably damaging to enzymatic and functional activity of Pendrin protein.

DISCUSSION

In this study common mutation in *SLC26A4 gene* is reported in four consanguineous Pakistani families with PDS. Initial exclusion analysis localized critical interval to a region on chromosome 7q31 that harbors *SLC26A4* gene. A>G transition (c.1667A>G) in exon 15 of *SLC26A4* was identified by gene sequencing that penetrated with the disease phenotype in all families, resulting in a p.Y556C change. All six families showed same alleles, and haplotype analysis sturdily predicted the causal mutation from a common ancestor inheritance (Table II).

Table II.- STR haplotype linked to p.Y556C Mutation.

Family designation	Ethnicity	Cast	STR haplotypes linked to Y556C			Variant	Protein change
PKDF172	**Punjabi**	**Bhatti**	**282**	**140**	**242**	**c. 1667A>G**	**p.Y556C**
PKDF804	**Punjabi**	**Changer**	**282**	**140**	**242**	**c. 1667A>G**	**p.Y556C**
PKDF1116	Punjabi	Rajput	282	140	242	c. 1667A>G	p.Y556C
PKDF1375	Punjabi	Ansari	282	140	242	c. 1667A>G	p.Y556C
PKDF1532	Punjabi	Rajpoot	282	140	242	c. 1667A>G	p.Y556C
PKDF1547	Punjabi	Rajpoot	282	140	242	c. 1667A>G	p.Y556C

The information in bold shows the previously reported families (17) with the haplotype similar to presently reported families.

	S_{551}	S_{552}	P_{553}	I_{554}	F_{555}	Y_{556}	G_{557}	N_{558}	V_{559}	D_{560}	G_{561}
Human	S	S	P	I	F	**Y**	G	N	V	D	G
Chimp	S	S	P	I	F	**Y**	G	N	V	D	G
Gorilla	S	S	P	I	F	**Y**	G	N	V	D	G
Orangutan	S	S	P	I	F	**Y**	G	N	V	D	G
Rhesus	S	S	P	I	F	**Y**	G	N	V	D	G
Marmoset	S	S	P	I	F	**Y**	G	N	V	D	G
Tarsier	S	S	P	I	F	**Y**	G	N	V	D	G
Mouse lemur	S	S	P	I	F	**Y**	G	N	V	D	G
Mouse	S	S	P	I	F	**Y**	G	N	V	D	G
Rat	S	S	P	I	F	**Y**	G	N	V	D	G
Kangaroo rat	S	S	P	I	F	**Y**	G	N	V	D	G
Guinea pig	S	G	P	I	F	**Y**	G	N	V	D	G
Dog	S	S	P	I	F	**Y**	G	N	V	D	G
Elephant	S	S	P	I	F	**Y**	G	N	I	D	G
Opossum	Y	S	P	I	F	**Y**	G	N	I	D	G
Chicken	S	S	P	I	F	**Y**	A	N	I	D	G
Xtropicalis	Y	S	G	M	F	**Y**	G	N	I	D	G

Fig. 3. Sequence alignment of amino acid of pendrin subunit showing conservation of amino acids among other primates, placental mammals and vertebrates.

Evolutionary conservation analysis of substituted amino acid showed that tyrosine at position 556 is highly conserved among different species. Zhanga *et al*. (2012) showed that p.Y556C mutation belongs to a group of mutations that incompletely translocate to cell membrane but ion transport function is lost or reduced thus the protein formed as a result is nonfunctional.

Hauwe *et al*. (1998) suggested that identification of frequent mutations in PDS gene will facilitate the diagnosis of PDS. As Pakistan belongs to a third world country, the perchlorate discharge and temporal bone radiology test are expensive and inaccessible for clinical diagnosis in such case only available diagnostic test is of *SLC26A4* mutation analysis since blood can be collected locally and sent anywhere for testing.

Different ethnic groups have variable prevalence of recurring mutations (Park *et al*., 2003). In European population three specific mutations p.L236P, p.T416P, p.IVS8+1G>A occur frequently (Campbell *et al*., 2001). In Japanese and Korean population p.H723R mutation accounts for 53% (Tsukamoto *et al*., 2003) and 40% (Park *et al*., 2005) in PDS. As in Pakistani population the most commonly reported mutations of *SLC26A4* are p.V239D and p.Q446R. p.Y556C mutation has been previously reported in two families (Anwar *et al*., 2009). In the present study the same mutation in four Pakistani families with the same haplotype as reported before were found. Our study defines more clearly the mutation spectrum of *SLC26A4* in Pakistani population.

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Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Anwar, S., Riazuddin, S. and Ahmed, Z.M., 2009. *SLC26A4* mutation spectrum associated with DFNB4 deafness and Pendred's syndrome in Pakistanis. *J. Hum. Genet*., **54**: 266-270. https://doi.org/10.1038/jhg.2009.21

Campbell, C., Cucci, R.A., Prasad, S., Green, G.E., Edeal, J.B., Galer, C.E., Karniski, L.P., Sheffield, V.C. and Smith, R.J., 2001. Pendred syndrome, DFNB4, and *PDS/SLC26A4* identification of eight novel mutations and possible genotype-phenotype correlations. *Hum. Mutat*., **17**: 403-411. https://doi.org/10.1002/humu.1116

Everett, A.L., Morsli, H., Wu, K.D. and Green, D.E., 1999. Genetics expression pattern of the mouse ortholog of the Pendred's syndrome gene (Pds) suggests a key role for pendrin in the inner ear. *Proc. natl. Acad. Sci*., **96**: 9727-9732. https://doi.org/10.1073/pnas.96.17.9727

Everett, L.A., Glaser, B., Beck, J.C., Idol, J.R., Buchs, A., Heyman, M., Adawi, F., Hazani, E., Nassir, E., Baxevanis, A.D., Sheffield, V.C. and Green, E.D., 1997. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (*PDS*). *Nat. Genet*., **17**: 411-422. https://doi.org/10.1038/ng1297-411

Grimberg, J., Nawoschik, S., Belluscio, L., McKee, R., Turck, A. and Eisenberg, A., 1989. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucl. Acids Res*., **17**: 8390. https://doi.org/10.1093/nar/17.20.8390

Hauwe, P.V., Everett, L.A., Coucke, P., Scott, D.A., Kraft, M.L., Ris-Stalpers, C., Bolder, C., Otten, B., de Vijlder, J.J., Dietrich, N.L., Ramesh, A., Srisailapathy, S.C., Parving, A., Cremers, C.W., Willems, P.J., Smith, R.J., Green, E.D. and Van-Camp, G., 1998. Two frequent missense mutations in Pendred syndrome. *Hum. Mol. Genet*., **7**: 1099-1104. https://doi.org/10.1093/hmg/7.7.1099

Jamal, B.S., Anwar, Z., Khan, R.U., Jamil, T., Iram, A., Mir, A., Waqar, M. and Khan, J.Z., 2012. Haplotype analysis for DFNB4/PDS locus in hearing impaired families of Punjab (Pakistan). *Curr. Res. J. biol. Sci*., **4**: 407-413.

Li, X.C., Everett, L.A., Lalwani, A.K., Desmukh, D., Friedman, T.B., Green, E.D. and Wilcox, E.R., 1998. A mutation in *PDS* causes non-syndromic recessive deafness. *Nat. Genet*., **18**: 215-217. https://doi.org/10.1038/ng0398-215

Park, H.J., Lee, S.J., Jin, H.S., Lee, J.O., Go, S.H. and Jang, H.S., 2005. Genetic basis of hearing loss associated with enlarged vestibular aqueducts in Koreans. *Clin. Genet*., **67**: 160-165. https://doi.org/10.1111/j.1399-0004.2004.00386.x

Park, H.J., Shaukat, S. and Liu, X.Z., 2003. Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: Global implications for the epidemiology of deafness. *J. med. Genet*., **40**: 242-248. https://doi.org/10.1136/jmg.40.4.242

Pendred, V., 1896. Deaf-mutism and goitre. *Lancet*, **148**: 532. https://doi.org/10.1016/S0140-

6736(01)74403-0

Prasad, S., Kolln, K.A., Cucci, R.A., Trembath, R.C., Camp, G.V. and Smith, R.H., 2004. Pendred syndrome and DFNB4-mutation screening of SLC26A4 by denaturing high-performance liquid chromatography and the identification of eleven novel mutations. *Am. J. med. Genet. A*., **124A**: 1-9. https://doi.org/10.1002/ajmg.a.20272

Rehman, A.U., Friedman, T.B. and Griffith, A.J., 2016. Unresolved questions regarding human hereditary deafness. *Oral Dis*., **23**: 551-558. https://doi.org/10.1111/odi.12516

Tsukada, K., Nishio, S., Hattori, M. and Usami, S., 2015. Ethnic-specific spectrum of GJB2 and SLC26A4 mutations: Their origin and a literature review. *Ann. Otol. Rhinol. Laryngol.,* **124**: 61S-76S. https://doi.org/10.1177/0003489415575060

Tsukamoto, K., Suzuki, H., Harada, D., Namba, A., Abe, S. and Usami, S., 2003. Distribution and frequencies of PDS (SLC26A4) mutations in Pendred syndrome and non-syndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. *Eur. J. Hum. Genet*., **11**: 916-922. https://doi.org/10.1038/sj.ejhg.5201073

Yan, D., Sundhari, K.A., Vishwanath, S., Qing, J., Mittal, R., Kameswaran, M. and Liu, X.Z., 2015. The genetic basis of non-syndromic hearing loss in Indian and Pakistani populations. *Genet. Test Mol. Biomark*., **19**: 512-527. https://doi.org/10.1089/gtmb.2015.0023

Zhanga, H., Feng, Y., Xia, K., Jiang, Lu., He, C. and Mei, Li., 2012. Functional analysis of pendrin (SLC26A4) and its pathogenic mutations in cos-7 cells. *J. Int. Adv. Otol*., **8**: 195-200.