

# A novel missense mutation in the *ACTG1* gene in a family with congenital autosomal dominant deafness: A case report

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**Abstract.** The *ACTG1* gene encodes the cytoskeletal protein  $\gamma$ -actin, which functions in non-muscle cells and is abundant in the auditory hair cells of the cochlea. Autosomal dominant missense mutations in *ACTG1* are associated with DFNA20/26, a disorder that is typically characterized by post-lingual progressive hearing loss. To date, 17 missense mutations in *ACTG1* have been reported in 20 families with DFNA20/26. The present study described a small family with autosomal dominant nonsyndromic hearing loss. A novel heterozygous missense mutation, c.94C>T (p.Pro32Ser), in *ACTG1* was identified using the TruSight One sequencing panel. Notably, congenital hearing loss in our proband was identified by newborn hearing screening at birth. *In silico* predictions of protein structure and function indicate that the p.Pro32Ser mutation may result in conformational changes in  $\gamma$ -actin. The present study expands the understanding of the phenotypic effects of heterozygous missense mutations in the *ACTG1* gene. In specific, the present results emphasize that mutations in *ACTG1* result in a diverse spectrum of onset ages, including congenital in addition to post-lingual onset.

## Introduction

More than 70% of hereditary hearing loss is nonsyndromic and exhibits extremely high genetic heterogeneity. Nonsyndromic hearing impairment (NSHI) exhibits autosomal recessive inheritance in ~75-80% of cases, autosomal dominant inheritance in 20-25% of cases, and X-linked inheritance in 1-1.5%

of cases (1). Mutations in at least 30 genes have been identified in patients with autosomal dominant sensory NSHI (ADNSHI).

*ACTG1* was initially identified as a causative gene for ADNSHI linked to the *DFNA20/26* locus (MIM #604717) on chromosome 17q25.3 in 2003 (2,3). The *ACTG1* gene encodes a protein called  $\gamma$ -actin, which is part of the actin protein family. The  $\gamma$ -actin is a cytoskeletal protein, which makes up the structural framework inside cells. This protein is particularly abundant in the specialized cells of inner ear called hair cells, which are essential for normal hearing. Thus *ACTG1* is predicted to be crucial for the shape and function of the stereocilia of the cochlear hair cells (4-6). To date, 17 missense mutations in *ACTG1* have been reported in 20 families (Fig. 1A; Table I) (2-5,7-15). The majority of these patients present progressive post-lingual hearing loss with ages of onset ranging between the first and fourth decades (13).

The present study presented a small family exhibiting autosomal dominant, congenital, sensorineural hearing loss with an autosomal dominant inherited novel heterozygous C-to-T transition at nucleotide 94 (p.Pro32Ser) of the *ACTG1* gene identified using the TruSight One sequencing panel. The proband was the first case of congenital hearing loss identified by newborn hearing screening. The present study provided a new insight into the genotype-phenotype correlation for *ACTG1* mutations in ADNSHI.

## Case report

A small family with ADNSHI was identified at the Eulji Medical Center (Seoul, Korea). The pedigree chart is illustrated in Fig. 1B, and the results of Sanger sequencing are presented in Fig. 1C.

The Institutional Review Board of Eulji General Hospital in Seoul, Korea (IRB approval no. 2014-06-007-001) approved the use of medical records and 2 ml peripheral blood samples in the present study. Written informed consent for genetic testing and medical photography was obtained from all subjects prior to participation.

**Patient 1 (proband).** A boy was born at term with a birth weight of 2,770 g (10-25th percentile) and an occipitofrontal circumference of 34.5 cm (75-90th percentile) as the first child of a non-consanguineous 33-year-old Filipina mother and a

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Table I. *ACTG1* variants in autosomal dominant sensorineural hearing impairment and Baraitser-Winter cerebrofrontofacial syndrome 2 (BWCF2).

No.	Author, year	Phenotype	AA change	Exon	Age of onset	N	(Refs.)
1	Present study	DFNA20/26	p.Pro32Ser	2	At birth	1 family	
2	Miyagawa <i>et al</i> , 2015	DFNA20/26	p.Gly48Arg	3	First decade	1 family	(7)
3	Verloes <i>et al</i> , 1993-2016	DFNA20/26	p.Arg51Asn	3	First decade	1 family	(14)
4	Zhu <i>et al</i> , 2003	DFNA20/26	p.Thr89Ile	3	Third decade	3 families	(2)
5	Zhu <i>et al</i> , 2003; Miyagawa <i>et al</i> , 2015	DFNA20/26	p.Lys118Asn	3	First-third decade	1 family	(2,7)
6	Morín <i>et al</i> , 2009	DFNA20/26	p.Lys118Met	3	Second-third decade	1 family	(4)
7	Liu <i>et al</i> , 2008	DFNA20/26	p.Ile122Val	4	First decade	1 family	(8)
8	Baek <i>et al</i> , 2012	DFNA20/26	p.Asp187His	4	First decade (1 year old)	1 family	(9)
9	Yuan <i>et al</i> , 2016	DFNA20/26	p.Lys213Arg	4	Second decade	1 family	(5)
10	Morín <i>et al</i> , 2009; Miyagawa <i>et al</i> , 2015	DFNA20/26	p.Glu241Lys	4	First decade	1 family	(4,7)
11	Zhu <i>et al</i> , 2003	DFNA20/26	p.Pro264Leu	4	First-second decade	1 family	(2)
12	Mutai <i>et al</i> , 2013	DFNA20/26	p.Gly268Ser	4	First-fourth decade	1 family	(10)
13	van Wijk <i>et al</i> , 2003	DFNA20/26	p.Thr278Ile	5	First-second decade	1 family	(3)
14	Miyagawa <i>et al</i> , 2015	DFNA20/26	p.Leu299Val	5	Second decade	2 families	(7,11)
15	Park <i>et al</i> , 2013	DFNA20/26	p.Met305Thr	5	Third-fourth decade	1 family	(12)
16	Vona <i>et al</i> , 2014	DFNA20/26	p.Met325Lys	5	At birth	1 family	(13)
17	Zhu <i>et al</i> , 2003	DFNA20/26	p.Pro332Ala	6	Second decade	1 family	(2)
18	Rendtorff <i>et al</i> , 2006	DFNA20/26	p.Val370Ala	6	First-second decade	1 family	(15)
1	Di Donato <i>et al</i> , 2016	BWCF2	p.Asn12Asp	2		1 patient	(16)
2	Rivière <i>et al</i> , 2012	BWCF2	p.Thr120Ile	3		1 patient	(17)
3	Rivière <i>et al</i> , 2012; Verloes <i>et al</i> , 2015	BWCF2	p.Ala135Val	4		2 patients	(17,18)
4	Poirier <i>et al</i> , 2015	BWCF2	p.Met153Ile	4		1 patient	(19)
5	Rivière <i>et al</i> , 2012	BWCF2	p.Ser155Phe	4		3 patients	(17)
6	Di Donato <i>et al</i> , 2016	BWCF2	p.Asp179Tyr	4		1 patient	(16)
7	Rivière <i>et al</i> , 2012	BWCF2	p.Thr203Lys	4		1 patient	(17)
8	Di Donato <i>et al</i> , 2016; Rivière <i>et al</i> , 2012	BWCF2	p.Arg254Trp	4		2 patients	(16,17)
9	Di Donato <i>et al</i> , 2016; Rivière <i>et al</i> , 2012	BWCF2	p.Arg256Trp	4		2 patients	(16,17)
10	Di Donato <i>et al</i> , 2016	BWCF2	p.Glu334Gln	6		1 patient	(16)
11	Di Donato <i>et al</i> , 2016	BWCF2	p.Arg335His	6		1 patient	(16)

AA, amino acid.

49-year-old Korean father (Fig. 2A and B). He had one sibling who was healthy. At birth, he had a complete bilateral cleft lip and palate. He did not pass the initial screening test with otoacoustic emissions at birth. He exhibited bilateral severe hearing loss on auditory brainstem response at threshold 90 dB above normal adult hearing level (nHL; Fig. 2C) and auditory steady-state response audiometry at threshold 80 dB

nHL (Fig. 2D). He underwent surgical repair of the cleft lip (cheiloplasty) at 4 months and cleft palate (palatoplasty) at 12 months. He received cochlear implant (CI) surgery in the right ear at 22 months and in the left ear at 4 years. When he visited our clinic (Nowon Eulji Medical Center, Eulji University, Seoul, Republic of Korea) at 4 years and 6 months old, his body weight was 17.8 kg [+0.00 standard deviation

(SD)], height was 99.9 cm (-1.53 SD), and head circumference was 53 cm (+1.44 SD). On examination, he presented a distinctive face owing to the reconstructed philtrum and upper lip by cheiloplasty (Fig. 2A). He had no coloboma or ptosis in an eye examination performed by an ophthalmologist. There were no visible abnormalities of the external ear. There were no skeletal abnormalities. He exhibited no seizures, movement problems, or other behavioral problems. He revealed developmental language delay based on the sequenced language scale of infants at 4 years and 5 months of age; expressive language function was at the 21-month-old level, and receptive language function was at the 23-month-old level. This was attributed to congenital deafness with relatively delayed cochlear implants. The results of brain magnetic resonance imaging (MRI) were normal. There were no associated structural ear abnormalities in temporal bone computer tomography findings. Electrocardiography and chest radiography results were normal. Echocardiograms exhibited a very small patent ductus arteriosus. No evidence of malformation of the kidneys was observed in an abdominal ultrasound evaluation. The laboratory tests, including a complete blood count, chemistry panel, lipid profile, thyroid function test, and urinalysis, were all normal.

**Patient 2 (father).** The patient's father first visited our clinic at 53 years of age with his son. He was the second child of healthy non-consanguineous Korean parents. There was no family history of neurologic disease, deafness, or developmental delay, with the exception of his son. At this visit, he exhibited profound pre-lingual deafness (Fig. 2B). He did not acquire speech and language. On examination, he had no specific dysmorphic craniofacial features. Brain MRI was normal.

## Results

**Cytogenetic analysis.** To identify the causative genetic alterations in the proband (III-1), cytogenetic and analysis were performed. Peripheral blood lymphocytes were cultured for 72 h according to routine cytogenetic protocols. G-banding normal chromosomes (46, XY) was found in the proband. A genomic microarray of DNA from the proband's peripheral blood using a 750K High-resolution Genotyping SNP Microarray (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) revealed no abnormalities.

**Mutation analysis.** Considering the genetic heterogeneity of the disease and for differential diagnosis, clinical sequencing of regions containing Mendelian disease-causing genes in the proband (III-1) was performed for genetic confirmation. Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification kit following the manufacturer's protocols (Promega Corporation, Madison, WI, USA). Library preparation was conducted using TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA), which included 125,395 probes targeting a 12-Mb region spanning 4,813 genes. Massively parallel sequencing was performed using Illumina MiSeq platform (Illumina, Inc.). Sequence reads were aligned to hg19 using Burrow-Wheeler Aligner (version 0.7.12). Duplicate reads were removed by Picard (version 1.96, <http://picard.sourceforge.net>). Local

Table II. Summary of the disease-causing novel mutation in *ACTG1*.

Gene	<i>ACTG1</i>	(Refs.)
Position (hg19)	chr17q25 (79,479,287 base pair)	
Exome	2/7	
Accession number	NM_001614.3	
Nucleotide change	c.94C>T, hetero	
Amino acid change	p.Pro32Ser	
HGMD®	None	(20)
Classification by ACMG guidelines	Likely pathogenic	(21)
Allele frequency in controls		
dbSNP 142	No rsID	
1000Gp3	None	
ESP6500	None	
ExAC	None	
KRGdb	None	
Missense prediction		
SIFT_pred	Deleterious	
LRT_pred	Unknown	
PolyPhen2 HDIV_pred	Probably damaging	
Polyphen2_HVAR_pred	Probably damaging	
MutationTaster_pred	Disease causing	
MutationAssessor_pred	Medium predicted functional	
MetaSVM_pred	Deleterious	
MetaLR_pred	Deleterious	
FATHMM_pred	Deleterious	
PROVEAN_pred	Damaging	
CADD_phred	16.96	
Nucleotide conservation prediction		
GEFF++_RS	3.99	
PhyloP7way_vertibrate	0.917	
phastCons7way_vertibrate	0.98	
SiPhy_29way_logOdds	15.0358	

realignment and base quality recalibration were conducted using Genome Analysis Toolkit (GATK version 3.5). Variant calling was performed using GATK HaplotypeCaller. Variants were annotated using Variant Effect Predictor and dbNSFP (version 3.0b2a). Sanger sequencing was used to determine the genotypes of affected and unaffected family members at candidate loci. From sequencing data of the proband with the TruSight One Panel, it was expected to discover a heterozygote ADNSHI-causing mutation in this family. Mutations in known genes causing autosomal recessive in addition to autosomal dominant syndromic/nonsyndromic hearing loss were also evaluated in the exome data of the proband. Two missense mutations were detected: c.94C>T (p.Pro32Ser) in *MYO6* and c.94C>T (p.Pro32Ser) in *ACTG1*. The mutation c.94C>T

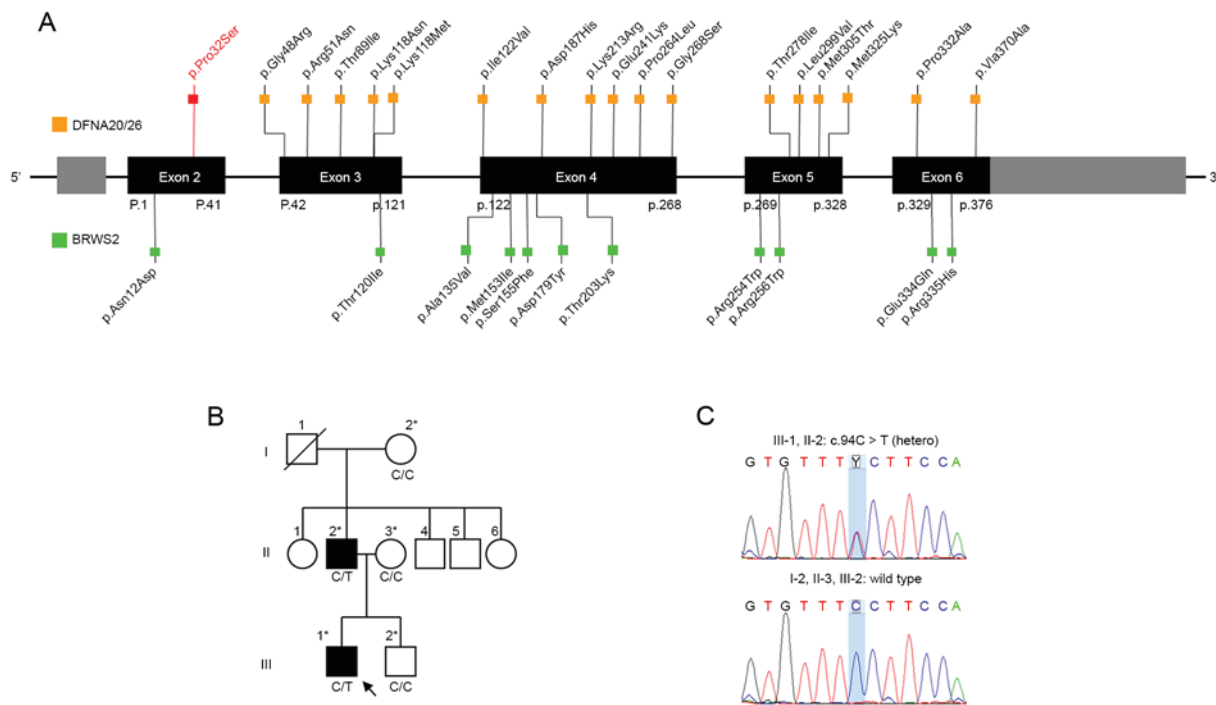


Figure 1. Summary of gene and pedigree analyses for a family with DFNA20/26. (A) A schematic of the *ACTG1* gene showing the location of previously published mutations. Mutations associated with DFNA20/26 are indicated above the gene with orange squares. The mutation detected in the present study is indicated with a red square. Mutations associated with Baraitser-Winter cerebrofrontofacial syndrome 2 (BRWS2) are indicated below the gene with green squares. (B) Pedigree of the family and identification of the novel heterozygous missense mutation c.94C>T in *ACTG1*. The black arrow indicates the proband patient III-1. Darkened symbols represent the affected members. Asterisks indicate sampled subjects. (C) Sanger sequencing confirmed the mutation in the patients (II-2 and III-1) and the wild-type genotype in unaffected family members (I-2, II-3, and III-2).

(p.Pro32Ser) in *MYO6* displayed lack of segregation in affected and unaffected members of this family. The mutation was not observed in another affected family member (II-2) and was observed in two unaffected family members (II-3 and III-2). In contrast, the heterozygous novel missense mutation, c.94C>T (p.Pro32Ser), in the *ACTG1* gene was supported as a disease-causing mutation based on segregation in the family. Sanger sequencing confirmed that the affected family members (III-1 and II-2) were heterozygous for the mutation, while the mutation was not observed in the unaffected family members (I-2, II-3 and III-2; Fig. 1C). This mutation is absent from controls in NHLBI GO Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), 1000 Genomes Project Phase 3 (<http://browser.1000genomes.org>), Exome Aggregation Consortium v0.3 (<http://exac.broadinstitute.org/>), and Korean Reference Genome Database (<http://152.99.75.168/KRGDB/menuPages/introKor.jsp>). Multiple lines of evidence from *in silico* prediction indicated that the mutation was potentially deleterious. The characteristics of the novel missense mutation are summarized in Table II.

In addition, genes known to serve a role in craniofacial development including cleft lip and palate were examined. A missense mutation c.251A>T (p.Glu84Val) in the *MSX1* gene was identified but lacked segregation in this family, as the mutation was also observed in two unaffected family members (II-3 and III-2).

**Structural modelling of p.Pro32Ser.** The effect of the p.Pro32Ser mutation on the structure and function of

the *ACTG1* protein product,  $\gamma$ -actin, was next assessed. The p.Pro32Ser region of *ACTG1* was checked against the protein feature view of the Protein Data Bank (<http://www.wwpdb.org/>) entries mapped to a UniProt Knowledgebase (<http://www.uniprot.org>) sequence. The native amino acid at position 32 is hydrophobic and in a predicted potentially disordered region (Fig. 3A). The substitution of proline (nonpolar, hydrophobic) by serine (uncharged polar, hydrophilic) is likely to change the hydrophobicity of the hydrophobic region and influence hydrophobic interactions. It may affect protein folding or decrease the stability of the native protein structure of  $\gamma$ -actin. Pro32Ser is also close to a phosphorylation site (Fig. 3A). Serine is one of most commonly phosphorylated amino acids. A substitution by serine could affect protein phosphorylation and post-translational modifications. *In silico* predictions were performed to infer the three-dimensional structure of  $\gamma$ -actin using UCSF chimera (<https://www.cgl.ucsf.edu/chimera>). The 32.Pro in wild-type  $\gamma$ -actin participates in two hydrogen bonds (distances, 2.804 Å and 2.864 Å), as demonstrated in Fig. 3B. Structural modeling of mutant 32.Ser identified a new hydrogen bond (distance, 3.367 Å) that interacted with 15.Gly (Fig. 3C). Hydrogen bonding serves a particularly important role in secondary structure formation and the determination of three-dimensional structures. Prolines are flexible amino acids and are often in  $\beta$  turns with glycine. In a  $\beta$  turn, there is a tight 180° reversal in the direction of the polypeptide chain, and proline can readily assume the *cis* configuration, which facilitates a tight turn. Accordingly,



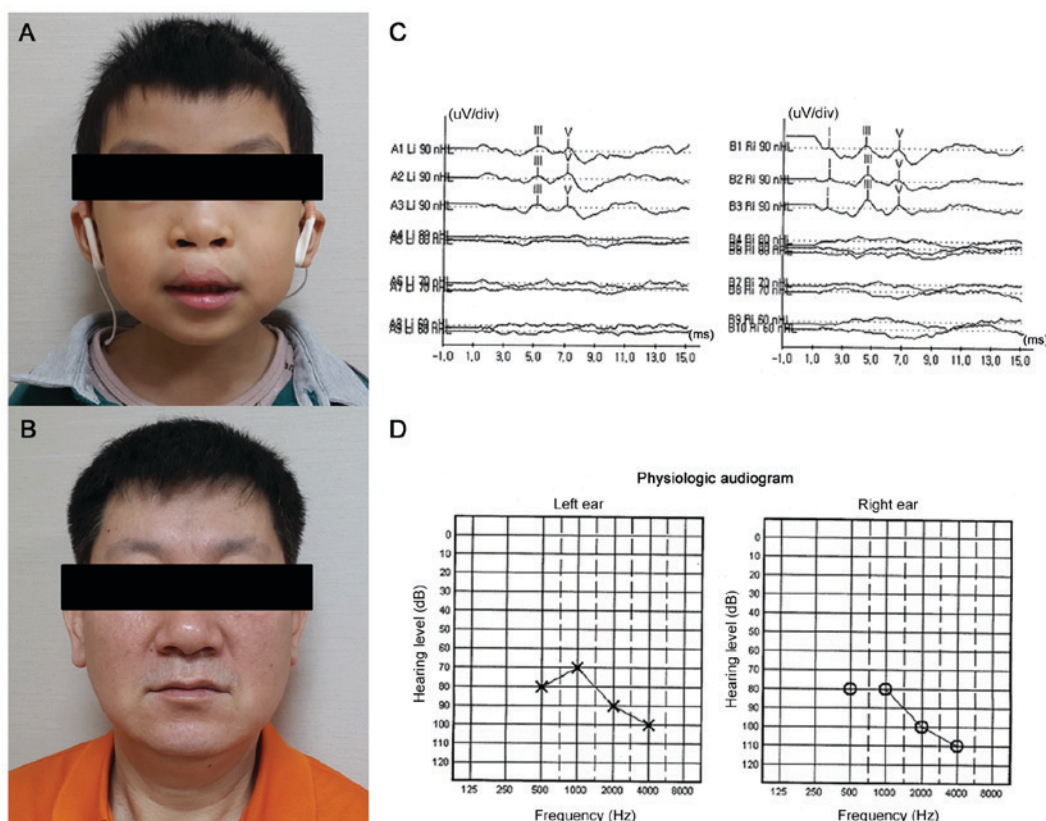


Figure 2. Facial and auditory features of patients. (A) Photograph of the proband (III-1). At his age of 4 years 6 months, he exhibits a distinctive face owing to the reconstructed philtrum and upper lip by cheiloplasty. (B) Photograph of the patients father (II-2). At 53-years-old, he reveals no distinctive craniofacial features. (C) ABR threshold was recorded in the proband (III-1) bilaterally using click stimuli at the age of 20 days. The ABR demonstrated severe sensorineural hearing loss with threshold at 90 dB nHL. (D) Auditory steady-state response audiometry of the proband (III-1) also exhibited severe sensorineural hearing loss with hearing threshold 80 dB nHL in right and 70-80 dB nHL in left at 500-1,000 Hz. ABR, auditory brainstem response.

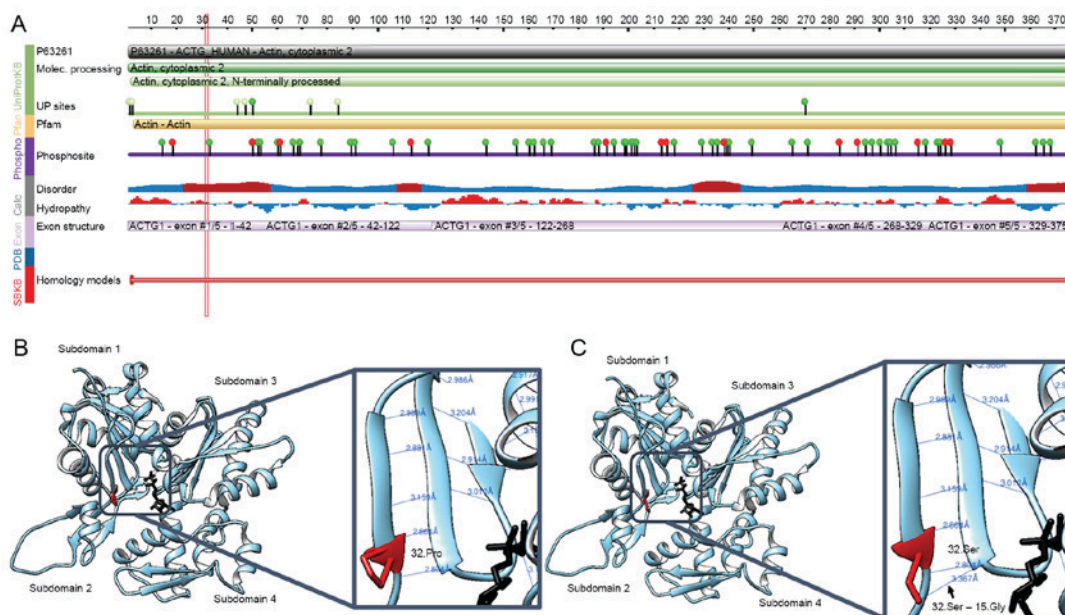


Figure 3. p.P32S mutation in *ACTG1*. (A) Structural summary of  $\gamma$ -actin obtained using the protein feature view of the Protein Data Bank (<http://www.wwpdb.org/>). Vertical red bar indicates the location of the P32S mutation. P32S is close to the phosphorylation site in the purple bar. P32S was located in a potentially disordered region (red), and grey indicates protein disorder predictions. The hydrophobic region is indicated in red. (B) 32.Pro in wild-type actin has two hydrogen bonds. (C) Structural modeling of mutant 32.Ser showing a new hydrogen bond that interacts with 15.Gly.

the Pro32Ser mutation may result in a conformational change in  $\gamma$ -actin. Molecular modelling analyses therefore predicted

alterations in  $\gamma$ -actin and actin-based structures following this mutation.

## Discussion

The auditory hair cells are located within the spiral organ of Corti on the thin basilar membrane in the cochlea of the inner ear. The inner hair cells transform the sound vibrations in the fluids of the cochlea into electrical signals that are then relayed via the auditory nerve to the auditory brainstem and to the auditory cortex. Humans and other mammals are generally incapable of regrowth of the inner ear cells that convert sound into neural signals when those cells are damaged by age or disease. Actins are highly conserved proteins involved in cell motility and cytoskeletal maintenance.  $\gamma$ -Actin is encoded by the *ACTG1* gene and is identified in the cytoplasm of non-muscle cells. It is abundant in the auditory hair cells of the cochlea. Actin filaments are essential for the shape and function of the stereocilia of hair cells. An alteration in actin filament regulation caused by actin-binding proteins is a major factor in deafness caused by *ACTG1* mutations (6). The DFNA20/26 phenotype includes hearing loss with post-lingual onset and an increasing degree of hearing loss with age (DFNA20/26, MIM# 604717). Impaired actin structures may be more susceptible to age-dependent degeneration, resulting in progressive late-onset, post-lingual hearing loss. In patients with very early-onset hearing loss, suspected hearing loss is rarely reported (13). The proband in the present study was the first case of congenital hearing loss to be identified by a newborn hearing screening test. His father exhibited pre-lingual profound hearing impairment. It is hypothesised that congenital and pre-lingual-onset hearing loss is possible in patients with ADNSHI associated with *ACTG1* mutations.

A missense mutation in *ACTG1* has also been identified in patients with Baraitser-Winter cerebrofrontofacial syndrome 2 (BWCFF2, MIM# 614583), which is a multiple congenital malformation syndrome including specific facial gestalt, short status, brain malformation, and sensorineural hearing loss (16). Eleven mutations in 16 patients have been detected in BWCFF2 to date (Fig. 1A; Table I). All mutations in BWCFF2, in addition to DFNA20/26, are heterozygous missense mutations. The spectrum of pathogenic mutations observed in DFNA20/26 does not overlap with the spectrum of pathogenic mutations observed in BWCFF2 (Fig. 1A). Mutations causing DFNA20/26 and BWCFF2 are distributed over the entire exome without regional clusters, as shown in Fig. 1A. The molecular genetic mechanisms of *ACTG1* heterozygous missense mutations in DFNA20/26 and BWCFF2 are unclear; this is an important open question for future research. Up to 83% of patients with BWCFF2 have congenital hearing loss. Riviere *et al* (17) suggested that BWCFF2 represents the severe end of a spectrum of cytoplasmic actin-associated phenotypes that begins with ADNSHI and extends to BWCFF2. In the present study, the father (II-2) carried a *de novo* mutation in *ACTG1* and presented with non-syndromic pre-lingual deafness. The proband (III-1) had additional features of cleft lip and cleft palate. While cleft lip and cleft palate may be identified in various craniofacial syndromes, environmental influences may also cause or interact with genetics to produce orofacial clefting. In addition, the proband exhibited no other minor or major anomalies suggestive of BWCFF2 with the exception of cleft lip and palate.

Advances in the acquisition of clinical and molecular data may improve molecular genetic diagnoses, including those based on *ACTG1* mutations, and may contribute to therapeutic decisions. Previous studies have suggested that CI surgery is a good therapeutic option for patients with *ACTG1* mutations, as the etiology involves the cochlea and normal brain structures; therefore, comparatively improved outcomes are predicted (7,11). In the present study, the proband with congenital deafness underwent CI surgery. Although cochlear implantation was performed late, at 2 and 4 years old, he received language therapy and has the ability to communicate. The present findings support the recommendation for early CI surgery in patients with *ACTG1* mutations.

In summary, a novel heterozygous missense mutation P32S in the *ACTG1* gene was identified in a small family with autosomal dominant nonsyndromic hearing loss. The present findings expand our understanding of the phenotypes associated with *ACTG1*. Specifically, the results of the present study emphasized that mutations in *ACTG1* result in a diverse spectrum of onset ages, including congenital in addition to post-lingual onset.

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