OPEN

Citation: Human Genome Variation (2016) **3**, 16023; doi:10.1038/hgv.2016.23
Official journal of the Japan Society of Human Genetics

www.nature.com/hgv

ARTICLE

A novel nonsense mutation in the *NOG* gene causes familial *NOG*-related symphalangism spectrum disorder

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The human *noggin* (*NOG*) gene is responsible for a broad spectrum of clinical manifestations of *NOG*-related symphalangism spectrum disorder (*NOG*-SSD), which include proximal symphalangism, multiple synostoses, stapes ankylosis with broad thumbs (SABTT), tarsal–carpal coalition syndrome, and brachydactyly type B2. Some of these disorders exhibit phenotypes associated with congenital stapes ankylosis. In the present study, we describe a Japanese pedigree with dactylosymphysis and conductive hearing loss due to congenital stapes ankylosis. The range of motion in her elbow joint was also restricted. The family showed multiple clinical features and was diagnosed with SABTT. Sanger sequencing analysis of the *NOG* gene in the family members revealed a novel heterozygous nonsense mutation (c.397A > T; p.K133*). In the family, the prevalence of dactylosymphysis and hyperopia was 100% while that of stapes ankylosis was less than 100%. Stapes surgery using a CO_2 laser led to a significant improvement of the conductive hearing loss. This novel mutation expands our understanding of *NOG*-SSD from clinical and genetic perspectives.

Human Genome Variation (2016) **3,** 16023; doi:10.1038/hgv.2016.23; published online 4 August 2016

INTRODUCTION

The human *noggin* (*NOG*) gene consists of a single exon and encodes a secreted protein that is critical for normal bone and joint development.[1] Noggin binds to bone morphogenetic protein (BMP) of the transforming growth factor-β superfamily and prevents its binding to the cognate receptor.[1,2] This interaction affects a number of developmental processes such as morphogenesis and body patterning,[1,3] middle ear formation,[4,5] and apoptosis in digital and interdigital regions.[4,6,7] Mutations in the *NOG* gene result in aberrant functioning of the noggin protein, which is linked to various autosomal dominant syndromes characterized by proximal symphalangism (SYM1: MIM #185800),[8] multiple synostosis syndrome (SYNS1: MIM#186500),[8] tarsal–carpal coalition syndrome (TCC: MIM#186570),[9,10] brachydactyly type B2 (BDB2: MIM#611377),[11] and stapes ankylosis with broad thumb and toes (SABTT: MIM#184460) (i.e., Teunissen-Cremers syndrome).[12–14] Precise diagnosis is complicated by the overlapping clinical features of these syndromes. Given the variable phenotypic manifestations within and among families with the same mutations, the term *NOG*-related symphalangism spectrum disorder (*NOG*-SSD) has been proposed.[15]

In the present study, we describe a novel nonsense mutation in the *NOG* gene causing familial *NOG*-SSD and report the associated clinical and molecular findings as well as the results of surgery for conductive hearing loss.

MATERIALS AND METHODS

Patients

Medical history including hearing loss, symphalangism, dactylosymphysis, brachydactyly, and hyperopia as well as results of a clinical examination were obtained for four members of a Japanese family, three of whom were affected (proband, father, and grandmother), and one who was unaffected (mother). Auditory function was assessed by pure tone audiometry, tympanometry, and the stapedius reflex test. High-resolution computed tomography scans were carried out to identify any middle and inner ear abnormalities, and X-rays images of the hand were obtained to identify any fusion of the bones. Stapes surgery was performed in the proband to restore hearing. The father had undergone stapes surgery in the right ear at a different hospital in his childhood.

Study participants and the parents of the child provided written, informed consent. The research protocol was approved by the Ethical Review Committee of Sapporo Medical University, Japan.

Genetic analysis

Genomic DNA was extracted from blood samples using the Gentra Puregene Blood kit (Qiagen, Hamburg, Germany). PCR primers specific for the *NOG* exon (GenBank NG_011958.1) and the amplification program were as previously reported.[16] Sanger sequencing data were analyzed using SeqScape software v.2.6 (Applied Biosystems, Foster City, CA, USA) and DNASIS Pro (Hitachisoft, Tokyo, Japan). The variant allele frequency was evaluated using the dbSNP 146 public database (http://www.ncbi.nlm.nih.gov/snp/), 1000 Genome Browser (http://browser.1000genomes.org/index.html), Human Genetic Variation Database (HGVD) (http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html), NHLBI Exome Sequencing Project (ESP6500) (http://evs.gs.washington.edu/EVS/), and Exome Aggregation Consortium v.0.3 (ExAC) (http://exac.broadinstitute.org). The Human Gene Mutation Database (HGMD) v.2015.4 (BIOBASE, Beverly, MA, USA) was searched to determine whether the variant had been previously reported as being associated with diseases.

RESULTS

Clinical features of the family

The family had five affected individuals (Figure 1). The proband (IV: 1) was a 6-year-old girl of non-consanguineous Japanese

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Received 11 April 2016; revised 23 May 2016; accepted 27 June 2016

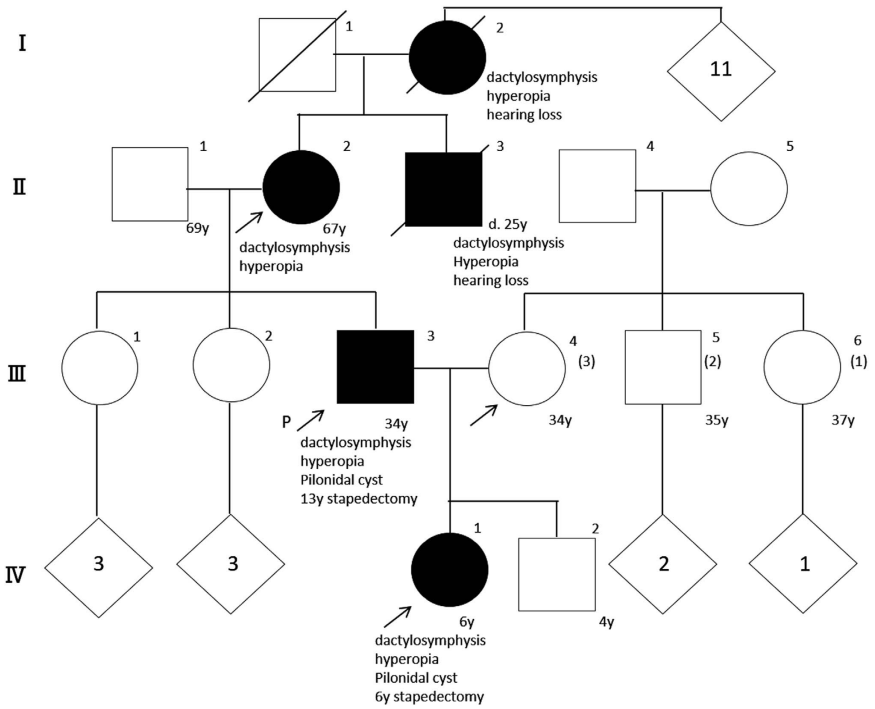

Figure 1. Pedigree of a family with *NOG*-SSD. The family included five affected individuals (I: 2, II: 2, II: 3, III: 3, and IV: 1 (proband)). Arrows indicate subjects who participated in the genetic analysis.

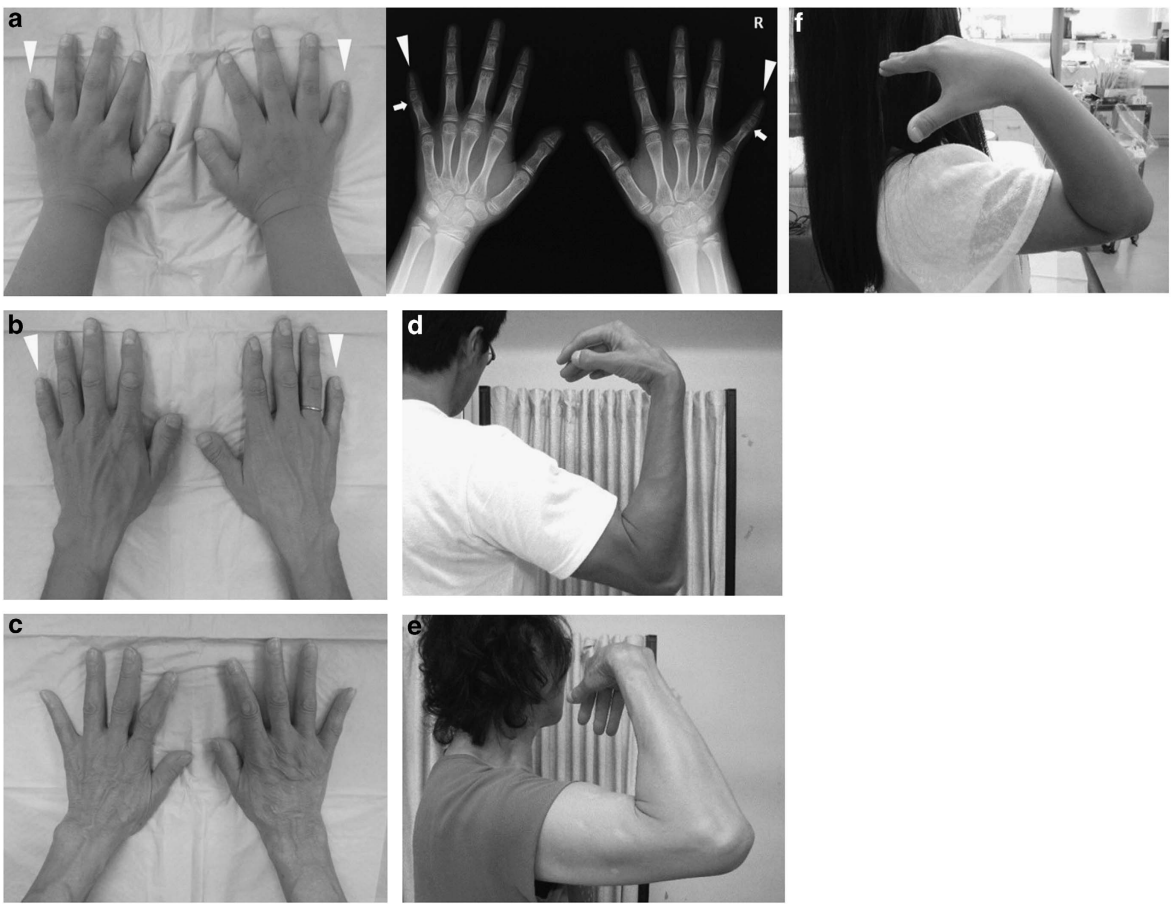

Figure 2. Photographs of the hands of the proband (**a**), her father (**b**), and her grandmother (**c**). Arrows indicate symphalangism and arrowheads indicate brachydactyly. (**d**–**f**) Photograph illustrating the restricted range of motion of the elbow joint in the affected individuals and their inability to touch their shoulders with their hands.

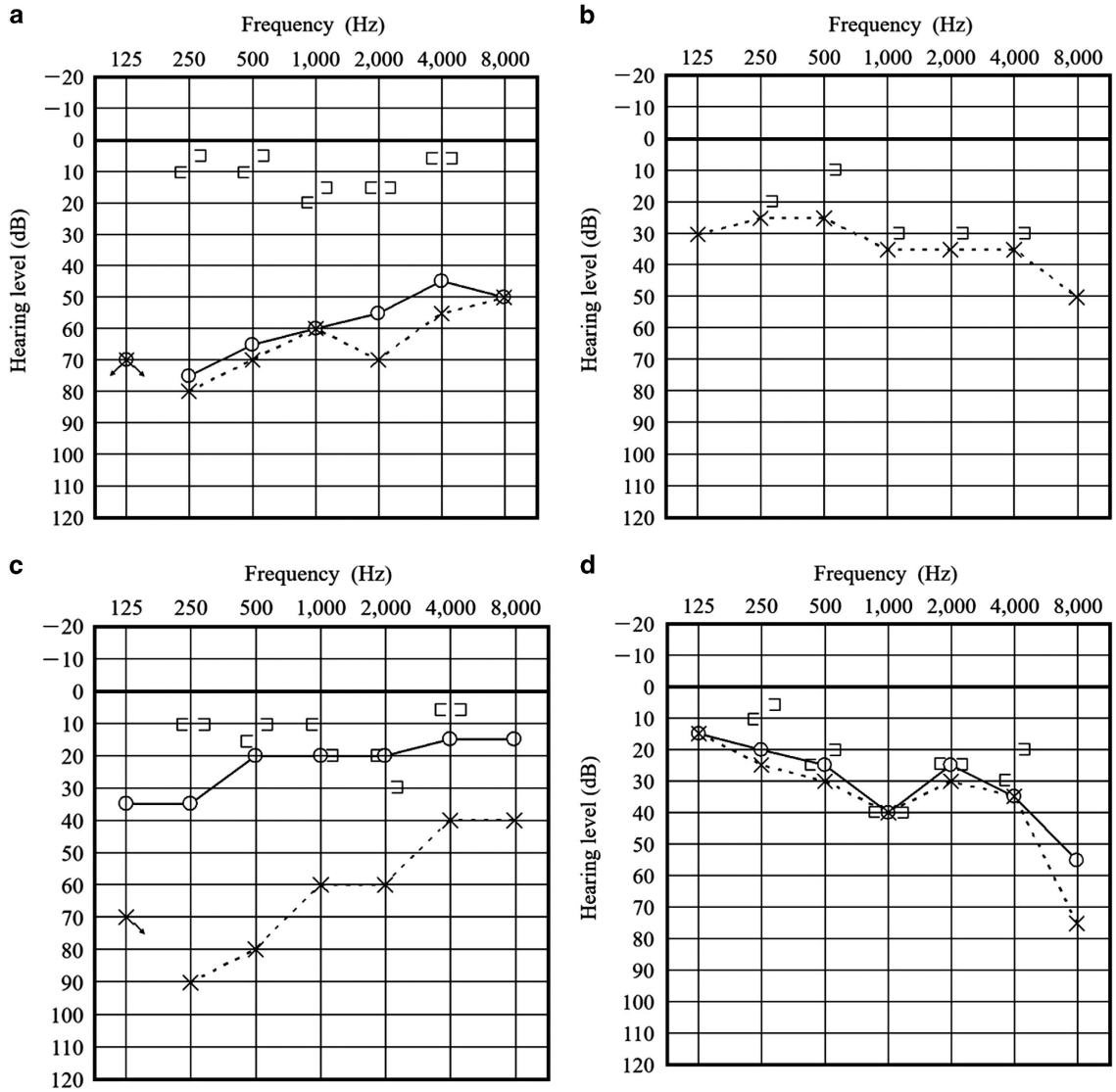

Figure 3. (**a**) Preoperative pure tone audiometry of the proband showing bilateral conductive hearing loss. (**b**) Postoperative pure tone audiometry demonstrating improvement in hearing levels in the operated ear. (**c**) Audiograms from III: 3 showed conductive hearing loss in the left ear, while the right ear treated by stapes surgery showed an improvement in hearing threshold. (**d**) Audiograms from II: 2 did not reveal conductive hearing loss.

parents. She was referred to our hospital at the age of 5 years because of bilateral hearing loss that had starting in early childhood. Physical and X-ray examinations of the hands showed symphalangism and short intermediate phalanges (brachydactyly) in both fifth fingers (Figure 2a). The range of motion in her elbow joint was restricted, and she was unable to touch her shoulders with her hands (Figure 2f).

She had undergone surgical treatment for dactylosymphysis in the second and third toes of both feet in her early childhood. An ophthalmologic examination revealed hyperopia. She had experienced bilateral progressive hearing loss from early childhood, and pure tone audiometry at age 6 showed bilateral conductive hearing loss (Figure 3a). At this time, she also underwent stapedotomy using a Teflon piston, which detected ankylosis of the stapes footplate with hypertrophy of the anterior and posterior crus; the footplate was also distant from the facial nerve (Figure 4). The patient's postoperative hearing threshold improved to 25 dB in the operated ear, and her hearing level has remained stable for more than 3 years since the surgery (Figure 3b).

The proband's father (III: 3), who underwent right stapedotomy at 13 years old at another hospital, had bilateral hearing loss since early childhood, and pure tone audiometry showed conductive hearing loss on the left side and an improvement of hearing level in the operated ear (Figure 3c). His hearing condition had not worsened for 15 years. Physical examination of his hands showed brachydactyly in both fifth fingers (Figure 2b), and he could not touch his shoulders with his hands due to a restricted range of motion in his elbow joints (Figure 2d). He had undergone surgical treatment in his early childhood for dactylosymphysis in the second and third toes of both feet. An ophthalmologic examination revealed hyperopia.

The proband's grandmother (II: 2) did not show conductive hearing loss (Figure 3d); however, she had also had surgery during childhood for dactylosymphysis in the second and third toes of the both feet. Physical examination of her hands did not reveal brachydactyly (Figure 2c). As in the case of the other two patients,

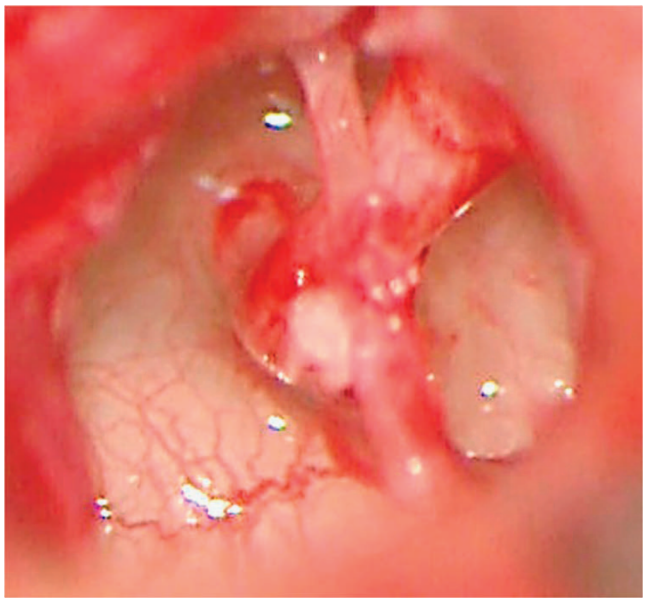

Figure 4. Operative findings in the proband showing ankylosis of the stapes footplate with hypertrophy of the anterior and posterior crus. The facial nerve was distant from the footplate.

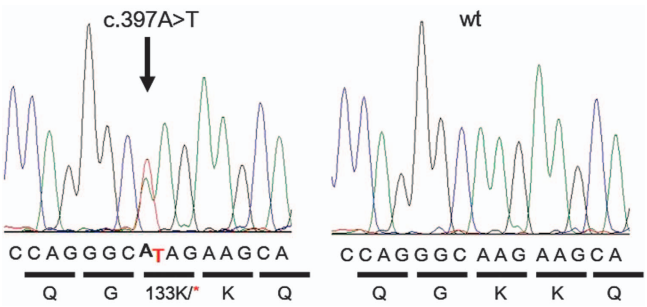

Figure 5. Partial electropherograms of *NOG* from a patient with c.397A>T (p.K133*) mutation (left) and a control subject with normal hearing (right). The mutated nucleotide is indicated by an arrow.

she was unable to touch her shoulders with her hands due to restricted range of motion of the elbow joint (Figure 2e). An ophthalmologic examination showed hyperopia.

Pilonidal cysts were found in the proband (IV: 1) and her father (III: 3).

Genetic analysis

A genetic analysis detected a heterozygous c.397A>T (p.K133*) variant of the *NOG* gene in the proband (IV: 1) as well as in II-2 and III-3 (Figure 5), which has not been previously reported according to the HGMD and is not registered in other databases such as dbSNP, 1000 Genome Browser, HGVD, ESP6500, or ExAC. Given that other nonsense mutations such as p.Q110* (rs104894614)[14] and p.L129* (rs104894613)[17] have been reported to be pathogenic, the p.K133* variant is presumed to produce a truncated noggin protein (132 of 232 amino acid residues) with disrupted function.

DISCUSSION

The present study identified a novel nonsense mutation in the *NOG* gene in a family with *NOG*-SSD. The clinical features included proximal symphalangism in one of the fingers, dactylosymphysis of the toes, brachydactyly, pilonidal cyst, hyperopia, and conductive hearing loss as a result of stapes ankylosis. The most common phenotypes in the family were dactylosymphysis (5/5), hyperopia (5/5), and hearing loss (4/5).

Heterozygous *NOG* mutations have been identified in several syndromes including SYM1,[8] SYNS1,[8] TCC,[9,10] BDB2,[11] and SABTT.[12–14] To date, a total of 45 human variations in *NOG* have been reported; the term *NOG*-SSD was put forth to describe these syndromes,[15,17] which exhibit shared but also some distinct clinical features. In our patients, the prevalence of dactylosymphysis and hyperopia was 100% while that of stapes ankylosis was less than 100%.

Mutations reported in the literature to date are shown in Table 1. *NOG* gene mutations including frameshift, missense, and nonsense mutations as well as deletions and insertions have been previously identified in patients with *NOG*-SSD. *NOG* gene mutations are mainly dominant; however, *de novo* mutations have also been reported in sporadic cases.[8,18] Therefore, genetic investigations are sometimes needed to clarify the pathogenesis of conductive hearing loss due to stapes ankylosis with stiffness of the proximal interphalangeal joints in patients with no familial history. *NOG* gene mutations are autosomal dominant, and is presumed to be manifested as either haploinsufficiency—which can lead to an aberrant gradient during development—or may have a dominant-negative effect due to the defective protein.[19] The *NOG* gene has a critical role in joint formation and bone development, and mutations in noggin compromise the folding stability of the protein and cause defective binding to BMP.[6,20] Noggin-mediated inhibition of BMP signaling is regulated by a two-step process:[21] noggin binds to BMP and prevents its binding to the BMP receptor, with the complex binding instead to heparin sulfate proteoglycan, a major cell surface and extracellular matrix proteoglycan. Sulfate induces the release of the noggin–BMP complex at the cell surface, increasing the accessibility of BMP to its receptor and thereby activating BMP signaling. A docking simulation of noggin to heparin analog and estimation of the change in interaction with p.R136C mutation demonstrated that the positively charged R136 in the heparin-binding site is required for retention of the noggin–BMP complex by negatively charged heparin sulfate proteoglycan at the plasma membrane.[16] The altered binding of mutant noggin and heparin sulfate proteoglycan may lead to hyperactivation of BMP signaling, ultimately leading to ankylosis of the joints and stapes.[16]

Stapes surgery for conductive hearing loss due to *NOG* mutations leads to an improvement in hearing for most patients,[10,19,22] as confirmed in the present study. However, it is necessary to exercise caution when performing stapes surgery for this syndrome due to the risk of bony reclosure of the oval window after surgery. It was previously reported that the hearing level of patients who underwent stapes surgery deteriorated during the follow-up period for this reason, which resulted in a dislocated piston.[18,23,24] Therefore, partial or total stapedectomy has been proposed as an alternative procedure to prevent reclosure of the oval window.[7,23] In the present case (IV: 1), we performed stapedotomy using a CO_2 laser. There have been no reports to date of CO_2 laser-assisted stapedotomy for treatment of stapes ankylosis due to *NOG* mutations; therefore, the surgical outcome must be carefully assessed after long-term follow-up.

In conclusion, we identified a novel nonsense mutation in the *NOG* gene (p.K133*) in a *NOG*-SSD family. *NOG* gene mutations lead to aberrant functioning of the noggin protein, giving rise to a large spectrum of clinical features. Our patients exhibited a phenotype that included proximal symphalangism, dactylosymphysis, brachydactyly of the toes, pilonidal cyst, hyperopia, and conductive hearing loss. Stapes surgery for

Table 1. *NOG* mutations reported in the literature

Nucleotide change	*Type of mutation*	*Protein*	*Phenotype*	*Authors (year)*	*Reference*
c.58del	Frameshift	p.Leu20fs	Multiple synostoses syndrome	Takahashi *et al.* (2001)	18
c.103C>G	Missense	p.Pro35Ala	Brachydactyly type B	Lehmann *et al.* (2007)	11
c.103C>T	Missense	p.Pro35Ser	Teunissen-Cremers syndrome	Hirshoren *et al.* (2008)	25
c.103C>T	Missense	p.Pro35Ser	Proximal symphalangism	Mangino *et al.* (2002)	26
c.103C>T	Missense	p.Pro35Ser	Brachydactyly type B	Lehmann *et al.* (2007)	11
c.104C>G	Missense	p.Pro35Arg	Proximal symphalangism	Gong *et al.* (1999)	8
c.104C>G	Missense	p.Pro35Arg	Tarsal–carpal coalition syndrome	Dixon et at. (2001)	27
c.106G>C	Missense	p.Ala36Pro	Brachydactyly type B	Lehmann *et al.* (2007)	11
c.110C>G	Missense	p.Pro37Arg	Tarsal–carpal coalition syndrome	Debeer *et al.* (2004)	28
c.[124C>G;149C>G]	Missense	p.(Pro42Ala; Pro50Arg)	Multiple synostoses syndrome	Debeer *et al.* (2005)	29
c.125C>G	Missense	p.Pro42Arg	Multiple synostoses syndrome	Oxley *et al.* (2008)	30
c.129_130dup	Frameshift	p.Val44fs	Teunissen-Cremers syndrome	Weekamp *et al.* (2005)	7
c.142G>A	Missense	p.Glu48Lys	Brachydactyly type B	Lehmann *et al.* (2007)	11
c.142G>A	Missense	p.Glu48Lys	Proximal symphalangism Premature ovarian failure	Kosaki *et al.* (2004)	31
c.149C>G	Missense	p.Pro50Arg	Tarsal–carpal coalition syndrome	Debeer *et al.* (2005)	29
c.304del	Frameshift	p.Ala102fs	Proximal symphalangism	Thomeer *et al.* (2011)	19
c.252dup	Frameshift	p.Glu85fs	Stapes ankylosis with broad thumb and toes	Brown *et al.* (2002)	14
c.328C>T	Nonsense	p.Gln110X	Stapes ankylosis with broad thumb and toes	Brown *et al.* (2002)	14
c.386T>A	Nonsense	p.Leu129X	Proximal symphalangism	Takahashi *et al.* (2001)	18
c.397A>T	Nonsense	p.Lys133X	Stapes ankylosis with broad thumb and toes	Present study	
c.406C>T	Missense	p.Arg136Cys	Proximal symphalangism	Masuda *et al.* (2014)	16
c.450G>C	Missense	p.Trp150Cys	Proximal symphalangism	Pan *et al.* (2015)	31
c.463T>A	Missense	p.Cys155Ser	Proximal symphalangism	Usami *et al.* (2012)	17
c.499C>G	Missense	p.Arg167Gly	Brachydactyly type B	Lehmann *et al.* (2007)	11
c.499C>T	Missense	p.Arg167Cys	Proximal symphalangism	Liu *et al.* (2015)	32
c.551G>A	Missense	p.Cys184Tyr	Proximal symphalangism	Takahashi 2001	18
c.551G>T	Missense	p.Cys184Phe	Proximal symphalangism	Usami 2012	17
c.559C>T	Missense	p.Pro187Ser	Brachydactyly type B	Lehmann *et al.* (2007)	11
c.559C>G	Missense	p.Pro187Ala	Proximal symphalangism	Ganaha *et al.* (2015)	22
c.561del	Frameshift	p.Glu188fs	Teunissen-Cremers syndrome	Weekamp *et al.* (2005)	7
c.565G>T	Missense	p.Gly189Cys	Proximal symphalangism	Gong *et al.* (1999)	8
c.568A>G	Missense	p.Met190Val	Multiple synostoses syndrome	Oxley *et al.* (2008)	30
c.608T>C	Missense	p.Leu203Pro	Teunissen-Cremers syndrome	Weekamp *et al.* (2005)	7
c.611G>T	Missense	p.Arg204Leu	Tarsal/carpal coalition syndrome	Dixon *et al.* (2001)	27
c.614G>A	Nonsense	p.Trp205X	Multiple synostoses syndrome	Dawson *et al.* (2006)	33
c.615G>C	Missense	p.Trp205Cys	Facioaudiosymphalangism syndrome	van den Ende *et al.* (2005)	34
c.615G>C	Missense	p.Trp205Cys	Stapes ankylosis with broad thumb and toes	Emery *et al.* (2009)	35
c.645C>A	Nonsense	p.Cys215X	Stapes ankylosis with broad thumb and toes	Usami *et al.* (2012)	17
c.649T>G	Missense	p.Trp217Gly	Multiple synostoses syndrome	Gong *et al.* (1999)	8
c.659T>A	Missense	p.Ile220Asn	Proximal symphalangism	Gong *et al.* (1999)	8
c.659_660delinsAT	Frameshift	p.Ile220Asn	Proximal symphalangism	Gong *et al.* (1999)	8
c.664T>G	Missense	p.Tyr222Asp	Proximal symphalangism	Gong *et al.* (1999)	8
c.665A>G	Missense	p.Tyr222Cys	Proximal symphalangism	Gong *et al.* (1999)	8
c.665A>G	Missense	p.Tyr222Cys	Tarsal–carpal coalition syndrome	Dixon *et al.* (2001)	27
c.668C>T	Missense	p.Pro223Leu	Proximal symphalangism	Gong *et al.* (1999)	8
c.682T>G	Missense	p.Cys228Gly	Stapes ankylosis with broad thumb and toes	Ishino *et al.* (2015)	36
c.682T>A	Missense	p.Cys228Ala	Multiple synostoses syndrome	Ganaha *et al.* (2015)	22
c.696C>G	Missense	p.Cys232Trp	Multiple synostoses syndrome	Rudnik-Schöneborn *et al.* (2010)	37
17q22 long deletion			Multiple synostoses syndrome	Shimizu *et al.* (2008)	38
17q22 microdeletion			proximal symphalangism	Pang *et al.* (2015)	39

Nucleotic numbering is based on GenBank reference sequence NM_005450.4.

conductive hearing loss is a good therapeutic option; however, patients should be carefully monitored over the long term. This novel mutation and clinical manifestations contribute to a better understanding of *NOG*-SSD.

ACKNOWLEDGEMENTS

This research was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant no. 25861575).

COMPETING INTERESTS

The authors declare no conflict of interest.

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