Stickler syndrome is a clinically and genetically heterogeneous group of collagenopathies characterized by ocular, auditory, orofacial and skeletal abnormalities. At least three subtypes of an autosomal dominant form of STL have been delineated: types 1 (STL 1, OMIM 120140), 2 (STL 2, OMIM 604841) and 3 (STL 3, OMIM 184840); these subtypes are a result of heterozygous mutations in COL9A1 (OMIM 120140), coding type II fibrillar collagen, COL11A1 (OMIM 120280), coding the α1 chain of type XI fibrillar collagen and COL11A2 (OMIM 120290), coding the α2 chain of type XI fibrillar collagen, respectively. Mutations in other genes, including biallelic mutations in COL9A1, COL9A2 and COL9A3 for an autosomal recessive form, are also known to be associated with this disease. Variable phenotypic expression of STL occurs both within and among families.

The diagnosis of STL is clinically based, but no consensus has been reached on the minimal clinical diagnostic criteria. As differential diagnosis is sometimes difficult owing to phenotypic variability, genetic testing facilitates diagnosis in some cases. Serial single-gene molecular genetic testing using standard Sanger sequencing based on an individual’s clinical findings and family history is routinely used to identify pathogenic mutations in clinical testing. However, the limitations of this genetic testing strategy, including low throughput and high cost, make the use of multigene panels laborious, time consuming and expensive. These limitations are particularly prominent when a target gene contains multiple exons (e.g., collagen genes), multiple genes are responsible for one disease due to genetic heterogeneity (e.g., STL) or multiple diseases with overlapping features caused by different genes exist. Disease-related gene panels or targeted-exome sequencing (TES) of candidate and/or known pathogenic genes using next-generation sequencing (NGS) technology can change the landscape of medical sequencing to diagnose patients with congenital disorders such as STL. We report a novel heterozygous and possibly pathogenic variation in COL11A1, NM_001854.3:n.4838G>A [NM_001854.3 (COL11A1_v001):c.4520G>A], detected by targeted-NGS in our study of a Japanese patient with STL using a targeted-exome panel comprising the coding regions of 4,813 clinical phenotype-associated genes, including STL-related genes.

The proband (IV:2, Figure 1) was a 2-year-old girl of healthy, non-consanguineous parents who was born after 40 weeks of gestation. At birth, her weight, height and occipitofrontal circumference (OFC) were 3,002 g (+0 s.d), 50 cm (+0.8 s.d) and 32.5 cm (+0.3 s.d), respectively. She was born with a cleft palate, and automated auditory brainstem response (AABR) testing suggested bilateral hearing impairment. At 2 months of age, she visited the Tokyo Medical and Dental University Hospital for cleft palate treatment. At this time, Pierre Robin sequence (U-shaped cleft palate, micro/retrognathia and glossoptosis) and her brother’s cleft palate were indicated. At 6 months of age, she was clinically diagnosed with STL according to her craniofacial anomalies as well as her older brother’s clinical characteristics associated with this disease, as described below. Plastic surgery for cleft palate was successfully performed. Eardrum tube was placed to alleviate otitis media with effusion. Her motor and mental development was normal. Bilateral mild high-frequency sensorineural hearing loss (HFSNHL) was revealed by audiometric testing, but no abnormality was revealed on ophthalmological evaluation up to 2 years of age.

Her older brother (IV:1, Figure 1) was a 4-year-old boy who was born after 40 weeks of gestation. At birth, his weight, height and OFC were 3,060 g (+0.1 s.d), 50 cm (+0.8 s.d) and 35 cm (+1.2 s.d), respectively. He was born with a cleft palate, and AABR testing suggested bilateral hearing impairment. At 1 year of age, plastic surgery for cleft palate was successfully performed. Eardrum tube was placed to alleviate otitis media with effusion. His motor and mental development was normal. Owing to his younger sister’s cleft palate, physical and radiographic examinations were...
performed at 34 months of age. A flat midface with nasal bridge hypoplasia was indicated at that age. Radiographs taken at that age revealed metaphyseal widening of the femoral neck and mild platyspondyly. Although the otitis media improved, audiometric testing at that age revealed bilateral HFSNHL. No ophthalmological abnormalities had been observed up to 4 years of age. His clinical characteristics pertaining to orofacial, auditory and skeletal abnormalities were compatible with those of STL. Detailed information on the orofacial, ocular, auditory and skeletal characteristics of the patients’ parents was not available (Figure 1).

The ethics committees of the Tokyo Medical and Dental University and Tokushima University approved this study. After obtaining written consent from the mother of the patients, molecular diagnosis was performed using genomic DNA extracted from the patients’ whole blood. We first performed TES using a TruSight One Sequencing Panel (Illumina, San Diego, CA, USA) and an MiSeq benchtop sequencer (Illumina) for the proband (IV:2). The alignments of sequencing reads to the human reference genome (hg19), duplicate read removal, local realignment around indels, base quality score recalibration, variant calling and annotation were performed as described elsewhere. To identify single-nucleotide variations (SNVs), we excluded minor sequence variants with low-allele frequencies, i.e., >0.05 included in the 1000 Genomes Project database (http://www.1000genomes.org/), NHLBI GO Exome Sequencing Project (ESP6500, http://evs.gs.washington.edu/EVS/), Human Genetic Variation Database (HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/) and integrative Japanese Genome Variation Database (iJGVD, https://igvdata.mega-bank.tohoku.ac.jp/).

The detection of copy-number variations (CNVs) using NGS data with a resolution of a single exon to several CNVs, were detected in genes potentially responsible for STL or related diseases. This mutation was neither present in human genome variation databases, such as dbSNP138, 1000 Genomes Project database (http://www.1000genomes.org/), NHLBI GO Exome Sequencing Project (ESP6500, http://evs.gs.washington.edu/EVS/), Human Genetic Variation Database (HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/) and integrative Japanese Genome Variation Database (iJGVD, https://igvdata.mega-bank.tohoku.ac.jp/).

Four of 15 mutations were detected in family members who were considered healthy in the STL family, mutations in collagen genes may not necessarily result in the full STL phenotype, but may cause a mild phenotype or remain below the clinical expression level. At present, it cannot be determined whether the same mutation observed in the siblings had been inherited or de novo. As accurate radiographic and clinical examinations, including formal testing of hearing and vision, and genetic testing of the parents were not performed, it remains unclear whether the same COL11A1 mutation observed in the siblings had been inherited or produced de novo. Although the penetrance of STL is known to be high, variable clinical expression of STL complicates the genetic counseling scenario. As it was reported that the COL2A1 frameshift mutation leading to a premature stop codon was detected in family members who were considered healthy in the STL family, mutations in collagen genes may not necessarily result in the full STL phenotype, but may cause a mild phenotype or remain below the clinical expression level.
be ruled out that, among the carriers of mutations, subjects may frequently have an atypical phenotype rather than the typically known phenotype of STL.\(^{15}\)
Although instances of germline mosaicism have been reported only in STL with COL2A1 mutation, this also remains a possibility.\(^{16}\) Such insights would be helpful for counseling and parental decision making.

HGV DATABASE
The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.780.

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COMPETING INTERESTS
The authors declare no conflict of interest.

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