DATA REPORT

Novel and recurrent *COL11A1* and *COL2A1* mutations in the Marshall–Stickler syndrome spectrum

Long Guo[1,8], Nursel H Elcioglu[2,3,8], Zheng Wang[1,4], Yasemin K Demirkol[2], Pinar Isguven[5], Naomichi Matsumoto[6], Gen Nishimura[1,7], Noriko Miyake[6] and Shiro Ikegawa[1]

Marshall–Stickler syndrome represents a spectrum of inherited connective tissue disorders affecting the ocular, auditory, and skeletal systems. The syndrome is caused by mutations in the *COL2A1*, *COL11A1*, *COL11A2*, *COL9A1*, and *COL9A2* genes. In this study, we examined four Turkish families with Marshall–Stickler syndrome using whole-exome sequencing and identified one *COL2A1* mutation and three *COL11A1* mutations. Two of the *COL11A1* mutations were novel. Our findings expand our knowledge of the *COL11A1* mutational spectrum that causes Marshall–Stickler syndrome.

Marshall and Stickler syndromes are phenotypically overlapping connective tissue disorders characterized by ocular, auditory, skeletal, and orofacial abnormalities.[1,2] Most patients develop eye manifestations, including myopia, vitreoretinal degeneration, retinal detachment, and cataracts. Additional features include cleft palate, micrognathia, flat midface, hearing loss, and skeletal abnormalities.[3]

Marshall and Stickler syndromes are clinically variable and genetically heterogeneous. Based on the causal genes, Stickler syndromes are classified into five different subtypes: type I (STL1; MIM 108300), type II (STL2; MIM 604841), type III (STL3; MIM 184840), type IV (STL4; MIM 614134), and type V (STL5; MIM 614284). STL1, STL2, and STL3 are autosomal dominant disorders caused by mutations in the *COL2A1* (MIM 120140),[4,5] *COL11A1* (MIM 120280),[6,7] and *COL11A2* genes (MIM 120290),[8,9] respectively. STL4 and STL5 are autosomal recessive disorders caused by mutations in *COL9A1* (MIM 120210)[10,11] and *COL9A2* (MIM 120260),[12] respectively. Marshall syndrome (MIM 154780) and STL2 have the same causal gene, *COL11A1*,[13,14] and are considered to constitute an identical clinical spectrum.[6]

In this study, we conducted whole-exome sequencing and identified two novel and one recurrent *COL11A1* splice-site mutations, and one recurrent *COL2A1* mutation in four unrelated Turkish patients with the Marshall–Stickler syndrome spectrum.

The study protocol was approved by the Ethics Committee of RIKEN and participating institutions, and informed consent was obtained from all participants. The clinical manifestations are described below and summarized in Table 1. More detailed information is available in Supplementary Material.

Patient 1 from Family A was a baby boy who was referred to our genetics clinic because of his familial degenerative myopia, mild deafness, and mild hypermobility. He is the younger child of a non-consanguineous couple who had two children (Supplementary Figure S1). After birth, he presented with severe hypotonia and contractures of the hands. Physical examination at 1.5 years of age revealed that his weight was 12.6 kg (50–75th percentile), height 84 cm (75–90th percentile), and occipital frontal circumference (OFC) 48.2 cm (50th percentile). He had a mildly depressed nasal bridge, mild micrognathia, hypermobility of the finger joints, and phimosis (Figure 1a,b). Eye examination revealed degenerative myopia (−5.75/− 5.25) and tigroid retinae. He had mild hearing loss (10 dB, mixed type).

Patient 2 from Family B was a newborn girl who was referred to the genetics clinic because of her Pierre Robin sequence (the triad of micro-retrognathia, cleft palate, and glossoptosis). She was the only child of unrelated healthy parents (Supplementary Figure S1). The first physical examination at the age of 1 month revealed that her weight was 3,080 g (10–25th percentile), length 49 cm (10–25th percentile), and OFC 35 cm (10–25th percentile). She presented with a flat face with a depressed profile, buphthalmic eyes, hypertelorism, bilateral epicanthus, depressed nasal bridge, short stubby nose, and Pierre Robin sequence (Figure 1c). She also had axial hypotony and pectus excavatum. Echocardiography revealed atrial septal defect (ASD). Strabismus, myopia, and megalocornea (corneal diameter 11 mm/11 mm) were followed later with growth retardation and deafness, which needed ear tube insertion. Her height fell under the third percentile at age 4 years.

Patient 3 from Family C was a baby girl who was referred to us because of cleft palate, deafness, and facial dysmorphism. She was born as the second child of a Turkish family consisting of non-consanguineous parents and a healthy elder boy (Supplementary Figure S1). Cleft palate was recognized at birth, and deafness was observed at the age of 4 months. When she was first examined

[1]Laboratory for Bone and Joint Diseases, RIKEN Center for Integrative Medical Sciences, Tokyo, Japan; [2]Department of Pediatric Genetics, Marmara University Medical School, Istanbul, Turkey; [3]Eastern Mediterranean University Medical School, Mersin, Turkey; [4]Department of Medical Genetics, Institute of Basic Medical Sciences, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China; [5]Department of Pediatric Endocrinology, Sakarya University Medical School, Sakarya, Turkey; [6]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan and [7]Department of Pediatric Imaging, Tokyo Metropolitan Children's Medical Center, Fuchu, Japan.
Correspondence: S Ikegawa (sikegawa@ims.u-tokyo.ac.jp)
[8]These authors contributed equally to this work.
Received 5 June 2017; revised 28 July 2017; accepted 2 August 2017

Table 1. Overview of the four individuals with Marshall–Stickler syndrome

Phenotype and genotype	Case 1	Case 2	Case 3	Case 4
Gene	COL11A1	COL11A1	COL11A1	COL2A1
Mutation	c.1845+1G>A	c.2808+1G>C	c.3816+1G>A	c.2710C>T
Phenotype				
Myopia	++	+	+++	++
Vitreoretinal degeneration	++	−	++	+
Retinal detachment	++	−	−	−
Cataracts	++	++	+	−
Midfacial hypoplasia	+	+++	+++	+++
Micrognathia/retrognathia	+	++	++	−
Cleft palate/bifid uvula	−	++	++	+
Hearing loss	+	+++	+++	+++
Joint hypermobility	++	−	−	−
Congenital heart defect	−	+	−	−
Short stature	−	+++	+++	++
Skeletal abnormalities	NA	NA	+	+

Abbreviation: NA, not assessed. The number of plus signs indicates the severity of the abnormality.
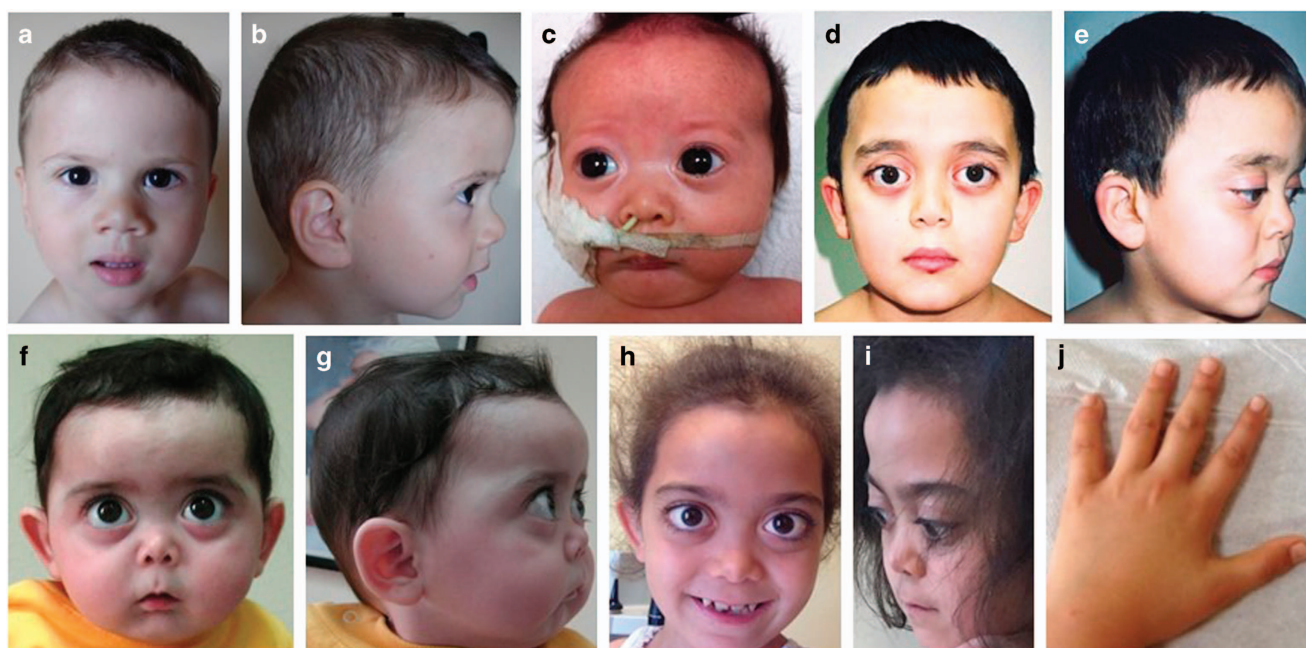

Figure 1. Physical appearance of the patients. (**a**, **b**) Case 1, age 1.5 years. Mild depression of the nasal bridge and micrognathia. (**c**) Case 2, age 1 month. Buphthalmic eyes, hypertelorism, bilateral epicanthus, flat face, depressed nasal bridge, short stubby nose, and micro-retrognathia. (**d**, **e**) Case 4, age 8.5 years. Proptotic eyes, flat face with mild frontal bossing, depressed nasal bridge, and short nose. (**f**, **g**) Case 3, age 9 months. Buphthalmic eyes, flat face with frontal bossing, midfacial hypoplasia, depressed nasal bridge, short nose with anteverted nares, long philtrum, and micro-retrognathia. (**h**–**j**) Case 3, age 9 years. (**h**, **i**) High-frontal area, big proptotic eyes, long palpebral fissures, depressed nasal bridge, short nose, long philtrum, irregular teeth order, micrognathia, and dry rough hairs. (**j**) Small hands with brachydactyly.

at the age of 9 months, she had a very flat face with frontal bossing, buphthalmic eyes, midfacial hypoplasia, flat nasal bridge, hypoplastic depressed short nose with anteverted nares, long philtrum, cleft palate, bifid uvula, small mouth and chin, and micro-retrognathia (Figure 1f,g). Audiological evaluation confirmed severe sensorineural deafness bilaterally. Eye examination revealed tigroid retinae, 10.5 mm corneal diameter, and 11 mmHg intraocular pressure. Her follow-up examination at the age of 9 years revealed that her weight was 21.5 kg (3rd–10th percentile), height 113 cm (<3rd percentile), and OFC 51 cm (2nd–50th percentile). Her main problems were large proptotic eyes, long palpebral fissures, degenerative high myopia (−22/−22), cone dystrophy (detected by electroretinography), retinal pigmentation,

right cataract, deafness (wearing a hearing aid), high-frontal area, depressed nasal bridge, hypoplastic short nose, repaired cleft palate, irregular teeth order, long philtrum, micrognathia, short stature, small hands with brachydactyly, and dry rough hairs (Figure 1h–j).

Patient 4 from Family D was an 8.5-year-old boy who suffered from high myopia, deafness, short stature, and facial dysmorphism. His consanguineous parents and his elder brother were healthy (Supplementary Figure S1). The prenatal and postnatal course were uneventful. Physical examination at the age of 8.5 years revealed that his weight was 32.5 kg (75–90th percentile), height 122 cm (10th percentile), and OFC 57 cm (97th percentile). He had macrocephaly, flat face with midfacial

hypoplasia, mild frontal bossing, depressed nasal bridge, short nose, anteverted nares, proptotic eyes, severe myopia (−17/ − 17), glaucoma with 14 mmHg intraocular pressure, bifid uvula, sensorineural deafness, and short hands with brachydactyly (Figure 1d,e).

Whole-exome sequencing was performed as previously described (Supplementary Methods, Supplementary Table S1).[15–18] The identified variants were confirmed by Sanger sequencing. We identified three heterozygous variants in COL11A1 (NM_001854: c.1845+1G > A, c.2808+1G > C, c.3816+1G > A) and one heterozygous variant in COL2A1 (NM_001844: c.2710C > T) in the four unrelated patients, respectively. COL11A1 and COL2A1 mutations are known to cause Marshall–Stickler syndrome.[4–7] No potential mutations in any known disease genes, including other disease genes of Stickler syndrome (COL11A2, COL9A1, and COL9A2), were identified.[4,5] By Sanger sequencing, we confirmed the variants in the patients. We also verified that c.2808+1G > C, c.3816+1G > A and c.2710C > T are de novo variants due to their absence in the patients' parents' genomes (Supplementary Figure S2).

c.3816+1G > A[13] and c.2710C > T[19] are recurrent mutations in the Marshall–Stickler syndromes and have been annotated in The Human Gene Mutation Database (HGMD) as mutations (CS982120 and CM980396, respectively). By contrast, both c.1845+1G > A and c.2808+1G > C have not been deposited in any available public databases, including dbSNP, 1,000 genomes, ExAC, ESP6500, and HGMD. Similar to the known mutation c.3816+1G > A, both of the novel variants also located at splice donor sites of the intronic sequences of COL11A1 (intron 18, 36, and 50, respectively; Supplementary Figure S2). According to the GT–AG rule, the two novel variants would certainly disrupt the donor-site motifs. Several in silico splice-site prediction programs indicated that the donor splice sites around these variants disappeared or were weakened greatly, suggesting aberrant splicing (Supplementary Table S2). Thus, the two novel splice-site variants in our study would be pathogenic mutations. Until now, more than 20 splice-site mutations in COL11A1 related to Marshall-STL2 Stickler syndrome have been reported. Our results will further expand our knowledge of the mutational spectrum of the COL11A1 gene.

The characteristics of Marshall–Stickler syndrome spectrum caused by COL11A1 are different from those caused by COL2A1.[14] The most important aspects of the differential diagnosis are that the patients with COL11A1 mutations more commonly had severe hearing impairment than those with COL2A1 mutations. In addition, the patients with COL11A1 mutations seldom had vitreoretinal degeneration and retinal detachment compared with the high incidence in those with COL2A1 mutations.[14] However, in our study, Case 4 with COL2A1 mutation presented with severe hearing impairment; Cases 1 and 3 with COL11A1 mutations had retinal detachment and/or retinal degeneration to a certain extent. Additionally, Marshall–Stickler syndrome caused by COL11A1 is considered to have a higher incidence of midfacial hypoplasia than that caused by COL2A1.[20] By contrast, Case 1, who carries a novel mutation in COL11A1, not only manifested very mild hearing loss and severe retinal detachment but also showed a nearly normal facial appearance. All of the clinical characteristics of Case 1 are compatible with the common phenotypes caused by COL2A1 but not COL11A1. The contradictory manifestations in our patients suggest a more complex genotype–phenotype association than previously reported. The highly overlapping phenotypes of STL1 and STL2 are reasonable because of the structural nature of the collagen molecules. COL2A1, COL11A1, and COL11A2 encode each of the three alpha chains. These alpha chains are finally assembled into one protein, type XI collagen characterized by triple helix.

Molecular genetic analysis is necessary for confirming the clinical diagnosis of patients with Marshall–Stickler syndrome spectrum due to the phenotypic variability. Candidate gene-based screening by Sanger sequencing is usually laborious and time-consuming, particularly when applied to the analysis of multiple large genes, such as COL11A1 and COL2A1, which have more than 50 exons. In this study, we used whole-exome sequencing to identify the causal mutations in different collagen genes. The method based on next-generation sequencing seems to be highly effective in the molecular diagnosis of Marshall–Stickler syndrome.

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.1639; http://dx.doi.org/10.6084/m9.figshare.hgv.1642; http://dx.doi.org/10.6084/m9.figshare.hgv.1645; http://dx.doi.org/10.6084/m9.figshare.hgv.1648.

COMPETING INTERESTS

The authors declare no conflict of interest.

PUBLISHER'S NOTE

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1 Stickler GB, Belau PG, Farrell FJ, Jones JD, Pugh DG, Steinberg AG et al. Hereditary progressive arthro-ophthalmopathy. Mayo Clin Proc 1965; 40: 433–455.
2 Marshall D. Ectodermal dysplasia; report of kindred with ocular abnormalities and hearing defect. Am J Ophthalmol 1958; 45: 143–156.
3 Baraitser M. Marshall/Stickler syndrome. J Med Genet 1982; 19: 139–140.
4 Ahmad NN, Ala-Kokko L, Knowlton RG, Jimenez SA, Weaver EJ, Maguire JI et al. Stop codon in the procollagen II gene (COL2A1) in a family with the Stickler syndrome (arthro-ophthalmopathy). Proc Natl Acad Sci USA 1991; 88: 6624–6627.
5 Ahmad NN, McDonald-McGinn DM, Zackai EH, Knowlton RG, LaRossa D, DiMascio J et al. A second mutation in the type II procollagen gene (COL2AI) causing stickler syndrome (arthro-ophthalmopathy) is also a premature termination codon. Am J Hum Genet 1993; 52: 39–45.
6 Majava M, Hoornaert KP, Bartholdi D, Bouma MC, Bouman K, Carrera M et al. A report on 10 new patients with heterozygous mutations in the COL11A1 gene and a review of genotype-phenotype correlations in type XI collagenopathies. Am J Med Genet A 2007; 143A: 258–264.
7 Richards A, Yates JRW, Williams R, Payne SJ, Pope FM, Scott JD et al. A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in alpha 1 (XI) collagen. Hum Mol Genet 1996; 5: 1339–1343.
8 Vikkula M, Mariman EC, Lui VC, Zhidkova NI, Tiller GE, Goldring MB et al. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. Cell 1995; 80: 431–437.
9 Brunner HG, van Beersum SE, Warman ML, Olsen BR, Ropers HH, Mariman EC. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. Hum Mol Genet 1994; 3: 1561–1564.
10 Van Camp G, Snoeckx RL, Hilgert N, van den Ende J, Fukuoka H, Wagatsuma M et al. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. Am J Hum Genet 2006; 79: 449–457.
11 Nikopoulos K, Schrauwen I, Simon M, Collin RWJ, Veckeneer M, Keymolen K et al. Autosomal recessive Stickler syndrome in two families is caused by mutations in the COL9A1 gene. Invest Ophthalmol Vis Sci 2011; 52: 4774–4779.
12 Baker S, Booth C, Fillman C, Shapiro M, Blair MP, Hyland JC et al. A loss of function mutation in the COL9A2 gene causes autosomal recessive Stickler syndrome. Am J Med Genet A 2011; 155A: 1668–1672.
13 Griffith AJ, Sprunger LK, Sirko-Osadsa DA, Tiller GE, Meisler MH, Warman ML. Marshall syndrome associated with a splicing defect at the COL11A1 locus. Am J Hum Genet 1998; 62: 816–823.

14 Annunen S, Körkkö J, Czarny M, Warman ML, Brunner HG, Kääriäinen H *et al.* Splicing mutations of 54-bp exons in the COL11A1 gene cause Marshall syndrome, but other mutations cause overlapping Marshall/Stickler phenotypes. *Am J Hum Genet* 1999; **65**: 974–983.

15 Guo L, Elcioglu NH, Iida A, Demirkol YK, Aras S, Matsumoto N *et al.* Novel and recurrent XYLT1 mutations in two Turkish families with Desbuquois dysplasia, type 2. *J Hum Genet* 2016; **62**: 447–451.

16 Guo L, Girisha KM, Iida A, Hebbar M, Shukla A, Shah H *et al.* Identification of a novel LRRK1 mutation in a family with osteosclerotic metaphyseal dysplasia. *J Hum Genet* 2016; **62**: 437–441.

17 Wang Z, Horemuzova E, Iida A, Guo L, Liu Y, Matsumoto N *et al.* Axial spondylometaphyseal dysplasia is also caused by NEK1 mutations. *J Hum Genet* 2017; **62**: 503–506.

18 Guo L, Elcioglu NH, Mizumoto S, Wang Z, Noyan B, Albayrak HM *et al.* Identification of biallelic EXTL3 mutations in a novel type of spondylo-epi-metaphyseal dysplasia. *J Hum Genet* 2017; **62**: 797–801.

19 Ballo R, Beighton PH, Ramesar RS. Stickler-like syndrome due to a dominant negative mutation in the COL2A1 gene. *Am J Med Genet* 1998; **80**: 6–11.

20 Poulson AV, Hooymans JM, Richards AJ, Bearcroft P, Murthy R, Baguley DM *et al.* Clinical features of type 2 Stickler syndrome. *J Med Genet* 2004; **41**: e107–e107.

Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv)