

Identification of three novel homozygous variants in *COL9A3* causing autosomal-recessive Stickler Syndrome

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Research Article

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Abstract

Background: Stickler syndrome (STL) is a rare, clinically and molecularly heterogeneous connective tissue disorder. Pathogenic variants occurring in a variety of genes cause STL, mainly inherited in an autosomal dominant fashion. Autosomal recessive STL is ultra-rare with only four families with biallelic *COL9A3* variants reported to date.

Results: Here, we report three unrelated families clinically diagnosed with STL carrying different novel biallelic loss of function variants in *COL9A3*. Further, we have collected *COL9A3* genotype-phenotype associations from the literature.

Conclusion: Our report substantially expands the molecular genetics and clinical basis of autosomal recessive STL and provides an overview about allelic *COL9A3* disorders.

Background

Stickler syndrome (STL) is a rare, clinically and genetically heterogeneous connective tissue disorder divided into six clinical subtypes with overlapping features, including ocular pathologies (myopia, retinal detachment, vitreoretinal degeneration, cataract), hearing impairment (sensorineural, mixed, and/or conductive), craniofacial abnormalities (midface hypoplasia, anteverted nares, depressed nasal bridge and either Pierre Robin sequence or cleft palate and micrognathia) and joint problems (mild spondyloepiphyseal dysplasia, and precocious osteoarthritis) (1). These features exhibit substantial variable expressivity according to clinical subtype (1). STL is molecularly diagnosed by the presence of pathogenic variants in six collagen-type genes including *COL2A1*, *COL11A1*, *COL11A2*, *COL9A1*, *COL9A2*, *COL9A3*, and two non-collagen genes consisting of *LRP2* and *LOXL3* (1-3), following a predominantly autosomal dominant inheritance pattern.

The heteropolymer collagen XI/IX/II are critical in the extracellular matrix of joints, bones, ligaments and connective tissues throughout the body (4). *COL2A1* encodes collagen type II alpha 1 chain. Heterozygous variants that cause functional haploinsufficiency are responsible for autosomal dominant STL type I (OMIM #108300), representing the most common subtype, accounting for roughly 80-90% of STL (5, 6). Pathogenic variants in *COL11A1* cause the second most common STL subtype, type II (OMIM #604841) (10-20%). Variants in this gene likewise typically follow a dominant inheritance pattern (7), although five families have been described with STL and biallelic *COL11A1* mutations (8-10). *COL11A2* pathogenic variants are very rare and cause autosomal dominant non-ocular Stickler syndrome (type III, OMIM#184840), also known as otospondylomegaepiphyseal dysplasia (OSMEDA, OMIM# 120290), as well as Weissenbacher-Zweymuller syndrome (WZS) (OMIM #184840) (11). Biallelic variants in *LOXL3*, a member of the lysyl oxidase family of genes, have recently been causally associated with STL in two unrelated families (2, 12). A biallelic missense variant in *LRP2* has likewise been suggested to cause STL (3).

Collagen IX proteins are encoded by *COL9A1*, *COL9A2* and *COL9A3* that together form fibril heterotrimer associated collagens and have been recently linked to autosomal recessive STL (13). Multiple epiphyseal dysplasia (MED) is an autosomal dominant allelic disorder caused by mutations in any of these three genes (1). Very recently, heterozygous *COL9A3* variants have been identified as causing peripheral vitreoretinal degeneration and retinal detachment (14). *COL9A1* and *COL9A2* are causally associated with autosomal recessive STL type IV (OMIM #614134) and V (OMIM #614284), respectively. The main clinical characteristics of individuals affected with biallelic *COL9A1* variants include moderate-to-severe sensorineural hearing loss, moderate-to-high myopia with vitreoretinopathy, and epiphyseal dysplasia, whereas *COL9A2* variants are associated with high myopia, vitreoretinal degeneration, retinal detachment, hearing loss, and short stature. Only very recently, biallelic mutations in *COL9A3* have been described to cause autosomal recessive STL in four unrelated families with seven patients (13, 15-17). The main phenotypes that are common in all these patients consisted of high myopia, moderate to severe sensorineural hearing loss, and spondylo/epiphyseal dysplasia. Here, we report three additional unrelated consanguineous STL families with five affected individuals in total who each present three novel biallelic *COL9A3* variants.

Results

Clinical assessments:

Three unrelated consanguineous families of Iranian descent were referred for genetic testing due to hearing and vision impairment (Fig. 1), as well as skeletal dysplasia that resulted in a clinical diagnosis of STL (Fig. 2).

The female proband (II1) from Family 1 is the oldest and only affected individual out of three children from first cousin parents. She had a normal delivery and birth, with a birth weight of 3.2 kg (-0.43 SD). She was 28 years old at last examination with a weight of 64 kg (+0.41 SD), height of 157 cm (-0.8 SD) and occipitofrontal circumference (OFC) of 55 cm (+0.62 SD). She suffers from high myopia in both eyes, in addition to vitreoretinal degeneration with empty vitreous, multiple lattice degenerations and retinal pigmentary changes. There was unilateral absence of the frontal sinus in her skull X-ray. She has severe and progressive sensorineural hearing loss. X-ray and detailed examination of her joints and bones, including mobility testing and examination for signs of osteoarthritis were normal, however she complained of pain in her knee joints. Typical STL craniofacial features such as midface hypoplasia, cleft palate, micrognathia, depressed nasal bridge and anteverted nares are absent.

Family 2 presented with two affected individuals out of four children who were born from a first cousin marriage. The proband (II1) and his affected sibling (II2) both had a normal delivery around term, measurements at birth could not be obtained. Weight, height and OFC at last clinical assessment (at 57 and 55 years-old) were 68 kg (-0.16 SD) and 66 kg (-0.39 SD), 166 cm (-1.4 SD) and 163 cm (-1.8 SD), 56 cm (+0.62 SD) and 57 cm (+1.32 SD), respectively. Both had a history of multiple vitreoretinal surgeries due to recurrent rhegmatogenous retinal detachments resulting from advanced vitreoretinal degeneration. The older patient is considered blind without light perception (NLP) in either eye. His sibling has 20/200 vision in one eye following multiple vitreoretinal operations due

to complex pseudophakic retinal detachment while NLP was noted for the other eye. Both suffer from severe and progressive sensorineural hearing loss. Likewise, both show a herniated cervical disc and muscular atrophy was noted in the older sibling. No radiologic documentation was available for review.

Family 3 presented with two affected and two healthy children from first cousin parents. Both affected individuals had normal delivery with a birth weight of 3.4 kg (-0.26 SD) and 3.75 kg (+0.71 SD), length of 49 cm (-0.6 SD) and 49.5 cm (0.1 SD), and OFC of 35 cm (-0.40 SD) and 36 cm (+0.62 SD). The most current weight, height and OFC measurements for the proband (II1) at age 11.8 years and his sister (II4) at age 3.1 years are 32 kg (-1.43 SD), 137 cm (0.9 SD), and 53 cm (-0.53 SD) and 12 kg (-0.05 SD), 84 cm (-0.6 SD), and 48 cm (+0.39 SD), respectively. Both affected individuals have myopia and congenital moderate to severe progressive sensorineural hearing impairment. The affected male complains of knee joint pain, especially when he runs. X-ray and detailed examination demonstrated spondyloepiphyseal dysplasia in both children. Anthropometric measurements indicated mild short stature for individual II4. Both individuals II1 and II4 have pes planus, depressed nasal bridge and anteverted nares, but only II4 showed midface hypoplasia and palpebral fissure. Detailed clinical features of all affected individuals are described in Table 1 and Supplementary Table 1.

Table 1
Summary of genetic and clinical findings in probands with biallelic COL

	p.(Pro36Argfs*49) Family 1	p.(Arg402*) Family 2, Patient1	p.(Arg402*) Family 2, Patient 2	p.(Leu119Serfs*10) Family 3, Patient 1	p.(Leu119Serfs*10) Family3, Patient 2	p.(Gln393Cysfs*25) Faletra et al. (2014)(15) Patient 1	p.(Gln393Cys Faletra et al (2014) Pati
Ethnicity	Iranian	Iranian	Iranian	Iranian	Iranian	Moroccan	Moroccan
Consanguinity	First cousin	First cousin	First cousin	First cousin	First cousin	First cousin	First cousin
Sex	Female	Male	Male	Male	Female	Female	Male
Age in years	28	57	55	11y, 8m	3y,1m	4	11
Birth	Uncomplicated (Normal Delivery)	Uncomplicated (Normal Delivery)	Uncomplicated (Normal Delivery)	Uncomplicated	Uncomplicated	NA	NA
Measurements							
OFC at last examination	55 cm (+ 0.62 SD)	56 cm (+ 0.62 SD)	57cm (+ 1.32 SD)	53 cm (-0.53 SD)	48 cm (+ 0.39 SD)	NA	NA
Weight at last evaluation	64 kg (+ 0.41 SD)	68 kg (-0.16 SD)	66 kg (-0.39 SD)	32 kg (-1.43 SD)	12 kg (-0.05 SD)	16 kg	38 kg
Height at last examination	157 cm (-0.8 SD)	166 cm (-1.4 SD)	163 cm (-1.8 SD)	137 cm (0.9 SD)	84 cm (-0.6 SD)	107 cm	144 cm
Visual system							
Myopia	Moderate-to-high	High	High	High	High	Moderate-to-high	Moderate-to
Vitreoretinal degeneration	No	Yes	Yes	No	No	No	No
Cataract	No	Yes	Yes	No	No	No	No
Retinal detachment	No	Yes	Yes	No	No	No	No
Auditory system							
Hearing loss	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Age at onset	NA	NA	NA	Early onset	Early onset	Early onset	NA
Type	Sensorineural	Sensorineural	Sensorineural	Sensorineural	Sensorineural	Sensorineural	Sensorineural
Degree of hearing loss	Severe	Profound	Profound	Moderate-to-severe	Moderate-to-severe	Moderate-to-severe	Moderate-to-severe
Progressive/stable	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive
Joints							
Short stature	No	No	No	Yes	Yes	No	No
Spondyloepiphyseal dysplasia	No	No	No	Yes	Yes	No	No
Epiphyseal dysplasia	No	No	No	Yes	Yes	Yes	Yes
Craniofacial structures							
Midface hypoplasia	No	No	No	No	Yes	Yes	Yes
Abbreviations: ID, intellectual disability; NA, (not ascertained), OFC, occipitofrontal circumference; PRS, Pierre Robin sequence; SD, standard deviation							

Genetic Analysis:

The DNA of probands from the three unrelated families (family 1 Proband II1, family 2 proband II1, family 3 proband II1) was subjected to Exome Sequencing (ES), revealing three different novel, homozygous loss of function (LOF) variants in *COL9A3*, NM_001853.3. The proband in Family 1 was found to have a *COL9A3* deletion (c.107_116del, p.(Pro36Argfs*49), rs1470627424), causing a frameshift in exon 2. The allele frequency in gnomAD is 0.00001390 with two carriers, while other public genomic databases such as Iranome and GME, and 1000 genomes have not reported this variant. The proband in family 2 disclosed a *COL9A3* nonsense variant (c.1204C > T, p.(Arg402*, rs989413835) in exon 23, while the proband in Family 3 showed a one base pair deletion in *COL9A3* (c.355delC, p.(Leu119Serfs*9)) in exon 7. Both variants have not been reported in public databases.

Discussion

Here, we report three families with five affected individuals clinically diagnosed with autosomal recessive STL due to biallelic LOF variants in *COL9A3*. Our report re-affirms previous studies that have described four families with biallelic LOF causing autosomal recessive STL, increasing the total number of families reported to date to seven (13, 15, 16). These *COL9A3* variants as well as other disease causing *COL9A3* variants submitted to HGMD are visualized in Fig. 3 for localization on cDNA and protein level.

COL9A3, along with two other heterodimers (*COL9A1* and *COL9A2*), belongs to the collagen IX complex, forming a fibril-associated collagen with interrupted triple (FACIT) helices and connecting with collagen II and XI fibrils. A *Col9a1* knockout mouse study previously demonstrated that absence of this protein in mice results in the loss of the entire collagen IX heterotrimer complex (18). Recent reports on the clinical phenotype of STL and MED syndromes that are caused by variants affecting different members of collagen IX have supported the hypothesis that each of the three proteins is essential for collagen IX function (13, 19).

While a variety of disorders have been described to result from heterozygous pathogenic variants in *COL9A3*, only four unrelated STL families and one family with nonsyndromic hearing loss have been reported to date carrying biallelic variants (Table 2)(13, 15–17, 20). Allelic disorders resulting from *COL9A3* variants include nonsyndromic hearing loss, MED, pseudoachondroplasia, cerebral palsy, and lumbar disc disease and severe peripheral vitreoretinal degeneration and retinal detachment (Table 2).

Table 2
Pathogenic *COL9A3* variants reported in HGMD and associated clinical phenotypes

-	-	99 bp duplication (CNV)	Het	NA	NA	Sensorineural hearing loss (27)
c.97C > T	p.(Pro33Ser)	2 Missense	Het	rs745914662	NA	Cerebral palsy (28)
c.104G > A	p.(Gly35Asp)	2 Missense	Het	rs1390736361	NA	Multiple epiphyseal dysplasia (29)
c.148-1G > A	p.?	2 Splicing	Het	rs606231367	NA	Multiple epiphyseal dysplasia (30)
c.148-2A > G	p.?	2 Splicing	Het	NA	NA	Multiple epiphyseal dysplasia(31)
c.148-2A > T	p.?	2 Splicing	Het	NA	P	Multiple epiphyseal dysplasia (32)
c.183 + 5G > A	p.?	3 Splicing	Het	NA	P	Multiple epiphyseal dysplasia (33)
c.268C > T	p.(Arg90Ter)	5 Nonsense	Comp het	rs763259234	NA	Stickler syndrome (17)
c.369 + 2T > C	p.?	7 Splicing	Het	rs1057518693	P	Multiple epiphyseal dysplasia (34)
c.369 + 8C > G	p.?	7 Splicing	Het	NA	NA	Multiple epiphyseal dysplasia (35)
c.388G > A	p.(Gly130Ser)	8 Missense	Het	rs139401633	VUS	Severe peripheral vitreoretinal degeneration and retinal detachment (14)
c.543_551del	p.(Pro185_Gly187del)	11 In frame	Hom	rs765392378	NA	Nonsyndromic hearing loss (20)
c.650dup C	p.(Gly217Trpfs*50)	13 Frameshift	Hom	NA	NA	Stickler syndrome (16)
c.971A > T	p.(Asn324Ile)	19 Missense	Het	NA	NA	Pseudoachondroplasia (36)
c.1107 + 1G > C	p.?	21 Splicing	Het			Severe peripheral vitreoretinal degeneration and retinal detachment (14)
c.1176_1198del	p.(Gln393Cysfs*25)	23 Frameshift	Hom	rs606231470	VUS	Stickler syndrome (15)
c.1277T > C	p.(Val426Ala)	24 Missense	Het	NA	NA	Pseudoachondroplasia (36)
c.1361G > A	p.(Gly454Glu)	26 Missense	Het	NA	NA	Nonsyndromic hearing loss (37)
c.1411C > T	p.(Arg471*)	28 Nonsense	Hom	rs747896279	P	Stickler syndrome (13)
c.1649C > T	p.(Pro550Leu)	30 Missense	Het	rs535230112	NA	Nonsyndromic hearing loss (37)
c.1729C > T	(p.Arg577Ter)	30 Nonsense	Comp het	rs1201247953	NA	Stickler syndrome (17)
c.1851C > A	p.(Asp617Glu)	31 Missense	Het	rs199577452	NA	Nonsyndromic hearing loss (20)

All variants are reported using the NM_001853.3 transcript. Abbreviations: Comp het, compound heterozygous; Het, heterozygous; Hom, homozygous; P, pathogenic; VUS, variant of uncertain significance; NA, (not ascertained)

Consistent clinical features among STL patients with biallelic *COL9A3* LOF alleles comprise moderate-to-profound progressive sensorineural hearing loss and moderate high myopia with vitreoretinal degeneration. Retinal detachment and cataract occasionally occur. In contrast, skeletal involvement seems to be more variable. For instance, Nixon et al 2019 reported a family with two affected siblings where the oldest affected sibling had severe arthropathy in the shoulders and hip, requiring a wheelchair(13). The X-ray of this patient showed spinal scoliosis and narrowing of the articular space in both knees, while the younger affected sibling did not show any of these signs. In line with this report, we also observed that the affected individuals in family 2, at the ages of 55 and 57 years-old, suffer only from myopia, hearing loss and each have a herniated cervical disc while the two much younger affected individuals in family 3, at ages 3 and 11 years-old, have more prominent skeletal findings that include radiological signs of spondyloepiphyseal dysplasia as well as craniofacial abnormalities including depressed nasal bridge and anteverted nares (Table 1). Moreover, Nixon et al 2019 observed that carrier parents can manifest mild STL phenotypes, while our report and others (15–17) have not observed mild phenotypes in heterozygous individuals. Besides Nixon’s report, Markova et al. 2021 introduced a more severe case with compound heterozygous variants with vitreoretinal degeneration, early onset osteoarthritis, midface hypoplasia, hip dysplasia, speech developmental delay, spina bifida, kyphosis, and eye pigment rearrangement (17).

Conclusion

In summary, our report consolidates that homozygous loss of function variants in *COL9A3* cause STL (type VI). We find high myopia and moderate-severe hearing loss to be consistent features amongst all cases while skeletal findings seem more variable.

Material And Methods

Subjects

Three unrelated Iranian families with syndromic phenotypes including hearing loss, vision impairment and skeletal dysplasia were referred for clinical genetic diagnostics. Blood samples were collected after obtaining informed consent from patients or their parents. Molecular genetic diagnostic testing was performed in Nijmegen via the Radboud innovative diagnostics programme and at the University of Tuebingen (197/2019B001). Informed consent from the parents or legal guardians of the patients/participants was obtained for the publication of their data.

Exome And Sanger Sequencing:

After extraction of DNAs from whole blood by standard protocol, proband DNA samples were subjected to exome capture using the Agilent SureSelect Human All Exon V6 Kit and exome sequencing (ES) was performed on an Illumina HiSeq 2500 sequencer for an average 50× sequencing depth, resulting in sequences of greater than 100 bases from each end of the fragments (Cambridge (Novogene UK)). Exome data were processed for analysis using a GATK-based pipeline (21) that uses Burrows-Wheeler alignment (22) to the GRCh37/UCSC hg19 (Families 1 and 2) and GRCh38/UCSC hg38 (Family 3). VarScan version 2.2.5, MuTec and GATK Somatic Indel Detector were used to detect SNV and InDels, respectively. The protocol to interpret potential pathogenic variants was previously described (23). For population-specific filtering, gnomAD (24), Iranome (25) and Greater Middle East (GME) Variome Project (26) databases were used.

Segregation analysis using Sanger sequencing was performed in available family members to confirm variant segregation after PCR amplification. Primers are available upon request.

List Of Abbreviations

DNA Deoxyribonucleic Acid

ES Exome sequencing

FACIT Fibril-associated collagen with interrupted triple helicies

GATK Genome Analysis Toolkit

GME Greater Middle East

LOF Loss of function

MED Multiple Epiphyseal Dysplasia

OFC Occipitofrontal circumference

PCR Polymerase Chain Reaction

SD Standard Deviation

SNV Single Nucleotide Variant

STL Stickler Syndrome

Declarations

Web Resources

ClinVar, <https://www.ncbi.nlm.nih.gov/clinvar/>

Exome Aggregation Consortium (ExAC), <http://exac.broadinstitute.org>

Genome Aggregation Database (*gnomAD*), <http://gnomad.broadinstitute.org/>

Ethics approval and consent to participate: Molecular genetic diagnostic testing was performed in Nijmegen via the Radboud innovative diagnostics programme and at the University of Tuebingen (197/2019B001).

Consent for publication: Informed consent from the parents or legal guardians of the patients/participants was obtained for the publication of their data.

Availability of data and materials: Data can be made available on personal request. Variants reported in this study have been deposited in the Leiden Open Variation Database (LOVD) and are available through the following variant accession numbers: 0000364418, 0000364419 and 0000364420.

Competing interests: The authors declare no competing interests.

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Authors' contributions:

FS, RM, EGK, ND, MD, AM, HA, HS, MR and NH recruited the probands and/or were involved in their clinical care. AR, MN, SL and DM conducted genetic data analysis. AR, MN, BV and MS conceived the study. AR, MN, BV and MS drafted the manuscript. BV and MS supervised the study. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Robin NH, Moran RT, Ala-Kokko L. Stickler syndrome. GeneReviews®[Internet]: University of Washington, Seattle; 2017.
2. Alzahrani F, Al Hazzaa SA, Tayeb H, Alkuraya FS. LOXL3, encoding lysyl oxidase-like 3, is mutated in a family with autosomal recessive Stickler syndrome. *Human genetics*. 2015;134(4):451-3.
3. Schrauwen I, Sommen M, Claes C, Pinner J, Flaherty M, Collins F, et al. Broadening the phenotype of LRP2 mutations: a new mutation in LRP2 causes a predominantly ocular phenotype suggestive of Stickler syndrome. *Clin Genet*. 2014;86(3):282-6.
4. Kadler KE, Hill A, Canty-Laird EG. Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Current opinion in cell biology*. 2008;20(5):495-501.
5. Liberfarb RM, Levy HP, Rose PS, Wilkin DJ, Davis J, Balog JZ, et al. The Stickler syndrome: genotype/phenotype correlation in 10 families with Stickler syndrome resulting from seven mutations in the type II collagen gene locus COL2A1. *Genetics in Medicine*. 2003;5(1):21-7.
6. Hoornaert KP, Vereecke I, Dewinter C, Rosenberg T, Beemer FA, Leroy JG, et al. Stickler syndrome caused by COL2A1 mutations: genotype–phenotype correlation in a series of 100 patients. *European journal of human genetics*. 2010;18(8):872-80.
7. 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. *American Journal of Medical Genetics Part A*. 2007;143(3):258-64.
8. Alzahrani F, Alshammari MJ, Alkuraya FS. Molecular pathogenesis of fibrochondrogenesis: is it really simple COL11A1 deficiency? *Gene*. 2012;511(2):480.
9. Richards AJ, Fincham GS, McNinch A, Hill D, Poulson AV, Castle B, et al. Alternative splicing modifies the effect of mutations in COL11A1 and results in recessive type 2 Stickler syndrome with profound hearing loss. *Journal of medical genetics*. 2013;50(11):765-71.
10. Nixon T, Richards AJ, Lomas A, Abbs S, Vasudevan P, McNinch A, et al. Inherited and de novo biallelic pathogenic variants in COL11A1 result in type 2 Stickler syndrome with severe hearing loss. *Mol Genet Genomic Med*. 2020:e1354.
11. Vuoristo MM, Pappas JG, Jansen V, Ala-Kokko L. A stop codon mutation in COL11A2 induces exon skipping and leads to non-ocular Stickler syndrome. *American Journal of Medical Genetics Part A*. 2004;130(2):160-4.
12. Chan TK, Alkaabi MK, ElBarky AM, El-Hattab AW. LOXL3 novel mutation causing a rare form of autosomal recessive Stickler syndrome. *Clinical Genetics*. 2019;95(2):325-8.

13. Nixon TR, Alexander P, Richards A, McNinch A, Bearcroft PW, Cobben J, et al. Homozygous Type IX collagen variants (COL9A1, COL9A2, and COL9A3) causing recessive Stickler syndrome—Expanding the phenotype. *American Journal of Medical Genetics Part A*. 2019;179(8):1498-506.
14. Nash BM, Watson CJG, Hughes E, Hou AL, Loi TH, Bennetts B, et al. Heterozygous COL9A3 variants cause severe peripheral vitreoretinal degeneration and retinal detachment. *Eur J Hum Genet*. 2021.
15. Faletra F, D'Adamo AP, Bruno I, Athanasakis E, Biskup S, Esposito L, et al. Autosomal recessive Stickler syndrome due to a loss of function mutation in the COL9A3 gene. *American Journal of Medical Genetics Part A*. 2014;164(1):42-7.
16. Hanson-Kahn A, Li B, Cohn DH, Nickerson DA, Bamshad MJ, Genomics UoWCFM, et al. Autosomal recessive Stickler syndrome resulting from a COL9A3 mutation. *American Journal of Medical Genetics Part A*. 2018;176(12):2887-91.
17. Markova T, Sparber P, Borovikov A, Nagornova T, Dadali E. Clinical and genetic characterization of autosomal recessive stickler syndrome caused by novel compound heterozygous mutations in the COL9A3 gene. *Mol Genet Genomic Med*. 2021:e1620.
18. Hagg R, Hedbom E, Möllers U, Aszódi A, Fässler R, Bruckner P. Absence of the $\alpha 1$ (IX) chain leads to a functional knock-out of the entire collagen IX protein in mice. *Journal of Biological Chemistry*. 1997;272(33):20650-4.
19. Jackson GC, Marcus-Soekarman D, Stolte-Dijkstra I, Verrips A, Taylor JA, Briggs MD. Type IX collagen gene mutations can result in multiple epiphyseal dysplasia that is associated with osteochondritis dissecans and a mild myopathy. *American Journal of Medical Genetics Part A*. 2010;152(4):863-9.
20. Asamura K, Abe S, Fukuoka H, Nakamura Y, Usami S. Mutation analysis of COL9A3, a gene highly expressed in the cochlea, in hearing loss patients. *Auris Nasus Larynx*. 2005;32(2):113-7.
21. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297-303.
22. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2010;26(5):589-95.
23. Rad A, Altunoglu U, Miller R, Maroofian R, James KN, Çağlayan AO, et al. MAB21L1 loss of function causes a syndromic neurodevelopmental disorder with distinctive cerebellar, ocular, craniofacial and genital features (COFG syndrome). *Journal of medical genetics*. 2019;56(5):332-9.
24. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-43.
25. Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Sellars E, Nezhadi SH, et al. Iranome: A catalog of genomic variations in the Iranian population. *Hum Mutat*. 2019;40(11):1968-84.
26. Scott EM, Halees A, Itan Y, Spencer EG, He Y, Azab MA, et al. Characterization of Greater Middle Eastern genetic variation for enhanced disease gene discovery. *Nat Genet*. 2016;48(9):1071-6.
27. Ji H, Lu J, Wang J, Li H, Lin X. Combined examination of sequence and copy number variations in human deafness genes improves diagnosis for cases of genetic deafness. *BMC Ear Nose Throat Disord*. 2014;14:9.
28. Pingel J, Andersen JD, Christiansen SL, Børsting C, Morling N, Lorentzen J, et al. Sequence variants in muscle tissue-related genes may determine the severity of muscle contractures in cerebral palsy. *Am J Med Genet B Neuropsychiatr Genet*. 2019;180(1):12-24.
29. Jeong C, Lee JY, Kim J, Chae H, Park HI, Kim M, et al. Novel COL9A3 mutation in a family diagnosed with multiple epiphyseal dysplasia: a case report. *BMC Musculoskelet Disord*. 2014;15:371.
30. Lohiniva J, Paassilta P, Seppänen U, Vierimaa O, Kivirikko S, Ala-Kokko L. Splicing mutations in the COL3 domain of collagen IX cause multiple epiphyseal dysplasia. *Am J Med Genet*. 2000;90(3):216-22.
31. Jackson GC, Mittaz-Crettol L, Taylor JA, Mortier GR, Spranger J, Zabel B, et al. Pseudoachondroplasia and multiple epiphyseal dysplasia: a 7-year comprehensive analysis of the known disease genes identify novel and recurrent mutations and provides an accurate assessment of their relative contribution. *Hum Mutat*. 2012;33(1):144-57.
32. Paassilta P, Lohiniva J, Annunen S, Bonaventure J, Le Merrer M, Pai L, et al. COL9A3: A third locus for multiple epiphyseal dysplasia. *Am J Hum Genet*. 1999;64(4):1036-44.
33. Nakashima E, Kitoh H, Maeda K, Haga N, Kosaki R, Mabuchi A, et al. Novel COL9A3 mutation in a family with multiple epiphyseal dysplasia. *Am J Med Genet A*. 2005;132a(2):181-4.
34. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, et al. Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. *N Engl J Med*. 2017;376(1):21-31.
35. Lord J, Gallone G, Short PJ, McRae JF, Ironfield H, Wynn EH, et al. Pathogenicity and selective constraint on variation near splice sites. *Genome Res*. 2019;29(2):159-70.
36. Jung WW, Balce GC, Cho JW, Jung SC, Hong SJ, Song HR. COMP and Col9A3 mutations and their relationship to the pseudoachondroplasia phenotype. *Int J Mol Med*. 2010;26(6):885-91.
37. Miyagawa M, Naito T, Nishio SY, Kamatani N, Usami S. Targeted exon sequencing successfully discovers rare causative genes and clarifies the molecular epidemiology of Japanese deafness patients. *PLoS One*. 2013;8(8):e71381.

Figures

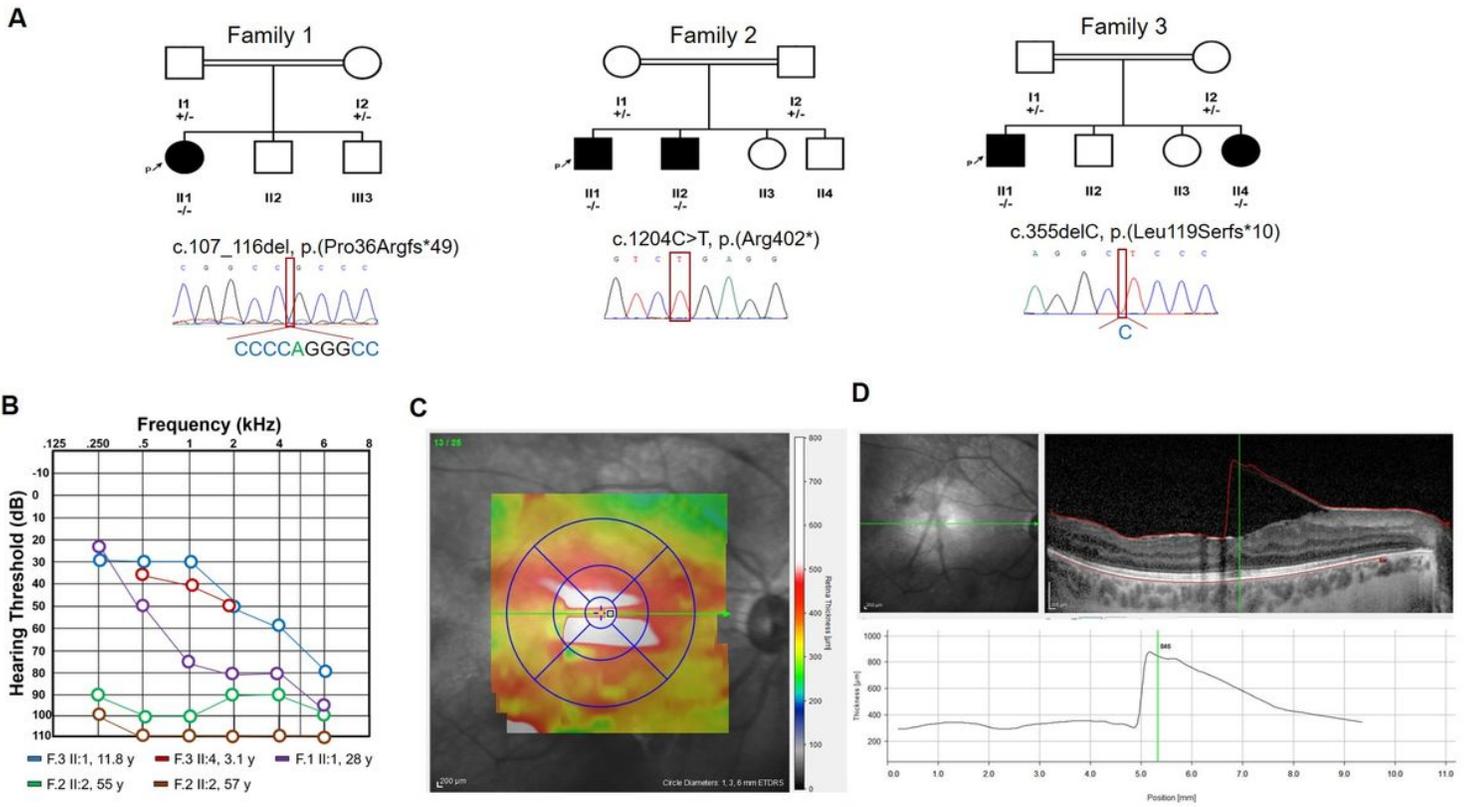


Figure 1
 A: Pedigrees and electropherogram of affected individuals with biallelic COL9A3 variants, B: audiograms of right ears of all affected individuals from three families. C and D: Optical coherence tomography (OCT) imaging in proband II1 from family 1 showing retinal detachment.

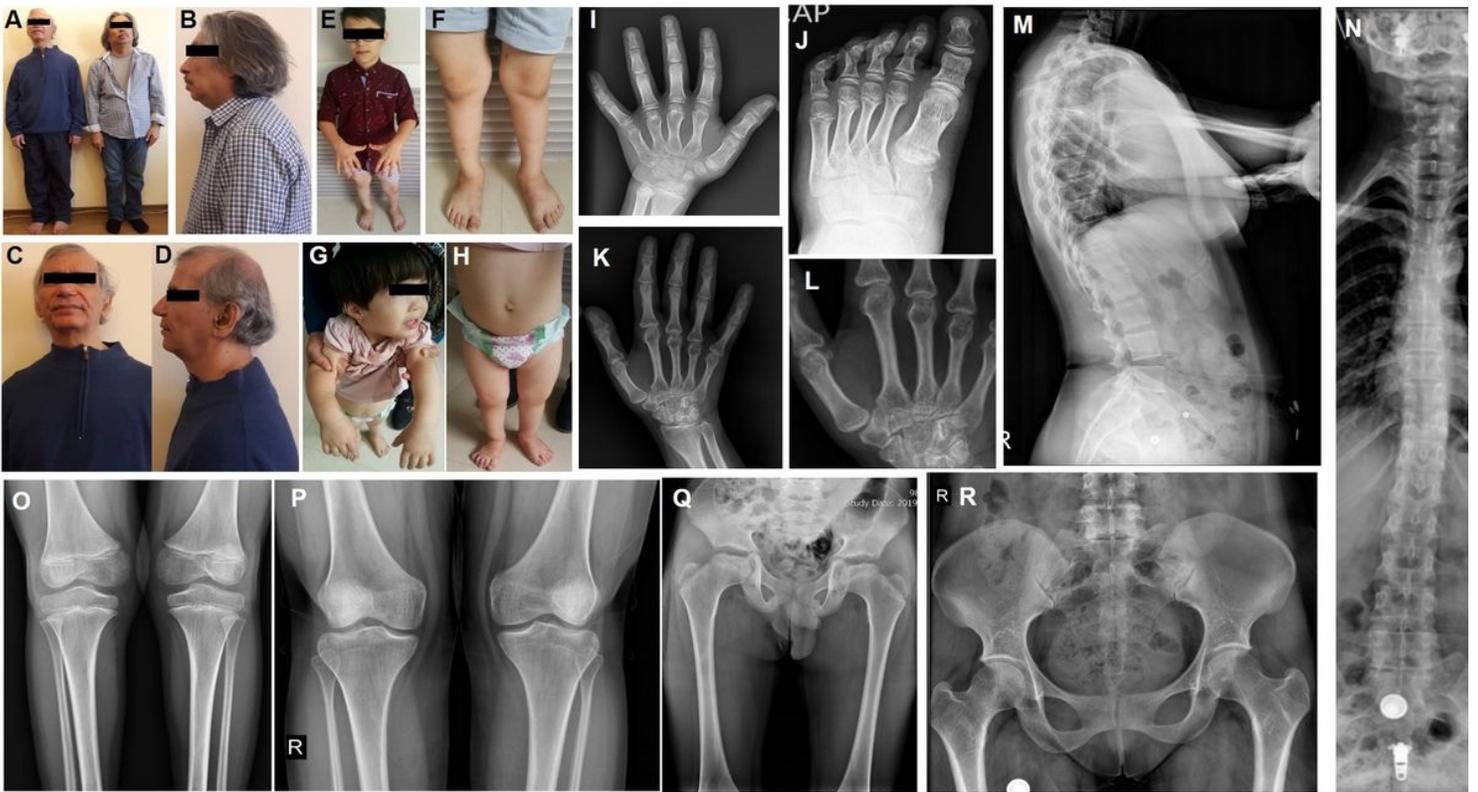


Figure 2
 Picture of four affected individuals and standard radiographs of the spine, pelvis and limbs of two patients. A-D: Pictures of two affected individuals from family 2 who both have herniated cervical disc. E,F: Pictures of affected individual II1 from family 3 showing short stature, pes planus, bowed tibia, genu valgum

and rotated distal femura distal femur. G,H: Clinical appearance of proband II1 from family 1 showing pes planus but a non-remarkable facial gestalt. I,J: Hand and Foot radiographs showing short metacarpalia and a broad big toe for individual II1 from family 3 at the age of 10 years. K,L: Radiograph images of the right hand and wrist joint of individual II1 from family 1 at the age of 28 years, showing short metacarpalia with widened epiphyses and an irregular radius epiphysis. M,N: Radiographs of the spine of II1 from family 1, showing mild platyspondyly of the thoracic spine as well as signs of ankylosing spondylitis. O: Knee radiograph of proband II1 from family 3 showing genua valga and irregular femur epiphyses. P: Knee radiograph of proband II1 from family 1 demonstrating genua valga and widened femur epiphyses. Q: Pelvis radiograph individual II1 of from family 3 showing a flat acetabular roof with irregularities and flattened capiti femori, as well as broadened and shortened necks. R: Radiograph of the pelvis of individual II1 from family 1 showing a relatively narrow intraarticular space but well developed capiti femori and no flattening of the acetabular roof.

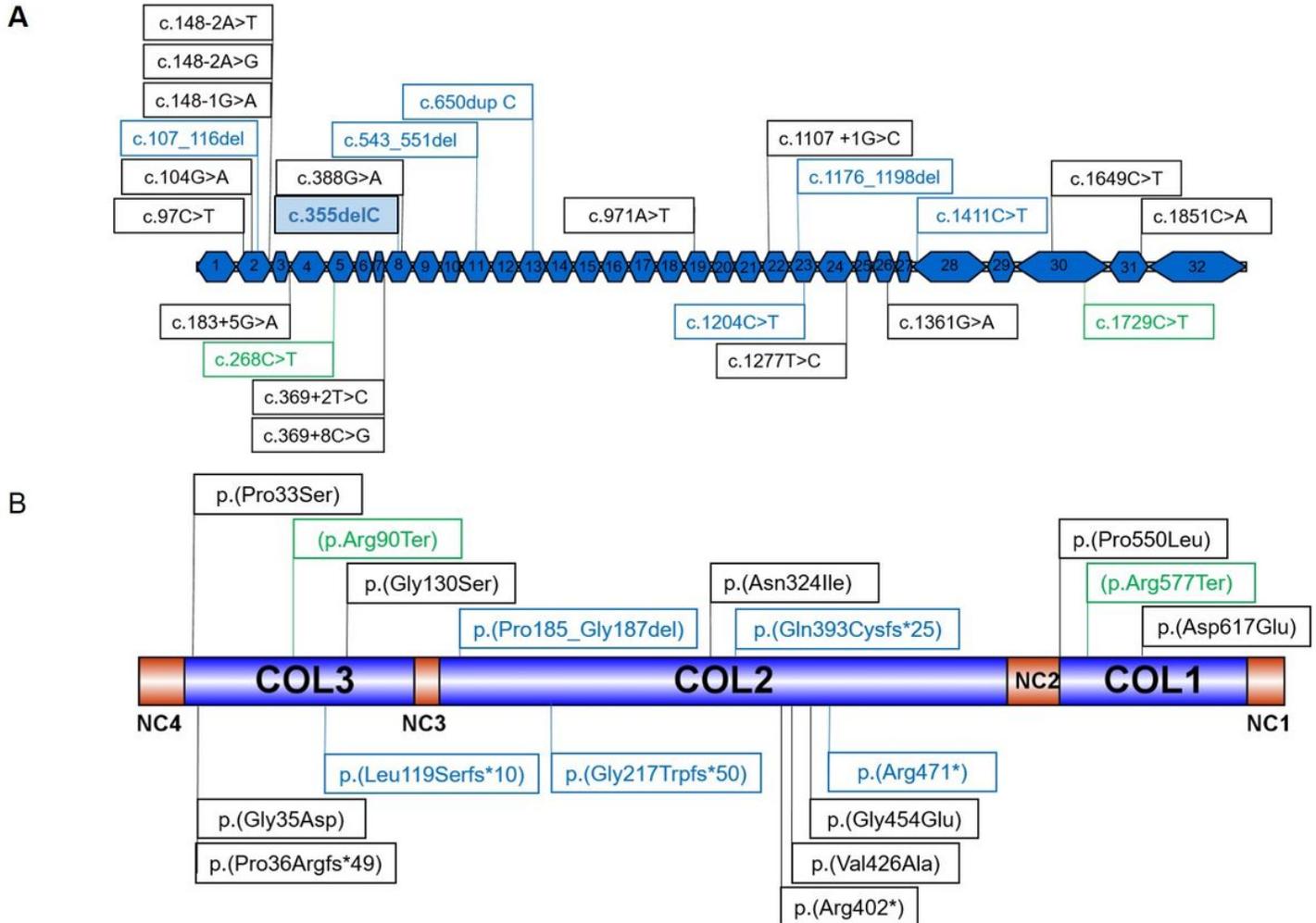


Figure 3
 Overview of known and novel variants in COL9A3 at the cDNA and protein levels. All variants have been reported by HGMD Professional 2020.1 and classified as pathogenic in ClinVar or are reported but not yet classified in ClinVar. Black text indicates heterozygous variants, blue text shows homozygous variants and green text shows the compound heterozygous variants. (A) variants on cDNA level; (B) variants on protein level.

Supplementary Files

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