Mutation in collagen II alpha 1 isoforms delineates Stickler and Wagner syndrome phenotypes

Khanh-Nhat Tran-Viet,1 Vincent Soler,1,2 Valencia Quiette,1 Caldwell Powell,1 Tammy Yanovitch,3 Ravikanth Metlapally,1 Xiaoyan Luo,4 Nicholas Katsanis,4 Erica Nading,1,5 Terri L. Young1,5

(The first two authors contributed equally to this work.)

1Duke Center for Human Genetics, 905 S LaSalle Street, 27705, Durham, North Carolina; 2UMRS 563, Centre de Physiopathologie de Toulouse Purpan, Université Paul Sabatier, Toulouse, France; 3The Department of Ophthalmology, The University of Oklahoma/Dean McGee Eye Institute, Oklahoma City, OK; 4Departments of Cell Biology and Pediatrics, Center for Human Disease Modeling, Duke University, Durham, NC; 5The Department of Ophthalmology, Duke University Eye Center, Durham, NC; 6Vision Science Group, School of Optometry and Departments, University of California, Berkeley, CA

Purpose: Stickler syndrome is an arthro-ophthalmopathy with phenotypic overlap with Wagner syndrome. The common Stickler syndrome type I is inherited as an autosomal dominant trait, with causal mutations in collagen type II alpha 1 (COL2A1). Wagner syndrome is associated with mutations in versican (VCAN), which encodes for a chondroitin sulfate proteoglycan. A three-generation Caucasian family variably diagnosed with either syndrome was screened for sequence variants in the COL2A1 and VCAN genes.

Methods: Genomic DNA samples derived from saliva were collected from all family members (six affected and four unaffected individuals). Complete sequencing of COL2A1 and VCAN was performed on two affected individuals. Direct sequencing of remaining family members was conducted if the discovered variants followed segregation.

Results: A base-pair substitution (c.258C>A) in exon 2 of COL2A1 cosegregated with familial disease status. This known mutation occurs in a highly conserved site that causes a premature stop codon (p.C86X). The mutation was not seen in 1,142 ethnically matched control DNA samples.

Conclusions: Premature stop codons in COL2A1 exon 2 lead to a Stickler syndrome type I ocular-only phenotype with few or no systemic manifestations. Mutation screening of COL2A1 exon 2 in families with autosomal dominant vitreoretinopathy is important for accurate clinical diagnosis.

Stickler syndrome is a genetically and clinically heterogeneous condition first described in 1965 by Gunnar Stickler as an inherited progressive arthro-ophthalmopathy [1]. Although Stickler syndrome is the most frequently inherited cause of retinal detachment in childhood, this disorder is rare, and the reported prevalence in the United States is 1:10,000 [2-4].

The autosomal dominant (AD) Stickler syndrome subtypes include Stickler syndrome type I (STL1, OMIM 108300), Stickler syndrome type II (STL2, OMIM 604841), and Stickler syndrome type III (STL3, OMIM 184840). STL1 results from altered type II collagen molecules encoded by the collagen type II alpha 1 (COL2A1) gene while STL2, caused by mutations in the collagen type XI alpha 1 (COL11A1) gene, and STL3, caused by mutations in the collagen type XI alpha 2 (COL11A2) gene, affect the type XI collagen molecules [5]. Since 2006, mutations in the collagen type IX alpha 1 (COL9A1) and collagen type IX alpha 2 (COL9A2) genes, encoding for type IX collagen, have been reported as causal for an autosomal recessive form of Stickler syndrome [6,7].

Type II, IX, and XI collagen molecules are major extracellular matrix components in the hyaline cartilage and the vitreous, and are structurally and functionally associated [8-10]. Affected individuals with mutations in STL1 and STL2 demonstrate a combination of ocular and systemic manifestations. In contrast, STL3 involves only non-ocular manifestations; affected individuals present with systemic malformations such as deafness and cleft palate [7,10-13]. Ocular features associated with AD Stickler syndrome consist of myopia, vitreous degeneration, radial perivascular retinal degeneration, presenile cataract, and high incidence of retinal detachments [14]. The vitreous phenotype depends on the AD Stickler syndrome type, since STL1 vitreous demonstrates a congenital retrolental membrane while patients with STL2 demonstrate vitreous with fibrillar or beaded aspect [15-17]. Non-ocular manifestations of AD Stickler syndrome include arthropathy, hearing impairment, flat midface, midline clefting, and skeletal abnormalities, but these findings can be
highly variable, even within a family [5]. Autosomal recessive forms of Stickler syndrome may present with ocular features, such as high myopia and vitreoretinal degeneration but no presenile cataract, and systemic features may include but are not limited to short stature, hearing impairment, facial structural abnormalities but no midline clefting, and causal gene-dependent spondyloepiphyseal dysplasia [7].

The expression of the COL2A1 gene, associated with STL1, is tissue-dependent, due to an alternative splicing of exon 2, which results in two isoforms of type II collagen [18]. Type IIB is the shorter form with 53 exons (exon 2 spliced), and is mainly expressed in the cartilage while type IIA is the longer form with 54 exons (including exon 2) and is predominantly present in the vitreous [18]. Thus, mutations in COL2A1 exon 2, such as a premature stop codon, may lead to the ocular variant of the STL1. This ocular condition shares typical STL1 ocular features, such as membranous vitreous and radial perivascular retinal degeneration, but shows none or few systemic manifestations [3,19-21]. To date, several families harboring COL2A1 exon 2 mutations have been reported [3,19,20,22-25]. In these families, the reported penetrance of vitreoretinal degeneration is more than 90% in affected patients at the age of 20 [19,20].

Wagner syndrome (OMIM 142000) is also associated with the extracellular matrix component gene versican (VCAN; alternatively called CSPG2), identified in 2005 by Miyamoto et al., which encodes for a large chondroitin sulfate proteoglycan versican [26-30]. We confirmed the involvement of this gene in 2009 by describing an intronic base pair splice site substitution in VCAN segregating with the disease in a multigenerational family resulting in a truncated protein affected by the splicing [31]. Wagner syndrome clinical features involve ocular manifestations close to those found in Stickler syndrome. However, patients also complain of nystagmatopia, where the vitreous phenotype is different as characterized by an optically empty aspect with avascular strands and veins or fibrillary condensations [32], retinal detachments occur rarely, and retinal features demonstrate retinitis pigmentosa–like “bone spicule” pigmentary atrophy [20] with accompanying electroretinogram abnormalities. Nevertheless, in some cases, distinguishing Wagner syndrome from the ocular-only variant of STL1 may be difficult. Erosive vitreoretinopathy syndrome (OMIM 143200) is another ocular-only disorder, first reported by Brown et al. [33], that can also lead to indeterminate diagnosis based on clinical features only [30]. The clinical features of erosive vitreoretinopathy syndrome include night blindness, visual field defects, and chorioretinal atrophy. The variability in the clinical presentations of vitreoretinal disease phenotypes underscores the importance of careful clinical assessment.

We clinically and genetically report a three-generation Caucasian family from the southeast United States demonstrating AD vitreous degeneration with variable phenotypes among affected members. Over the years, one affected member was initially diagnosed clinically with Stickler syndrome and then Wagner syndrome by her ophthalmologist. We conducted Sanger sequencing of the COL2A1 (NM_001844) and VCAN (NM_004385) genes to delineate the genetic etiology of disease in this family.

**METHODS**

**Study subjects:** Ten individuals (six affecteds, four unaffecteds) from a three-generation Caucasian family was recruited. All consenting family members (four males, six females) were recruited under the approval of the Duke University Institutional Review Board according to the principles of the Declaration of Helsinki, under the research protocol entitled “Clinical and Molecular Analysis of Genetic Eye Disorders”, to include molecular genetic testing (protocol number Pro00008040). Individuals underwent ophthalmic examinations that included health histories regarding systemic issues such as cleft palate, midline defects, skeletal or joint abnormalities, and early onset arthritis. The clinical evaluation included assessment tests of Early Treatment Diabetic Retinopathy Study visual acuity (Snellen equivalent) and intraocular pressure, slit-lamp inspection of the anterior segment, and indirect ophthalmoscopy to inspect the fundus [34,35].

Genomic DNA was extracted using AutoPure LS® DNA Extractor and PUREGENETM reagents (Gentra Systems Inc., Minneapolis, MN) from blood or saliva samples. DNA samples were also collected from 1,142 unrelated ethnically matched Caucasian healthy control participants.

**Gene screening and sequence analysis of collagen type II alpha 1 and versican genes:** Primers for PCR and sequencing were designed to cover coding and untranslated gene regions, including intron–exon boundaries, using the Exon Primer and Primer3 programs (Helmholtz Zentrum, Munich, Germany). Primers were selected to produce amplified product sizes not to exceed 900 bp for optimal sequence output and reading. Large exons or untranslated gene regions were covered with overlapping amplicons, with a minimal 50 bp of overlapped sequence. All 54 COL2A1 exons and 15 VCAN exons were examined. Appendix 1 displays the optimized primer sequences used for VCAN and COL2A1 screening.
Genomic DNA of two affected individuals (II:2 and II:3; Figure 1) of the study family was initially screened for sequence variations in the \textit{COL2A1} and \textit{VCAN} genes. The DNA of the remaining family members was subsequently screened to determine and confirm sequence variants segregation.

PCR was conducted using an Eppendorf Mastercycler Pro S® with a standard touchdown PCR protocol. PCR amplicons were visualized with 2% agarose gel electrophoresis. BigDye™ Terminator 3.1 was used to perform sequencing reactions, and ABI3730XL robotics was used to process the DNA fragments (Applied Biosystems Inc. [ABI], Foster City, CA). The Sequencher® 5.0 Software (Gene Codes, Ann Arbor, MI) was used to analyze the base pair calls. Sequences of affected and unaffected individuals were aligned to a known reference genomic sequence (UCSC Genome Browser) and compared for sequence variation. Sorting Intolerant From Tolerant (SIFT) [36] and Polymorphism Phenotyping (PolyPhen2) [37] software tools were used to predict mutational consequence of all \textit{COL2A1} and \textit{VCAN} variations segregating with disease.

Genotyping: Applied Biosystems (ABI) TaqMan® SNP Genotyping assays were designed and employed to measure the allelic frequencies in 1,142 ethnically matched control DNA samples. Variants of interest were screened with PCR assay technology using TaqMan probes according to the manufacturer’s protocol (Applied Biosystems). Alleles were detected and allelic discrimination were analyzed with ABI Prism® 7900HT Sequence Detection System and ABI Sequence Detection Systems 2.4 software, respectively (Applied Biosystems). For quality control, positive and negative controls were run in the same experiment.

\textit{Complementary deoxyribonucleic acid tissue expression:} We investigated the expression of the \textit{COL2A1} messenger ribonucleic acid (mRNA) construct(s) in fetal ocular tissues to verify the presence of type IIA and/or type IIB isoforms. Fetal ocular tissue panels not affected with disease were established internally by acquisition of whole eye globes from Advanced Bioscience Resources (Alameda, CA). Twenty-four-week fetal eyes were obtained and preserved in RNAlater® Foster City, CA within minutes of abortion and shipped overnight on ice. Whole globes were dissected the same day as they arrived, and specific ocular tissues were isolated by snap-freezing the samples and storing at −80 °C until RNA extraction. RNA was extracted from each tissue sample independently using the Ambion Foster City, CA mirVana Total RNA Extraction Kit per the protocol. The tissue samples were homogenized in Ambion’s lysis buffer using an Omni Bead Ruptor 24 Homogenizer per protocol. Reverse transcription reactions were performed with Invitrogen's SuperScript™ III First-Strand Synthesis kit to obtain cDNA (Life Technologies, Grand Island, NY).

In-house fetal eye cDNA was amplified using primers that spanned multiple exons, not to exceed 600 bp (Appendix 1). PCRs were run using a standard protocol. Visualization of the PCR products was done on a 2% agarose gel through electrophoresis at 120 V for 50 min. Products with exon 2 were expected to amplify at 510 bp, whereas those that did not contain the exon 2 were expected to produce a product of 303 bp. Band extraction, purification, and sequencing of the bands were conducted to verify the amplicon.

\textbf{RESULTS}

\textbf{Clinical features:} A three-generation family with affected member variable ocular-only diagnosis of either Stickler or Wagner syndrome was ascertained. The disease appeared to be transmitted in an AD inheritance pattern and showed variable expressivity with 100% penetrance (Figure 1). Six affected and four unaffected individuals participated in the study. Clinical data were obtained where available.

The proband, patient III:3, was a 40-year-old woman with moderate myopia and history of retinal detachment in the left eye (OS) at the age of 18 years. Periodically, she underwent prophylactic peripheral laser photocoagulation treatment bilaterally. She had cataract extraction surgery of both eyes at the age of 37 (right eye, OD) and 40 (OS). Fundus examination showed bilateral vitreous syneresis, with a vitreous

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Study family pedigree. The family consisted of 14 individuals in three generations with six affected and four unaffected participants. Solid symbols indicate affected individuals. Asterisks indicate participating individuals for whom DNA was available for genomic analysis.}
\end{figure}
membrane in the left eye, and bilateral radial extensive lattice degeneration. She had no history of systemic manifestations.

Patient III:2 was a 40-year-old woman and the monozygotic twin sister of III:3. Patient III:2 presented with moderate myopia and underwent retinal detachment surgeries at the age of 18 (OD) and 33 (OS) for retinal detachment (OD) and epiretinal membrane and large retinal tears (OS). Subsequently, she was treated with extensive peripheral prophylactic photocoagulation. She underwent cataract extractions bilaterally at the age of 37 (OS) and 38 (OD). Fundus examination revealed bilateral radial lattice degeneration and bilateral vitreous syneresis. She had no history of systemic manifestations.

Patient II:2 was the 65-year-old mother of the twins. She had a history of bilateral congenital cataract extractions. Her fundus examination demonstrated bilateral vitreous syneresis with vitreous membranes. Patient II:3 presented with a cleft palate at an early age. Patients IV:3 and IV:4 presented with a history of retinal detachments but no systemic manifestations. We were not able to collect clinical data from the deceased individuals I:1 and I:2, although historical accounts from II:1 revealed her mother (I:2) had a history of retinal problems.

Molecular genetic analysis: Initial genomic DNA sequencing of two affected individuals (II:2 and II:3) was conducted in the VCAN and COL2A1 genes. For VCAN, no sequence variants segregated with the disease status were identified compared to the control DNA and to published reference sequences.

For the COL2A1 gene, we identified 18 single nucleotide variations: one nonsense variant, one known missense variant, two known coding-synonymous variants, and 14 intronic single nucleotide polymorphisms (Appendix 1).

The nonsense mutation was a C to A change (Figure 2) in exon 2 (c.258C>A; NM_001844.4), converting codon TGC for cysteine at position 86 to codon TGA for a premature stop codon (Cys86Stop; NP_001835.3). Subsequent sequencing of the remaining family members showed mutation cosegregation with the disease phenotype (Figure 2).

Genotyping was performed for the c.258C>A mutation in the COL2A1 gene for an additional 1,142 unrelated ethnically matched controls (2,284 chromosomes). The mutation was not present in the control samples.

Tissue expression: We examined COL2A1 expression across normal fetal eye tissues to verify the presence or absence of type IIA and/or type IIB isoforms (Figure 3). Both COL2A1 mRNA isoforms (type IIA and type IIB) were expressed in the fetal retina/retinal pigment epithelium and choroid (Appendix 1). The COL2A1 mRNA type IIB isoform (excluding exon 2) was expressed in the sclera, optic nerve, and cornea. Gel extraction and Sanger sequencing of the specific product bands confirmed our findings of the isoforms except for the optic nerve, where the IIA isoform was not sequenced.

DISCUSSION

We report a nonsense mutation in a large Caucasian family consisting of six affected individuals variably diagnosed with Stickler and Wagner syndromes. A base pair change at c.258C>A leading to a premature stop codon in exon 2 of COL2A1 was cosegregated with the disease status. Sequencing of ocular tissues confirmed the presence or
absence of exon 2, demonstrating that isoforms may be ocular tissue specific. The mutation was not present in more than 2,000 chromosomes, validating the rarity of this mutation and confirming Stickler syndrome has a predominant ocular-only phenotype.

Two striking features of Stickler syndrome are, as in our reported family, the high penetrance and variable expressivity. In the literature, the same mutation as in our family (c.258C>A; NM_001844.4) demonstrated high penetrance [3,19,20]. In families harboring alternative COL2A1 exon 2 mutations, the ocular manifestation penetrance was also high—from 90% [20] to 100% [24,38]. The variable expressivity, even within the same family [39], contrasts with the high disease penetrance: In most reported COL2A1 exon 2 mutations, ocular features were variable as either myopia, retinal detachment [22], or retinal degeneration [23] could be absent in affected patients. Furthermore, two common ocular features in COL2A1 exon 2 mutations are vitreous degeneration and radial perivascular retinal degeneration [3,19,20]. Systemic manifestations were rarely associated with COL2A1 exon 2 mutations, as manifestations were present in few cases [3,19,20,22]. In our family, only one affected individual presented with cleft palate.

Underlying causes of variable expressivity in ocular-only STL1 are still undetermined. However, in recent years the phenotypic variability of exon 2 mutations has been hypothesized to be due to degradation of mRNA by nonsense-mediated decay (NMD) or synthesis of alternatively spliced protein [21].

NMD is a regulation pathway involving the targeted degradation of mRNA that contains a premature stop codon. In this way, NMD may play a role in phenotype variability by minimizing the potential damage caused by premature termination codons [40,41]. In achondrogenesis and hypochondrogenesis caused by COL2A1 mutations, a relationship has been proved between the severity of the phenotype and the amount of type II collagen within the cartilage extracellular matrix [42]. This implies that not only qualitative but also quantitative factors likely modulate phenotypes linked to the COL2A1 gene [43], as in haploinsufficiency due to NMD.

Haploinsufficiency due to NMD was reported by Kaarmiranta et al. [44], who found that heterozygous inactivation of COL2A1 gene in the murine model led to structural defects and alterations that resulted from haploinsufficiency in ocular tissues containing type II collagen. These alterations included vitreous changes similar to those seen in patients with Stickler syndrome, which included reduced immunostaining of type II collagen in the vitreous and retina, in addition to reduced density of vitreous filaments in COL2A1+/− mutant mice [45]. Furthermore, in COL2A1 exon 2 mutant mice with mutant allele encoding COL2A1 mRNA without exon 2 [46], IIA+/− mutant embryos demonstrated, at an early stage, craniofacial abnormalities of truncated frontonasal structures and hypoplasia of the midface tissues. These malformations were more frequent in IIA+/− mutants. These findings are consistent with COL2A1 type IIA mRNA expression in regions of active recruitment of cells for chondrogenesis and in areas of skeletal growth [41].

Alternatively, a second hypothesis is that nonsense-mediated altered splicing can be caused by disruption within the splicing cis element [21]. Minigene constructs created by McAlinden et al. demonstrated that disruptions in the enhancer sites in COL2A1 exon 2 favor the production of the procollagen type IIB isoform. The decrease in the ratio of type IIA compared to type IIB leads to variance in expression levels, perhaps one isoform predominating over another, but the less expressed is not completely absent. These studies highlight the alternative imbalance between the two isoforms, which may have adverse effects during ocular embryogenesis [21].

Systemic manifestations associated with STL1, particularly facial development abnormalities and midline clefting as reported here (individual II:3; Figure 1), have been observed in some cases of COL2A1 exon 2 mutations, with a frequency depending on the series, 1%, 4%, and 43% in the Donoso [3], Parma [19], and Richards [22] series, respectively. These findings are not inconsistent with exclusive expression of the longer type IIA isoform in the adult vitreous [47], as embryonic expression of this isoform has been demonstrated in chondroprogenitor tissues [48].

The Cys86Stop mutation has previously been reported in four families with Stickler syndrome whose genealogy was traced to the 16th century. Subsequently, Donoso et al. identified a member of the branch that migrated to the southeastern and mid-southern United States during the 19th century [3,19,20]. Some members of these families included direct descendants from the passengers of the 1620 Mayflower voyage [20] who arrived on the northeastern coast and then migrated to the south. Interestingly, an affected individual (III:2) reviewed her genealogy and traced her ancestry to the state of Georgia. If descendants from Donoso’s family indeed migrated south due to the Cherokee Land Grant of 1813, the similar geographical region of their cohort and ours would not exclude the possibility that the families could be related [20]. The apparent founder effect coupled with literature estimations of 50,000 to 100,000 descendants that could be related to the original family with this reported mutation demonstrates the importance of the genotype-phenotype...
relationship in patients with Stickler syndrome with this particular mutation [3].

The overarching similarities in phenotypes among vitreoretinal diseases make accurate diagnosis difficult clinically. Clinicians must understand and be updated on all allied conditions associated with Stickler syndrome to properly diagnose patients [47]. The characteristics of vitreous and retinal degeneration may guide molecular testing, but in case of doubt, a retinal specialist should be consulted [47]. Although concentrations of exon 2 mutations are for predominantly ocular-only phenotypes, family members with mutations can still have systemic manifestations, seen in individual II:3, who presented with a cleft palate at a young age. The broad phenotypic variation seen in families with Stickler syndrome underscores the importance of using clinical and genetic testing to properly diagnose and treat patients with Stickler and Wagner syndromes.

APPENDIX 1. SUPPLEMENTAL SECTION CONTAINS 4 TABLES AND 1 FIGURE.

Supplemental section contains 4 tables and 1 figure. Table S1 contains all variants identified in COL2A1. Table S2 and S3 contains primers used for COL2A1 and VCAN gene screening. Table S4 contains primers used for cDNA amplification. Figure S1 contains PCR products of COL2A1 cDNA tissue expression. To access the data, click or select the words “Appendix 1.”

ACKNOWLEDGMENTS

The authors would like to thank all family members for participation in the study. This research effort was supported by the National Institutes of Health Grant EY014685, The Lew Wasserman Award from Research To Prevent Blindness Inc., and the Duke-National University of Singapore core grant. (TLY) This research was also supported by the Toulouse Hospital Young Researcher Fellowship, the Fondation de France.

REFERENCES

1. Stickler GB, Belau PG, Farrell FJ, Jones JD, Pugh DG, Steinberg AG, Ward LE. Hereditary progressive arthro-ophthalmopathy. Mayo Clin Proc 1965; 40:433-5. [PMID: 14299791]
2. Carroll C, Papaioannou D, Rees A, Kaltenthaler E. The clinical effectiveness and safety of prophylactic retinal interventions to reduce the risk of retinal detachment and subsequent vision loss in adults and children with Stickler syndrome: a systematic review. Health Technol Assess 2011; iii:15-[PMID: 21466760].
3. Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad NN, Vrabec T, Rogers J, Meyer D. Identification of a stop codon mutation in exon 2 of the collagen 2A1 gene in a large stickler syndrome family. Am J Ophthalmol 2002; 134:720-7. [PMID: 12429249].
4. Körkkö J, Ritvaniemi P, Haataja L, Kaariainen H, Kivirikko KI, Prockop DJ, Ala-Kokko L. Mutation in type II procollagen (COL2A1) that substitutes aspartate for glycine alpha 1–67 and that causes cataracts and retinal detachment: evidence for molecular heterogeneity in the Wagner syndrome and the Stickler syndrome (arthro-ophthalmopathy). Am J Hum Genet 1993; 53:55-61. [PMID: 8317498].
5. Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. J Med Genet 1999; 36:353-9. [PMID: 10353778].
6. Van Camp G, Snoeckx RL, Hilgert N, van den Ende J, Fukuoka H, Wagatsima M, Suzuki H, Smets RM, Vanhoenacker F, Declau F, Van de Heyning P, Usami S. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. Am J Hum Genet 2006; 79:449-57. [PMID: 16909383].
7. Baker S, Booth C, Fillman C, Shapiro M, Blair MP, Hyland JC, Ala-Kokko L. A loss of function mutation in the COL9A2 gene causes autosomal recessive Stickler syndrome. Am J Med Genet A 2011; 155A:1668-72. [PMID: 21671392].
8. Eyre D. Collagen of articular cartilage. Arthritis Res 2002; 4:30-5. [PMID: 11879535].
9. Kleinberg TT, Tzekov RT, Stein L, Ravi N, Kaushal S. Vitreous vitreoretinal diseases make accurate diagnosis difficult clinically. Clinicians must understand and be updated on all allied conditions associated with Stickler syndrome to properly diagnose patients [47]. The characteristics of vitreous and retinal degeneration may guide molecular testing, but in case of doubt, a retinal specialist should be consulted [47]. Although concentrations of exon 2 mutations are for predominantly ocular-only phenotypes, family members with mutations can still have systemic manifestations, seen in individual II:3, who presented with a cleft palate at a young age. The broad phenotypic variation seen in families with Stickler syndrome underscores the importance of using clinical and genetic testing to properly diagnose and treat patients with Stickler and Wagner syndromes.

ACKNOWLEDGMENTS

The authors would like to thank all family members for participation in the study. This research effort was supported by the National Institutes of Health Grant EY014685, The Lew Wasserman Award from Research To Prevent Blindness Inc., and the Duke-National University of Singapore core grant. (TLY) This research was also supported by the Toulouse Hospital Young Researcher Fellowship, the Fondation pour la Recherche Médicale and Fondation de France (VS).

REFERENCES

1. Stickler GB, Belau PG, Farrell FJ, Jones JD, Pugh DG, Steinberg AG, Ward LE. Hereditary progressive arthro-ophthalmopathy. Mayo Clin Proc 1965; 40:433-5. [PMID: 14299791].
2. Carroll C, Papaioannou D, Rees A, Kaltenthaler E. The clinical effectiveness and safety of prophylactic retinal interventions to reduce the risk of retinal detachment and subsequent vision loss in adults and children with Stickler syndrome: a systematic review. Health Technol Assess 2011; iii:15-[PMID: 21466760].
3. Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad NN, Vrabec T, Rogers J, Meyer D. Identification of a stop codon mutation in exon 2 of the collagen 2A1 gene in a large stickler syndrome family. Am J Ophthalmol 2002; 134:720-7. [PMID: 12429249].
4. Körkkö J, Ritvaniemi P, Haataja L, Kaariainen H, Kivirikko KI, Prockop DJ, Ala-Kokko L. Mutation in type II procollagen (COL2A1) that substitutes aspartate for glycine alpha 1–67 and that causes cataracts and retinal detachment: evidence for molecular heterogeneity in the Wagner syndrome and the Stickler syndrome (arthro-ophthalmopathy). Am J Hum Genet 1993; 53:55-61. [PMID: 8317498].
5. Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. J Med Genet 1999; 36:353-9. [PMID: 10353778].
6. Van Camp G, Snoeckx RL, Hilgert N, van den Ende J, Fukuoka H, Wagatsima M, Suzuki H, Smets RM, Vanhoenacker F, Declau F, Van de Heyning P, Usami S. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. Am J Hum Genet 2006; 79:449-57. [PMID: 16909383].
7. Baker S, Booth C, Fillman C, Shapiro M, Blair MP, Hyland JC, Ala-Kokko L. A loss of function mutation in the COL9A2 gene causes autosomal recessive Stickler syndrome. Am J Med Genet A 2011; 155A:1668-72. [PMID: 21671392].
8. Eyre D. Collagen of articular cartilage. Arthritis Res 2002; 4:30-5. [PMID: 11879535].
9. Kleinberg TT, Tzekov RT, Stein L, Ravi N, Kaushal S. Vitreous
type 2 Stickler syndrome. J Med Genet 2004; 41:e107-
[PMID: 15286167].

17. Richards AJ, Laidlaw M, Whittaker J, Treacy B, Rai H, Bearcroft P, Baguley DM, Poulsen A, Ang A, Scott JD, Snead MP. High efficiency of mutation detection in type 1 stickler syndrome using a two-stage approach: vitreoretinal assessment coupled with exon sequencing for screening COL2A1. Hum Mutat 2006; 27:696-704. [PMID: 16752401].

18. Ryan MC, Sandell LJ. Differential expression of a cysteine-rich domain in the N-terminal propeptide of type II (cartilage) procollagen by alternative splicing of mRNA. J Biol Chem 1990; 265:10334-9. [PMID: 2355003].

19. Parma ES, Korkko J, Hagler WS, Ala-Kokko L. Radial perivascular retinal degeneration: a key to the clinical diagnosis of an ocular variant of Stickler syndrome with minimal or no systemic manifestations. Am J Ophthalmol 2002; 134:728-34. [PMID: 1249250].

20. Donoso LA, Edwards AO, Frost AT, Ritter R 3rd, Ahmad N, Vrabec T, Rogers J, Meyer D, Parma S. Clinical variability of Stickler syndrome: role of exon 2 of the collagen COL2A1 gene. Surv Ophthalmol 2003; 48:191-203. [PMID: 12686304].

21. McAlinden A, Majava M, Bishop PN, Perveen R, Black GC, Pierpont ME, Ala-Kokko L, Mannikko M. Missense and nonsense mutations in the alternatively-spliced exon 2 of COL2A1 cause the ocular variant of Stickler syndrome. Hum Mutat 2008; 29:83-90. [PMID: 17721977].

22. Richards AJ, Martin S, Yates JR, Scott JD, Baguley DM, Pope FM, Snead MP, COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes. Br J Ophthalmol 2000; 84:364-71. [PMID: 10729292].

23. Yoshida S, Yamaji Y, Kuwahara R, Yoshida A, Hisatomi T, Ueno A, Ishibashi T. Novel mutation in exon 2 of COL2A1 gene in Japanese family with Stickler Syndrome type I. Eye (Lond) 2006; 20:743-5. [PMID: 16021188].

24. Gupta SK, Leonard BC, Damji KF, Bulman DE. A frame shift mutation in a tissue-specific alternatively spliced exon of collagen 2A1 in Wagner’s vitreoretinal degeneration. Am J Ophthalmol 2002; 133:203-10. [PMID: 12429250].

25. Edwards TL, Burt BO, Black GC, Perveen R, Kearns LS, Staffieri SE, Toomes C, Buttery RG, Mackey DA. Familial retinal detachment associated with COL2A1 exon 2 and FZD4 mutations. Clin Experiment Ophthalmol 2012; 40:476-83. [PMID: 22574936].

26. Miyamoto T, Inoue H, Sakamoto Y, Kudo E, Naito T, Mikawa T, Mikawa Y, Isashiki Y, Osabe D, Shinohara S, Shioita H, Itakura M. Identification of a novel splice site mutation of the CSPG2 gene in a Japanese family with Wagner syndrome. Invest Ophthalmol Vis Sci 2005; 46:2726-35. [PMID: 16043844].

27. Kloockener-Gruissem B, Bartholdi D, Abdou MT, Zimmermann DR, Berger W. Identification of the genetic defect in the original Wagner syndrome family. Mol Vis 2006; 12:350-5. [PMID: 16636652].

28. Mukhopadhyay A, Nikopoulos K, Maugeri A, de Brouwer AP, van Nouhuys CE, Boon CJ, Perveen R, Zegers HA, Wittebol-Post D, van den Biesen PR, van der Velde-Visser SD, Brunner HG, Black GC, Hoyng CB, Cremers FP. Erosive vitreoretinopathy and wagner disease are caused by intronic mutations in CSPG2/Versican that result in an imbalance of splice variants. Invest Ophthalmol Vis Sci 2006; 47:3565-72. [PMID: 16877430].

29. Meredith SP, Richards AJ, Flanagan DW, Scott JD, Poulsen AV, Snead MP. Clinical characterisation and molecular analysis of Wagner syndrome. Br J Ophthalmol 2007; 91:655-9. [PMID: 17035272].

30. Brézin AP, Nedelec B, Barjol A, Rothschild P, Delpech M, Valleix S. A new VCAN/versican splice acceptor site mutation in a French Wagner family associated with vascular and inflammatory ocular features. Mol Vis 2011; 17:669-78. [PMID: 21738396].

31. Ronan SM, Tran-Viet KN, Burner EL, Metlapally R, Toth CA, Young TL. Mutational hot spot potential of a novel base pair mutation of the CSPG2 gene in a family with Wagner syndrome. Arch Ophthalmol 2009; 127:1511-9. [PMID: 19901218].

32. Graemiger RA, Niemeyer G, Schneeberger SA, Messmer EP. Wagner vitreoretinal degeneration. Follow-up of the original pedigree. Ophthalmology 1995; 102:1830-9. [PMID: 9098284].

33. Brown DM, Kimura AE, Weingeist TA, Stone EM. Erosive vitreoretinopathy, A new clinical entity. Ophthalmology 1994; 101:694-704. [PMID: 8152765].

34. Beck RW, Moke PS, Turpin AH, Ferris FL 3rd, SanGiovanni JP, Johnson CA, Birch EE, Chandler DL, Cox TA, Blair RC, Krakor RT. A computerized method of visual acuity testing: adaptation of the early treatment of diabetic retinopathy study testing protocol. Am J Ophthalmol 2003; 135:194-205. [PMID: 12566024].

35. Ferris FL 3rd, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol 1982; 94:91-6. [PMID: 7091289].

36. Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. Genome Res 2002; 12:436-46. [PMID: 11875032].

37. Adzhubei IA, Schmidt S, Peshkin L, Gerassimova A, Nikopoulos K, Maudner S, Koehl G, Loh MA, Ureta-Vidal A, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7:248-9. [PMID: 20354512].

38. Snead M, Richards A. A frame shift mutation in a tissue-specific alternatively spliced exon of collagen 2A1 in Wagner’s vitreoretinal degeneration. Am J Ophthalmol 2002; 134:473-479. [PMID: 12208278].

39. Richards AJ, Snead MP. The influence of pre-mRNA splicing on phenotypic modification in Stickler’s syndrome and other type II collagenopathies. Eye (Lond) 2008; 22:1243-50. [PMID: 18309338].
40. Chang YF, Chan WK, Imam JS, Wilkinson MF. Alternatively spliced T-cell receptor transcripts are up-regulated in response to disruption of either splicing elements or reading frame. J Biol Chem 2007; 282:29738-47. [PMID: 17693403].

41. Lui VC, Ng LJ, Nicholls J, Tam PP, Cheah KS. Tissue-specific and differential expression of alternatively spliced alpha I(II) collagen mRNAs in early human embryos. Dev Dyn 1995; 203:198-211. [PMID: 7655082].

42. Mortier GR, Wilkin DJ, Wilcox WR, Rimoin DL, Lachman RS, Eyre DR, Cohn DH. A radiographic, morphologic, biochemical and molecular analysis of a case of achondrogenesis type II resulting from substitution for a glycine residue (Gly691→Arg) in the type II collagen trimer. Hum Mol Genet 1995; 4:285-8. [PMID: 7757081].

43. Van Der Hout AH, Verlind E, Beemer FA, Buys CH, Hofstra RM, Scheffer H. Occurrence of deletion of a COL2A1 allele as the mutation in Stickler syndrome shows that a collagen type II dosage effect underlies this syndrome. Hum Mutat 2002; 20:236--[PMID: 12204008].

44. Kaarniranta K, Ihnamiaki T, Sahlman J, Pulkkinen H, Uusitalo H, Arita M, Tammi R, Lammi MJ, Helminen HJ. A mouse model for Stickler’s syndrome: ocular phenotype of mice carrying a targeted heterozygous inactivation of type II (pro)collagen gene (Col2a1). Exp Eye Res 2006; 83:297-303. [PMID: 16546167].

45. Kaarniranta K, Ihnamiaki T, Sahlman J, Pulkkinen H, Uusitalo H, Arita M, Tammi R, Lammi MJ, Helminen HJ. A mouse model for Stickler’s syndrome: ocular phenotype of mice carrying a targeted heterozygous inactivation of type II (pro)collagen gene (Col2a1). Exp Eye Res 2006; 83:297-303. [PMID: 16546167].

46. Leung AW, Wong SY, Chan D, Tam PP, Cheah KS. Loss of procollagen IIA from the anterior mesendoderm disrupts the development of mouse embryonic forebrain. Dev Dyn 2010; 239:2319-29. [PMID: 20730911].

47. Snead MP, McNinch AM, Poulson AV, Bearcroft P, Silverman B, Gomersall P, Parfect V, Richards AJ. Stickler syndrome, ocular-only variants and a key diagnostic role for the ophthalmologist. Eye (Lond) 2011; 25:1389-400. [PMID: 21921955].

48. Zhu Y, Oganesian A, Keene DR, Sandell LJ. Type IIA procollagen containing the cysteine-rich amino propeptide is deposited in the extracellular matrix of prechondrogenic tissue and binds to TGF-beta1 and BMP-2. J Cell Biol 1999; 144:1069-80. [PMID: 10085302].