COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes

Allan J Richards, Sam Martin, John R W Yates, John D Scott, David M Baguley, F Michael Pope, Martin P Snead

Abstract

Aims—To compare the clinical and molecular genetic features of two phenotypically distinct subgroups of families with type 1 Stickler syndrome.

Background—Stickler syndrome (hereditary arthro-ophthalmopathy, McKusick Nos 108300 and 184840) is a dominantly inherited disorder of collagen connective tissue, resulting in an abnormal vitreous, myopia, and a variable degree of orofacial abnormality, deafness, and arthropathy. Stickler syndrome is the commonest inherited cause of rhegmatogenous retinal detachment in childhood with a risk of giant retinal tear (GRT) which is commonly bilateral and a frequent cause of blindness.

Method—Pedigrees were identified from the vitreoretinal service database and subclassified according to vitreoretinal phenotype. Ophthalmic, skeletal, auditory, and orofacial features were assessed. Linkage analysis was carried out with markers for the candidate genes COL2A1, COL11A1, and COL11A2. The COL2A1 gene was amplified as five overlapping PCR products. Direct sequencing of individual exons identified mutations.

Results—Eight families exhibiting the type 1 vitreous phenotype were studied. Seven were consistent for linkage to COL2A1, with lod scores ranging from 2.1 to 0.3. In most instances linkage to COL11A1 and COL11A2 could be excluded. One family was analysed without prior linkage analysis. Three of the families exhibited a predominantly ocular phenotype with minimal or absent systemic involvement and were found to have mutations in exon 2 of COL2A1. Five other pedigrees with an identical ocular phenotype plus orofacial, auditory, and articular involvement had mutations in other regions of the COL2A1 gene. None of the pedigrees exhibited the characteristic lenticular, retinal pigment epithelial, or choroidal changes seen in Wagner syndrome.

Conclusions—These data confirm that type 1 Stickler syndrome is caused by mutations in the gene encoding type II collagen (COL2A1). In addition, data are submitted showing that mutations involving exon 2 of COL2A1 are characterised by a predominantly ocular variant of this disorder, consistent with the major form of type II procollagen in non-ocular tissues having exon 2 spliced out. Such patients are all at high risk of retinal detachment. This has important implications for counselling patients with regard to the development of systemic complications. It also emphasises the importance and reliability of the ophthalmic examination in the differential diagnosis of this predominantly ocular form of Stickler syndrome from Wagner’s vitreoretinopathy.

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II collagen. These pedigrees feature sparse and irregularly thickened bundles of “beaded” fibres (type 2 vitreous phenotype) throughout the vitreous cavity. It is not yet clear whether this is specific to mutations in COL11A1 or may also result from defects at other loci. The other polypeptide chain (α2) of type XI collagen is not expressed in the vitreous so that mutations in its encoding gene (COL11A2) produce a syndrome with the systemic features of Stickler syndrome but without eye involvement.

Evidence for another Stickler syndrome locus also exists. Type II procollagen exists in two alternatively spliced forms. A short form, which is expressed in cartilage, has exon 2 spliced out, resulting in a molecule with a smaller N-propeptide (αIIb). The short form also functions as a third α(XI) chain in cartilage. It co-assembles with products of the COL11A1 and COL11A2 genes, to form a α1(XI) α2(XI) α1(IIb) heterotrimer. Thus, mutations in COL2A1 can affect both type II and XI collagen in this tissue. In vitreous both transcripts exist but here the longer form predominates. It is unclear whether this longer form can also participate in heterotrimer formation with α1(XI) collagen.

Of the Stickler syndrome pedigrees exhibiting the type 1 vitreous phenotype, a subgroup has also been identified that is characterised by a predominantly ocular disorder without systemic skeletal or auditory involvement. Before the introduction of the subclassification of Stickler syndrome based on vitreous phenotype it is possible that some of these pedigrees might otherwise have been confused with another inherited vitreoretinopathy, Wagner syndrome (MIM 143200). However, although Wagner syndrome also appears to be a predominantly ocular vitreoretinopathy without systemic manifestations, it has distinct

### Table 1 Phenotypes resulting from COL2A1 mutations not in exon 2

<table>
<thead>
<tr>
<th>Family</th>
<th>Ocular phenotype</th>
<th>Articular phenotype</th>
<th>Aural phenotype</th>
<th>Oro-facial phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myopia</td>
<td>Retinal detachment</td>
<td>Joint hypermobility</td>
<td>Radiological joint abnormality</td>
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<tr>
<td>MS2</td>
<td></td>
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<tr>
<td>II-2</td>
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<td>MS20</td>
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<tr>
<td>Mean score</td>
<td>1.7</td>
<td>1.0</td>
<td>0.9</td>
<td>0.7</td>
</tr>
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</table>

NA = not applicable because of prophylactic treatment. ND = not determined.

### Table 2 Phenotypes resulting from COL2A1 exon 2 mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>Ocular phenotype</th>
<th>Articular phenotype</th>
<th>Aural phenotype</th>
<th>Oro-facial phenotype</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Radiological joint abnormality</td>
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<td>2</td>
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<td>0</td>
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<tr>
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<td>1.5</td>
<td>0.1</td>
<td>0.2</td>
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</table>

NA = not applicable because of prophylactic treatment. ND = not determined.
clinical differences from Stickler syndrome,22 23 and in some families linked to a separate locus on chromosome 5.22 24 25 The term “Wagner-Stickler” syndrome,26–29 is therefore confusing and should be abandoned.

Here we describe the clinical and molecular genetic features of eight families with Stickler syndrome. All eight families feature the same type 1 vitreous phenotype but three of the families have a predominantly ocular phenotype with minimal or absent systemic involvement and were found to have mutations in exon 2 of COL2A1. Five other pedigrees have, in addition to the type 1 ocular phenotype, auditory and articular involvement and have mutations in others regions of the same gene. None of the pedigrees exhibits the characteristic lenticular, retinal pigment epithelial, or choroidal changes seen in Wagner syndrome.22 23

Method

CLINICAL EXAMINATION

Pedigrees were identified from the vitreous clinic database at Addenbrooke’s Hospital and subclassified prospectively according to vitreoretinal phenotype before molecular genetic analysis. Informed written consent was received in all cases and prior ethical approval for the study was obtained (LRC92/019).

OPHTHALMIC EXAMINATION

A general ophthalmic history was recorded with particular attention to the age of onset, degree and progression of myopia, cataract, and vitreoretinal disease. A full ophthalmic examination including refraction, slit lamp biomicroscopy, indirect ophthalmoscopy, and B scan ultrasonography was carried out. In some of the younger patients applanation tonometry and gonioscopy were not possible. Anterior and posterior segment photographs were taken where appropriate.

PHYSICAL DEVELOPMENT

In order to assess the developmental morphology of the face and hands, all patients were examined subjectively and objectively. A subjective assessment was made of the non-quantifiable factors retrognathia and anteversion of nares. All other factors were measured objectively according to a standardised method.30 Anteroposterior and lateral facial photographs were taken at a standardised scale of 1:8, and of both hands at a scale of 1:10 using a Nikon FM2 camera with Micro Nikon 105 mm medical lens and Kodachrome 64 film at F16. A 1 cm grid was printed and then photographed at a scale of 1:8 to match the facial films and 1:10 to match the hand views. Clinical measurements of outer canthal distance, inner canthal distance, philtrum length, and middle finger length were also recorded at the time of initial examination. These were used subsequently to test the accuracy of the method of photographic calibration. Control measurements of inner and outer canthal distance, interpupillary distance, and philtrum length from 20 unaffected siblings and 60 age matched controls (recruited from the general ophthalmic clinic) were also recorded.

Using dual projection of identically calibrated projectors, the matched scales were superimposed over each facial view in turn so that the quantifiable facial measurements could be made. This was repeated using the matched scale for the hand views. All measurements were made according to published criteria.30
GENERALISED SKELETAL COMPLEX
Height and weight were measured and an assessment made of general body build. Joint hypermobility was assessed objectively using the Beighton scoring system. A score of one or zero is given for a series of joint manoeuvres and the total sum allocated up to a possible maximum score of 9/9. This method allows comparison with published age and sex matched populations.31 Plain anteroposterior and lateral radiographs were carried out on affected pedigree members.

AUDIOLOGY EXAMINATION
A specific inquiry was made regarding the date and progression of any subjective hearing loss and in particular whether this had been a congenital, sudden, or progressive deterioration. A record of the type and duration of noise exposure was made together with any contributory factors such as glue ear, grommets, or drug induced sensorineural damage. All affected patients underwent bilateral otoscopy and audiometry involving air and bone conduction testing according to standardised procedures.32

Molecular Genetic Analysis
Seven families with the type 1 vitreous phenotype were used for linkage analysis to markers for COL2A1, COL11A1, and COL11A2 as previously described.7 All were consistent for linkage to COL2A1, with lod scores ranging from 2.1 to 0.3. In most instances COL11A1 and COL11A2 could be excluded (data not shown). Another family with the type 1 vitreous phenotype was used without any linkage analysis. All eight were analysed for COL2A1 mutations.

COL2A1 Amplification and Sequencing
Database available DNA sequences were used to design primers for polymerase chain reaction (PCR) amplification. A 30.991 kb sequence contained exons 1–54 (Accession No L10347); 343 bp of 3’ untranslated region was contained in an EST Image clone (Accession No H62509). Five PCR products covering bases (1) 4052–6902, (2) 6028–11591, (3) 11331–19598, (4) 18342–26231, (5) 24171–30991+106 3’UTR were amplified using DNA from a second or third generation affected individual. In each case the primers...
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Table 3  Type and position of COL2A1 mutations. The position and type of mutations
found in COL2A1 are indicated by nucleotide location within the COL2A1 gene
(Accession No L10347). Amino acids are numbered by the usual convention in which the
first glycine of the triple helical region is 1. Amino acids in the N-propeptide are numbered
from the mutating methionine.

<table>
<thead>
<tr>
<th>Family</th>
</tr>
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<tbody>
<tr>
<td>MS62</td>
</tr>
<tr>
<td>MS61</td>
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<tr>
<td>MS113</td>
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<td>MS54</td>
</tr>
<tr>
<td>MS2</td>
</tr>
<tr>
<td>MS66</td>
</tr>
<tr>
<td>MS20</td>
</tr>
<tr>
<td>MS18</td>
</tr>
</tbody>
</table>

Lod score 
Family DNA Analysis

Small regions of COL2A1 containing potential mutation sites, identified by sequencing,
were amplified from each family member. Taq DNA polymerase (Life Technologies) was
used with amplification conditions described previously. Products were purified using QIAquick spin
columns (Qiagen), and eluted in water. Direct cycle sequencing of individual exons con-
tained within the PCR products was performed with BigDye terminator RR mix (PE Applied Biosystems).
Sequencing primers were situated around 50 bp up and downstream from each exon and were derived from the
published sequence for the COL2A1 gene (Accession No L10347). Reactions were
analysed using an ABI 377 machine.

FAMILY DNA ANALYSIS

Results

The type 1 vitreous anomaly was present in all affected individuals and clinical status was
confirmed on subsequent mutation analysis (see below). Although there was both inter-
familial and intrafamilial variation in systemic phenotype the vitreous phenotype remained
consistent throughout and this emphasises the importance of vitreous slit lamp examination in this
disorder. Five families (MS2, MS18, MS20, MS54, and MS66) expressed a predominantly ocular phenotype
in which skeletal, auditory changes were minimal and orofacial abnormalities were present
only in a minority of individuals. These differences are illustrated and quantified in Tables 1
and 2. In seven families linkage analysis was consistent with COL2A1 as the disease locus.

An additional family (MS54) was analysed solely on the basis of the vitreous phenotype.

The COL2A1 gene was amplified as five overlapping PCR products and individual exons
were directly sequenced. In all cases changes to the sequence which lead to premature
termination codons and haploinsufficiency were found (examples are shown in Fig 1). Five of
these were frame shift mutations and three were single base substitutions (Table 3). Mutations
in each family were confirmed by single dideoxynucleotide sequencing reactions designed
to detect the abnormality. In each case the status of affected and normal individuals, as
determined by slit lamp examination before molecular analysis, was confirmed by mutation
detection. All but one of these mutations are novel and, because they result in premature ter-
mation codons, they would be very unlikely to occur in the general population.

Three mutations were in exon 2. Two of these were frame shift mutations, a 2 bp
deletion, and a 4 bp duplication (GGAT) (Fig 2). Both lead to a premature termination
codon at the same position in exon 2. The third exon 2 mutation was a single base substitution,
which converted a codon for tryptophan to a premature termination codon. Two other frame shifts were both in exon 42 and were
insertions of a single cytosine residue into different poly C sequences. These lead to prema-
ture termination codons in different positions in the cDNA sequence. Another frameshift
mutation was caused by a 1 bp deletion in exon 21. This led to a downstream premature termina-
tion codon, in exon 28. Two single base sub-
stitutions converting codons for arginine to stop codons were seen in exons 35 and 42. The stop codon mutation in exon 42 was identical
to the first Stickler mutation characterised by Ahmad et al in 1991.

The three families with exon 2 mutations corresponded to those with minimal systemic changes (MS11, MS13, and MS62) (Table 2).
Articular changes were absent in all except an 80 year old member of family MS62. It is of
interest and importance to note, however, that the risk of ophthalmic complications (myopia, retinal detachment) is high in both groups of
families. Although midline clefting was less common in the families with exon 2 mutations
the number of patients is too small for statisti-
cal analysis.

Discussion

The Stickler and Wagner syndromes are both dominantly inherited vitreoretinopathies and
one of the main criteria often used to distinguish between the two is the lack of
systemic features in the Wagner syndrome. Wagner described a new ocular disease in a
three generation pedigree from the Kanton of Zurich with 13 affected individuals. It featured
autosomal dominant inheritance, low myopia (3.00 dioptres or less), fluid vitreous, cortical
cataract, and inconstant and variably affected dark adaptation. No affected individual suf-
f ered a retinal detachment. In a follow up study of Wagner’s original pedigree,10 further
affected individuals were identified. The cardi-
The association of retinal detachment and cleft palate was noted before Stickler’s description. The extra bands seen in affected members are arrowed.

Table 4  Comparison of ophthalmic features in type 1 Stickler syndrome and Wagner syndrome

<table>
<thead>
<tr>
<th></th>
<th>Stickler syndromes</th>
<th>Wagner syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myopia</td>
<td>Congenital, high degree</td>
<td>Usually mild</td>
</tr>
<tr>
<td>Anterior chamber angle dysgenesis</td>
<td>Occasional</td>
<td>Unreported</td>
</tr>
<tr>
<td>Cataract</td>
<td>Wedge-shaped, cortical</td>
<td>Early onset, nuclear, progressive, “cataracta complicata”</td>
</tr>
<tr>
<td>Type 1 vitreous anomaly</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Frequently normal</td>
<td>Progressive pigmentary and punched out chorioretinal atrophy</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>Common, frequently bilateral</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Dark adaptation</td>
<td>High risk of giant retinal tear</td>
<td>May be reduced</td>
</tr>
</tbody>
</table>

Figure 3  Family confirmation of premature termination codons. **P labelled single deoxynucleotide, sequencing reactions (as indicated) were performed on PCR products amplified from individual family members. Numbers indicate nucleotide sequence (Accession No L16347). The extra bands seen in affected members are arrowed.
tion of hereditary arthro-ophtalmopathy and controversy arose regarding the authenticity of the proposed new syndrome. Indeed, many subsequent publications referred to the “Wagner-Stickler” syndrome or “Wagner syndrome with arthropathy” implying phenotypic variation of the same basic underlying disorder. It has subsequently been shown that families with Stickler syndrome and a non-ocular variant have mutations in the genes COL2A1, COL11A1, and COL11A2; whereas, several families with Wagner syndrome (WGN1) including the original family have been linked to a locus at 5q14.3.

The mutations in COL2A1 exon 2 described here illustrate the critical importance of ophthalmic and particularly vitreous examination in distinguishing between these two disorders, especially if a pedigree is too small to exclude one or other loci on linkage analysis. The main clinical differences are summarised in Table 4.

There has been one report of a COL2A1 mutation causing Wagner syndrome. However, the clinical description including the mild facial hypoplasia and hypermobile joints more closely resembled that of Stickler syndrome. In our experience the ocular phenotype displayed by COL2A1 exon 2 mutation patients matches that seen in other COL2A1 Stickler syndrome families. Patients exhibit the membranous congenital anomaly and do not show the atrophy of the retinal pigment epithelium and choriocapillaris seen in 5q linked Wagner families (Table 4). In our view these clinical differences are sufficient to distinguish between the two disorders.

Our results also illustrate that the families with COL2A1 exon 2 mutations, even in the absence of obvious skeletal or auditory features are also at high risk of retinal detachment. This is particularly important since the diagnosis of Stickler syndrome may be overlooked or rejected on the basis of the minimal or absent skeletal changes. Although the midline clefting seen in some individuals might suggest Stickler syndrome, most patients have few if any non-ocular characteristics normally associated with this disorder.

Seven individuals with exon 2 mutations had some degree of midfacial hypoplasia or midline clefting, the latter being severe in two cases. This may indicate that the longer αIIa collagen plays a part during craniofacial development. Certainly, the level of αIIa mRNA is higher in fetal skeletal tissue. In these few individuals where facial hypoplasia or midline clefting is apparent the diagnosis of Stickler syndrome would be acceptable. However, for the majority of exon 2 mutants (including all members of MS11) the lack of extraocular characteristics might, in the view of some clinicians, exclude the designation of Stickler syndrome. A suggested alternative title might be “predominantly ocular” Stickler syndrome (POSS), commensurate with the non-ocular Stickler syndrome (NOSS) phenotype caused by mutations in COL11A2 and “full” Stickler syndrome (FSS) secondary to other mutations in either COL2A1 or COL11A1.

The absence of arthropathy, cleft palate, hearing loss, and cardiovascular abnormalities led Perveen et al to assign a diagnosis of Wagner syndrome in 18 families. However, 14 were too small to confirm linkage to 5q14.3. The lack of mutations in these families was used to exclude the versican (CSPG2) gene as the Wagner locus. The authors themselves were cautious as to whether all of their families might be linked to 5q14.3. We suggest that unless the retinal phenotype was the same as for families linked to 5q14.3, then COL2A1 should be considered a candidate for the disease locus in families with vitreoretinopathy without systemic changes, unless it could be excluded by linkage. It is not known if mutations in other regions of the COL2A1 gene can also lead to similar ocular only phenotypes.

Finally, our family studies emphasise the reliability of vitreous phenotype in the diagnosis of Stickler syndrome. Whereas systemic features vary between and within families the eye changes were remarkably consistent (Fig 4). Of 52 individuals examined (excluding unaffected spouses) mutation detection ratified the clinical diagnosis in 35 affected and 19 unaffected individuals.
The authors gratefully acknowledge the financial support of The Iris Fund for Prevention of Blindness, The Medical Research Council, The Stanley Thomas Johnson Foundation, and Michael and Elizabeth Greig.

We also wish to thank the following: Maureen Laidlaw (tissue culture), Katherine Haslam and Medical Illustration, Addenbrooke’s Hospital, Cambridge (photography), Chris Madren, Department of Genetics (sequencing), and our medical colleagues who have kindly referred patients to the vitreoretinal service at Addenbrooke’s Hospital.

1 Stickler GB, Belau PG, Farrell FJ, et al. Hereditary progres-


2 Stickler GB, Pugh DG. Hereditary progressive arthro-


10 Horton WA. Progress in human chondrodysplasia: molecu-


17 Bishop PN, Reardon AJ, McLeod D, et al. Identification of alternatively spliced variants of type II procollagen in vitre-


30 British Society of Audiology. Recommended procedures for pure tone audiometry using a manually operated instru-


35 Zorzi J, Rivasniani P, Haastaja L, et al. Mutation in type II procollagen (COL2A1) that substitutes aspartate for glycine 97–97 and that causes cataracts and retinal detachment: evidence for molecular heterogeneity in the Wagner syndrome and Stickler syndrome (Arthro-


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