



HAL
open science

Stickler syndrome caused by COL2A1 mutations: genotype-phenotype correlation in a series of 100 patients

Geert Mortier, Kristien P Hoornaert, Inge Vereecke, Chantal Dewinter,
Thomas Rosenberg, Frits A Beemer, Jules G Leroy, Laila Bendix, Erik Björck,
Dr. Bonduelle, et al.

► To cite this version:

Geert Mortier, Kristien P Hoornaert, Inge Vereecke, Chantal Dewinter, Thomas Rosenberg, et al..
Stickler syndrome caused by COL2A1 mutations: genotype-phenotype correlation in a series of 100
patients. *European Journal of Human Genetics*, 2010, n/a (n/a), pp.n/a-n/a. 10.1038/ejhg.2010.23 .
hal-00511182

HAL Id: hal-00511182

<https://hal.science/hal-00511182>

Submitted on 24 Aug 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Stickler syndrome caused by *COL2A1* mutations: genotype-phenotype correlation**
2 **in a series of 100 patients**

3

4 Kristien P Hoornaert¹, Inge Vereecke¹, Chantal Dewinter¹, Thomas Rosenberg², Frits A
5 Beemer³, Jules G Leroy¹, Laila Bendix⁴, Erik Björck⁵, Maryse Bonduelle⁶, Odile
6 Boute⁷, Valerie Cormier-Daire⁸, Christine De Die-Smulders⁹, Anne Dieux-Coeslier⁷,
7 Hélène Dollfus¹⁰, Mariet Elting¹¹, Andrew Green^{12, 13}, Veronica I Guerçi¹⁴, Raoul CM
8 Hennekam^{15, 16}, Yvonne Hilhorts-Hofstee¹⁷, Muriel Holder⁷, Carel Hoyng¹⁸, Kristi J
9 Jones^{19, 20}, Dragana Josifova²¹, Ilkka Kaitila²², Suzanne Kjaergaard²³, Yolande H
10 Kroes³, Kristina Lagerstedt⁵, Melissa Lees¹⁵, Martine LeMerrer⁸, Cinzia Magnani²⁴,
11 Carlo Marcelis²⁵, Loreto Martorell²⁶, Michèle Mathieu²⁷, Meriel McEntagart²⁸, Angela
12 Mendicino²⁹, Jenny Morton³⁰, Gabrielli Orazio³¹, Véronique Paquis³², Orit Reish³³,
13 Kalle O. J. Simola³⁴, Sarah F Smithson³⁵, Karen I Temple³⁶, Elisabeth Van Aken³⁷,
14 Yolande Van Bever³⁸, Jenneke van den Ende³⁹, Johanna M Van Hagen⁴⁰, Leopoldo
15 Zelante⁴¹, Riina Zordania⁴², Anne De Paepe¹, Bart P Leroy^{1, 37}, Marc De Buyzere⁴³,
16 Paul J Coucke¹, Geert R Mortier¹

17

18 ¹ Center for Medical Genetics, Ghent University Hospital, Gent, Belgium

19 ² National Eye Clinic, Gordon Norrie Centre for Genetic Eye Diseases, Hellerup,

20 Denmark

21 ³ Center for Medical Genetics, Utrecht, Netherlands

22 ⁴ Department of Clinical Genetics, University of Southern Denmark, Vejle Hospital,

23 Denmark

24 ⁵ Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden

25 ⁶ Center for Medical Genetics, UZ Brussel, Belgium

26 ⁷ Center for Clinical Genetics, Hopital Jeanne de Flandre, Lille, France

27 ⁸ Department of Medical Genetics, Université Paris Descartes, Necker Enfants Malades
28 Hospital, Paris, France

29 ⁹ Department of Clinical Genetics, University Hospital Maastricht, Maastricht, The
30 Netherlands

31 ¹⁰ Centre de reference pour les Affections Rares et Génétique Ophthalmologique
32 (CARGO), Hôpitaux Universitaires de Strasbourg, France

33 ¹¹ Department of Clinical Genetics, VU University Medical Center, Amsterdam, The
34 Netherlands

35 ¹² National Centre for Medical Genetics Our Lady's Hospital, Dublin, Ireland

36 ¹³ University College Dublin School of Medicine and Medical Science

37 ¹⁴ Metabolic Disease Unit, IRCCS Burlo Garofolo, Trieste, Italy

38 ¹⁵ Clinical and Molecular Genetics Unit, Institute of Child Health, University College
39 London, UK

40 ¹⁶ Department of Pediatrics, Academic Medical Center, University of Amsterdam,
41 Amsterdam, The Netherlands

42 ¹⁷ Department of Clinical Genetics, Leiden University Medical Center, Leiden, the
43 Netherlands

44 ¹⁸ Department of Ophthalmology, Radboud University Nijmegen Medical Centre,
45 Nijmegen, The Netherlands

46 ¹⁹ Western Sydney Genetics Program, The Children's Hospital at Westmead, Sydney,
47 Australia

48 ²⁰ Discipline of Paediatrics and Child Health, University of Sydney, Sydney, Australia

49 ²¹ Department of Clinical Genetics, Guy's Hospital, London, UK

50 ²² Department of Clinical Genetics, Helsinki University Central Hospital, Hus, Finland

51 ²³ Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark

52 ²⁴ Department of Paediatrics, University Hospital of Parma, Italy

53 ²⁵ Department of Human Genetics, Radboud University Medical Centre Nijmegen, the

54 Netherlands

55 ²⁶ Department of Genetics, Hospital Sant Joan de Déu, Barcelona, Spain

56 ²⁷ Clinical Genetics, CHU Nord, Amiens, France

57 ²⁸ Clinical Genetics, St George's University of London, London, UK

58 ²⁹ U.O.S. di Genetica, DTMI, ASL RME, Roma, Italy

59 ³⁰ Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham, UK

60 ³¹ Department of Clinical Genetics, Ospedale G. Salesi, Ancona, Italy

61 ³² Department of Clinical Genetics, Hopital de L'Arghet, Nice, France

62 ³³ Genetics Institute, Assaf Harofeh Medical Center, Zerifin, Israel

63 ³⁴ Department of Pediatrics, Tampere University Hospital, Tampere, Finland

64 ³⁵ Department for Clinical Genetics, St. Michael's Hospital, Bristol, UK

65 ³⁶ Academic Unit of Genetic Medicine, Division of Human Genetics, University of

66 Southampton, Southampton, UK

67 ³⁷ Department of Ophthalmology, Ghent University Hospital, Gent, Belgium

68 ³⁸ Department of Clinical Genetics, Erasmus MC, Rotterdam, the Netherlands

69 ³⁹ Center for Medical Genetics, University Hospital, Antwerp, Belgium

70 ⁴⁰ Department of Clinical Genetics, University Medical Center, Amsterdam, the

71 Netherlands

72 ⁴¹ Medical Genetics Service, IRCCS Casa Sollievo della Sofferenza, San Giovanni

73 Rotondo, Italy

74 ⁴² Clinical Genetics, Tallinn Children's Hospital, Tallinn, Estonia

75 ⁴³ Heart Center, Ghent University Hospital, Ghent, Belgium

76

77 **Correspondence:** Geert Mortier, MD, PhD

78 Center for Medical Genetics

79 Ghent University Hospital

80 De Pintelaan 185, B-9000 Ghent, Belgium

81 Tel 0032 9 332 36 03

82 Fax 0032 9 332 49 70

83 email: geert.mortier@ugent.be

84

85

86 **Keywords:** *COL2A1*, Stickler syndrome, genotype-phenotype correlation,

87 type II collagenopathies, splice site mutation

88

89 **ABSTRACT**

90 Stickler syndrome is an autosomal dominant connective tissue disorder caused by
91 mutations in different collagen genes. The aim of our study was to define more
92 precisely the phenotype and genotype of Stickler syndrome type 1 by investigating a
93 large series of patients with a heterozygous mutation in COL2A1. In 188 probands with
94 the clinical diagnosis of Stickler syndrome, the COL2A1 gene was analysed by either a
95 mutation scanning technique or bidirectional fluorescent DNA sequencing. The effect of
96 splice site alterations was investigated by analysing mRNA. MLPA analysis was used
97 for the detection of intragenic deletions. We identified 77 different COL2A1 mutations
98 in 100 affected individuals. Analysis of the splice site mutations revealed unusual RNA
99 isoforms, most of which contained a premature stop codon. Vitreous anomalies and
100 retinal detachments were found more frequently in patients with a COL2A1 mutation
101 compared to the mutation negative group ($p < 0.01$). 20 of the 23 sporadic patients with a
102 COL2A1 mutation had either a cleft palate or retinal detachment with vitreous
103 anomalies. The presence of vitreous anomalies, retinal tears or detachments, cleft palate
104 and a positive family history were shown to be good indicators for a COL2A1 defect. In
105 conclusion, we confirm that Stickler syndrome type 1 is predominantly caused by loss-
106 of-function mutations in the COL2A1 gene since more than 90% of the mutations were
107 predicted to result in nonsense mediated decay. Based on binary regression analysis we
108 developed a scoring system that may be useful when evaluating patients with Stickler
109 syndrome.

110

111

112

113 **INTRODUCTION**

114 Stickler syndrome (MIM# 108300) is a connective tissue disorder first described
115 by Stickler et al in 1965. It is characterized by ocular, orofacial, auditory and skeletal
116 manifestations with considerable intra- and interfamilial variability.[1, 2] The incidence
117 is estimated to range between 1 in 7,500 to 1 in 9,000 newborns
118 (<http://ghr.nlm.nih.gov/condition%3Dsticklersyndrome> US Dept of Health). The most
119 characteristic ocular features are congenital myopia, vitreous alterations, cataract,
120 glaucoma and a high risk of spontaneous retinal detachments. The orofacial changes
121 include cleft palate, midfacial hypoplasia, low nasal bridge and micrognathia. Joint pain
122 is common in childhood and osteoarthritis may be apparent from the third or fourth
123 decade. Radiographs may show signs of a spondyloepiphyseal dysplasia. Mild
124 sensorineural hearing loss, mainly for the high tones, can be present in Stickler
125 syndrome type 1 (*COL2A1* gene), more severe sensorineural hearing loss is usually
126 found in the other types of Stickler syndrome.[3, 4]

127 At present, at least 3 types of autosomal dominant Stickler syndrome have been
128 discerned. A correlation between these different types and their accompanying vitreous
129 anomalies has been suggested.[5] ‘Membranous’ or type 1 vitreous has been associated
130 with Stickler syndrome type 1 caused by heterozygous mutations in the *COL2A1* gene
131 (MIM# 108300).[6] Type 2 or ‘beaded’ vitreous is mainly found in patients with
132 Stickler syndrome type 2 which is due to a heterozygous mutation in the *COL11A1* gene
133 (MIM# 604841).[3, 7] Stickler syndrome type 3 or ‘non-ocular Stickler syndrome’
134 refers to the phenotype of patients with a mutation in the *COL11A2* gene that is not
135 expressed in the eye (MIM# 184840).[8] In addition to the different types of autosomal

136 dominant Stickler syndrome, recently also a recessive form of Stickler syndrome,
137 caused by a mutation in the *COL9A1* gene, has been described (MIM# 120210).[9]

138 Stickler syndrome type 1 is the most common form. The majority of *COL2A1*
139 mutations identified in patients with Stickler syndrome type 1 are predicted to result in
140 nonsense mediated decay (NMD). On the other hand, missense mutations (usually
141 glycine substitutions) in *COL2A1* usually result in short stature disorders such as
142 achondrogenesis type II/hypochondrogenesis, spondyloepiphyseal dysplasia (SEDC),
143 Kniest dysplasia, spondyloperipheral dysplasia (SPD) and Torrance dysplasia (MIM#
144 200610, 183900, 156550, 271700, 151210).[5, 10, 11]

145 The aim of this study was to define more precisely the phenotype and genotype
146 of Stickler syndrome type 1 by investigating a large series of Stickler syndrome patients
147 with a heterozygous mutation in the *COL2A1* gene.

148

149 **MATERIALS & METHODS**

150 **Evaluation of phenotype**

151 Over the past 10 years, blood or DNA samples from 278 individuals were
152 referred for mutation analysis of the *COL2A1* gene in order to confirm or exclude the
153 clinical diagnosis of Stickler syndrome.

154 Information on clinical and radiographic features of each patient was requested
155 by using a specific questionnaire (Supplementary Table 1). Ninety patients were
156 excluded from the study because insufficient clinical data were available (in 11 of those
157 patients a *COL2A1* mutation was identified). Each patient in the group of 188 remaining
158 subjects had two or more of the following features reminiscent of Stickler syndrome:
159 myopia, spontaneous retinal detachment, cleft palate, sensorineural hearing loss and
160 arthropathy. Informed consent was obtained from each enrolled patient.

161 **Analysis of genomic DNA**

162 Genomic DNA was extracted from blood samples by standard procedures,
163 followed by touchdown PCR amplification of the *COL2A1* gene using forward and
164 reverse primers located in the flanking introns. The PCR products were analysed by gel
165 electrophoresis and visualized by ethidium bromide staining on 2% agarose gels.

166 Mutation screening was performed by SSCP and CSGE (period 1997-2002) or
167 by DHPLC analysis (period 2003-2006) using the WAVE DNA fragment analysis
168 system (Transgenomic, Cheshire, UK).[12, 13, 14] All fragments showing an aberrant
169 pattern were directly sequenced on the ABI PRISM 3730 automated sequencer (Applied
170 Biosystems, Foster City, CA) using the BigDye terminator cycle sequencing chemistry.
171 From 2007 on, direct sequencing of all 54 exons was performed. These obtained
172 sequences were compared to the wild-type sequence as submitted to GenBank

173 Accession number NM_001844. The nucleotides were numbered starting from the first
174 base of the start codon (ATG) of the cDNA reference sequence. Amino acid residues
175 were numbered from the first methionine (start codon for translation) of the procollagen
176 α 1(II)-chain (GenBank Accession number L10347).

177 **RNA studies**

178 In patients with a splice site mutation, an EBV cell line or skin biopsy was
179 requested for analysis of mRNA splicing. In order to stabilize mutant *COL2A1* mRNA,
180 cycloheximide (Sigma, www.sigmaaldrich.com) was added to the cultures, followed by
181 mRNA isolation and cDNA preparation. Nested PCR was used to obtain sufficient PCR
182 fragments for direct sequencing.

183 **MLPA analysis**

184 Multiplex ligation-dependent amplification (MLPA) was set up, following the
185 directions provided by the manufacturer (MRC Holland, Amsterdam, The Netherlands)
186 (www.MPLA.com).[15] The probe set for COL2A1 (SALSA MLPA kit P214) covering
187 exons 1, 4, 6, 8, 10, 16, 17, 19, 20, 24, 27, 29, 31, 35, 39, 43, 46, 49, 51 and 54 was
188 used.

189 **Binary logistic regression analysis/statistics**

190 The formula for the proposed scoring system was developed using binary (mutation
191 positive or not) logistic regression analysis.[16, 17, 18] The parameters tested in the
192 model comprised: vitreous abnormalities, retinal abnormalities, flat face, micrognathia,
193 retinal tear and/or detachment, cataract, low nasal bridge, cleft palate, positive family
194 history, myopia, conductive hearing loss, premature arthropathy, hypermobility,
195 epiphyseal dysplasia on X-rays and sensorineural hearing loss. The weight (score) for
196 each characteristic in the scoring system was proportional to its regression coefficient in

197 the model. To simplify the scoring system the scores were rounded to positive integers
198 and the scores of the characteristics with lowest significant regression coefficients were
199 conventionally given a score value of one and the intercept of the linear predictor was
200 neglected. Otherwise, no recalibration, shrinkage factor or model revision or extension
201 seemed to be needed to study the whole study population. Calibration of the scoring
202 system was further evaluated with the Hosmer-Lemeshow test. The clinical applicability
203 of the obtained score was evaluated for several thresholds using conventional receiver
204 operating characteristics (positive and negative predictive value, sensitivity and
205 specificity). All statistical analyses were performed with SPSS 15.0 for Windows (SPSS
206 Inc. Chicago, IL).
207

208 **RESULTS**

209 In 100/188 individuals referred with a potential diagnosis of Stickler syndrome,
210 a heterozygous *COL2A1* mutation was identified. This panel of 77 different mutations
211 included 1 deletion of the entire gene [19], 13 nonsense mutations, 21 deletions, 1
212 insertion, 9 duplications, 2 combinations of an insertion and a deletion, 22 splice site
213 alterations, 1 synonymous mutation, 2 missense mutations resulting in an arginine-to-
214 cysteine substitution [20] and 5 missense mutations substituting a glycine residue in the
215 triple helical domain of the protein. The mutations were distributed over the entire gene
216 and no hot spot regions were apparent (Table 1). Thirteen mutations were observed in
217 more than one proband: c.625C>T, p.Arg209X and c.1833+1G>A, p.GlyfsX619 were
218 found in four patients each; c.3106C>T, p.Arg1036X occurred five times (Table 1).
219 Two mutations were located in the alternatively spliced exon 2. The first one, a
220 duplication of 23 nucleotides (c.211_233dup; p.Glu79ThrfsX2) causes a frameshift that
221 leads to a premature stop codon within the exon itself. The patient with this mutation
222 only had ocular features (retinal detachment) of Stickler syndrome as expected since
223 exon 2 is retained in the eye but spliced out in the cartilage.[21, 22, 23] The second
224 patient had the deletion c.264_276del; p.Cys89SerfsX24 that causes a frameshift with a
225 premature stop codon in exon 3. However, this patient had both ocular and extra-ocular
226 manifestations of Stickler syndrome including vitreal abnormalities, a retinal
227 detachment, flat face, sensorineural hearing loss, arthropathy and epiphyseal changes on
228 radiographs.

229 In the skin fibroblasts or the EBV cell line available from 13 patients with 12
230 different splice site alterations, cDNA analysis showed that each splice site alteration
231 resulted in a premature stop codon (data not shown). For the three splice site mutations,

232 multiple isoforms of mRNA were detected. In each case at least one isoform harboured
233 a premature stop codon (Supplementary Figure 1: isoforms A3, B2 and C2). In the
234 additional isoforms A1, B1 and C1 only skipping of the adjacent exon was observed. In
235 the C1 isoform skipping of even three consecutive exons (51-53) had occurred. These
236 exons constitute the carboxypropeptide of the procollagen $\alpha 1(\text{II})$ -chain, which is
237 necessary for chain association and initiation of the triple helix formation.[24]
238 Consequently, the resulting truncated protein will most likely be lost and not
239 incorporated into the collagen trimer. In the isoform A2, exon 7 was deleted but intron
240 5 retained, the latter containing an in-frame stop codon. Both patients harbouring the
241 c.430-1G>C and c.4074+1G>T splice site mutation suffered from myopia, vitreoretinal
242 abnormalities and spontaneous retinal detachments. They also showed a flat face. The
243 individual with the c.3003+5G>A splice site mutation was born with a Pierre-Robin
244 anomaly and had myopia, a retinal detachment and cataract. He also suffered from
245 conductive hearing loss. His affected father had a history of spontaneous bilateral retinal
246 detachments in childhood.

247 One patient was heterozygous for a synonymous mutation (c.2862C>T;
248 p.Gly954Gly) in exon 42. Since this mutation was cosegregating with Stickler
249 syndrome in the affected family, the pathogenic effect was further explored at the
250 mRNA level. cDNA analysis showed that this mutation generated a cryptic splice site
251 35 nucleotides upstream of the normal donor splice site in intron 42, resulting in a
252 frameshift with a premature stop codon (Supplementary Figure 2).

253 The nonsense (p.Trp1293X) and frameshift mutations (p.Cys1289ProfsX3,
254 p.Ile1300ThrfsX15, p.Asn1303ThrfsX9) residing in the carboxypropeptide were
255 predicted to result in NMD since they occur before the last 50 nucleotides of the last

256 exon-exon junction (Table 1).[25] In the patient with the splice site alteration in intron
257 53 (c.4317+2T>C), the splice site prediction program
258 (http://www.fruitfly.org/seq_tools/splice.html) computed an insertion of a part of the
259 intron 53 containing an in-frame stop codon.

260 Since only 100 mutations were identified in a series of 188 patients, we decided
261 to expand the molecular analysis with MLPA to explore the possibility of missed
262 intragenic deletions. For this analysis we selected 20 patients in whom we strongly
263 suspected the diagnosis of Stickler syndrome because of the presence of severe myopia,
264 retinal detachment and/or cleft palate. However, no additional mutations were identified
265 in these affected individuals.

266 In a next step we evaluated the clinical and radiographic features in our series of
267 188 patients and looked for differences between the mutation positive (n=100) and
268 mutation negative (n=88) group. The results are summarized in Figure 1. A positive
269 family history, orofacial anomalies (cleft palate, low nasal bridge, flat face,
270 micrognathia) and vitreoretinal changes were more frequently ($p \text{ value} \leq 0.05$) present
271 in the mutation positive group. On the other hand, sensorineural hearing loss was
272 observed more frequently in the mutation negative group ($p < 0.005$). 20/23 of the
273 sporadic patients with a *COL2A1* mutation had either a cleft palate or retinal
274 detachment(s) with vitreous anomalies and myopia.

275 To determine the discriminating power of these features, we performed a binary
276 logistic regression analysis. The following characteristics were most distinguishing
277 between both groups : a) vitreous abnormalities, b) retinal abnormalities, c) history of
278 retinal tear and/or detachment, d) low nasal bridge, e) cleft palate, f) micrognathia and
279 g) positive family history (Figure 2). Based on the regression coefficient of each

280 distinguishing characteristic, a specific scoring system was proposed. The highest score
281 (score 5) was attributed to retinal abnormalities and positive family history, a score of 4
282 was assigned to cleft palate and vitreous abnormalities, a retinal tear and/or detachment
283 represented a score of 3, whereas low nasal bridge and micrognathia received the lowest
284 score (score 1) (Table 2). When applying this scoring system to each patient, we
285 observed a higher median score for patients with a *COL2A1* mutation compared to those
286 without a mutation (11.5 versus 6). The calculated score ranges from 0 to 21 with a
287 theoretical maximum of 23. The distribution of the score for mutation positive and
288 mutation negative cases is shown in Figure 3. 75% of the patients with a *COL2A1*
289 mutation had a total score ≥ 9 (Figure 4). The presence of vitreoretinal anomalies and a
290 retinal detachment yields a total score of 12, illustrating the importance of a thorough
291 ophthalmological evaluation in patients with Stickler syndrome.
292

293 **DISCUSSION**

294 The past decade we have identified a large series of *COL2A1* mutations in a
295 group of patients referred with the diagnosis of Stickler syndrome. The availability of
296 these data prompted us to retrospectively analyse both genotype and phenotype of these
297 patients. With this study we aimed to define more precisely the phenotype of Stickler
298 syndrome type 1 and were interested in identifying discriminating features between
299 patients with and those without a *COL2A1* mutation. In addition, we wanted to
300 investigate in what respect Stickler syndrome type 1 mutations were different from
301 other *COL2A1* mutations causing the type II collagenopathies with short stature. More
302 precisely, we wanted to learn if all Stickler syndrome mutations were predicted to have
303 a loss-of-function effect on the procollagen $\alpha 1(\text{II})$ -chain.

304 Sufficient clinical and radiographic data were available on 188 probands and in
305 100 of these individuals a heterozygous *COL2A1* mutation was identified. The 77
306 different mutations were distributed over the entire gene and no regions of mutation
307 clustering were found. Thirteen mutations were observed in more than one proband,
308 with 10 involving a CpG dinucleotide. One patient was heterozygous for a deletion of
309 the entire gene and details have been published earlier.[19] The 34 smaller and
310 intragenic deletions, insertions, duplications and insertion-deletions were all out of
311 frame and therefore predicted to result in NMD. A similar effect was demonstrated for
312 the synonymous mutation (p.Gly954Gly) which created a cryptic splice site
313 (Supplementary Figure 2). This mutation is the second example of an apparently silent
314 *COL2A1* mutation that alters RNA splicing, illustrating the importance of studying the
315 effect of so-called synonymous mutations at the mRNA level.[26] Analysis of cDNA
316 also allowed us to study the effect of 12 different splice site alterations. In addition, it

317 gave us more insights into the complexity of mRNA splicing of the *COL2A1* gene. Each
318 splice site mutation was shown to create at least one isoform with a frameshift and
319 premature stop codon as a consequence (Supplementary Figure 1). In addition, some
320 unexpected splice site outcomes were observed with skipping of one or more
321 consecutive exons and even retention of introns more remote from the mutation. As
322 shown before for collagen types I and V, introns are not consecutively removed in a 5'
323 to 3' direction which may explain some unusual RNA isoforms observed in our
324 patients.[27, 28]

325 In addition to the above mentioned hypomorphic mutations, also 7 different
326 missense mutations were identified in this series of patients. Five mutations
327 (p.Gly216Asp; p.Gly219Arg; p.Gly222Val; p.Gly492Asp; p.Gly1131Ala) were
328 predicted to result in a glycine substitution. Glycine substitutions in the triple helical
329 domain usually have a dramatic effect by hampering proper triple helix formation of the
330 collagen trimer. They usually result in a type II collagen disorder with either lethal
331 outcome (achondrogenesis type 2/hypochondrogenesis) or severe short stature (SEDC,
332 Kniest dysplasia). Upon review of the literature and our own data, glycine substitutions
333 causing these short stature phenotypes never seem to occur amino-terminal to the
334 glycine residue at position 303.[29] Glycine substitutions upstream of this residue seem
335 to have a less deleterious effect on collagen trimer formation and function which may
336 explain the Stickler syndrome phenotype in our patients with the p.Gly216Asp,
337 p.Gly219Arg or p.Gly222Val substitution. For the more carboxy-terminally located
338 missense mutations, there is a less clear correlation between the location of the glycine
339 substitution and the phenotypic outcome. The nature of the substituting amino acid may
340 also play a role as is exemplified by the p.Gly492Val mutation that causes

341 spondyloepiphyseal dysplasia [30] and the Gly492Asp mutation that results in Stickler
342 syndrome (our series).

343 Not only missense mutations substituting a glycine residue were identified but
344 also two different missense mutations changing an arginine for a cysteine residue
345 (Arg565Cys; Arg904Cys) were found in a group of 5 patients. These substitutions
346 involve an arginine residue in the X position of the Gly-X-Y triplet.[20, 31] As we
347 reported before, substituting an arginine in the X position seems to cause Stickler
348 syndrome, whereas substituting an arginine in the Y position rather causes a type II
349 collagenopathy without ocular involvement.[20] Cysteine residues are normally not
350 present in the triple helical domain of the procollagen $\alpha 1(\text{II})$ -chain.[24] The insertion of
351 such a residue may generate aberrant disulphide bonds between mutant procollagen
352 chains and as such hamper proper chain alignment and trimer formation. In these
353 circumstances, the mutation may have a loss-of-function effect on the protein.

354 The second major goal of this study was to delineate the phenotype of Stickler
355 syndrome type 1 and to try and identify distinguishing characteristics between patients
356 with and without a *COL2A1* mutation. In the group of 100 patients with a mutation,
357 89% had myopia and 55% suffered from at least one episode of spontaneous retinal
358 detachment. Vitreous abnormalities were identified in 42% of the affected individuals,
359 but it proved difficult for most referring ophthalmologists to classify these anomalies
360 into either a type 1 or type 2 vitreous anomaly. Sixty per cent of the mutation positive
361 patients presented with a cleft palate at birth. Binary logistic regression analysis
362 revealed that the ocular and orofacial features were the most distinguishing clinical
363 characteristics between both groups. An affected first degree relative, the presence of
364 vitreoretinal anomalies and cleft palate were good indicators for Stickler syndrome type

365 1. Their presence in a patient with Stickler syndrome increases the likelihood of finding
366 a *COL2A1* mutation upon molecular analysis. On the other hand, severe sensorineural
367 hearing loss was more frequently observed in the mutation negative group (Figure 1).
368 The latter confirms the findings of previous studies indicating that hearing loss is more
369 prevalent and pronounced in type 2 Stickler syndrome.[3] Some features (e.g. myopia)
370 were not included in the scoring system because they were frequently reported in both
371 groups and thus only had a weak discriminating power. Interestingly, there was no
372 statistical difference in the occurrence of early-onset osteoarthritis and
373 spondyloepiphyseal anomalies between the group with and without a *COL2A1*
374 mutation. When applying the proposed score system, a higher total score was found in
375 the group of patients with a *COL2A1* mutation (Figures 3 and 4), which is in contrast to
376 previous studies in which no differences were observed.[32] Nevertheless, a
377 considerable overlap between both groups was present. This overlap is most likely due
378 to an age-of-onset effect in the mutation positive group and genetic heterogeneity in the
379 mutation negative group. In the latter group, individuals with a *COL11A1* mutation may
380 be present (especially those with severe hearing loss) as well as patients with an
381 undetected *COL2A1* mutation (false negative patients). Indeed, samples referred at the
382 beginning of the study were analysed with less sensitive mutation screening techniques
383 such as SSCP and CSGE. In addition, deletions involving one particular amplicon will
384 be missed by sequencing analysis. However, MLPA analysis in a selected group of
385 patients failed to unravel new mutations. Also, regions outside the coding sequences
386 such as the promoter were not analysed in this study. Lower scores in the mutation
387 positive group may be due to the young age of the affected individuals not yet showing all

388 features (such as retinal detachments) of Stickler syndrome type 1. Of the 16 cases with
389 a score of ≤ 8 , fourteen patients were less than 14 years of age (Table 1).

390 In conclusion, this study conducted in a large series of patients, confirms that
391 Stickler syndrome type 1 is predominantly caused by loss-of-function mutations in the
392 *COL2A1* gene. Only 10% of the gene alterations were missense mutations residing in
393 the triple helical domain, some of which may still exert a hypomorphic effect (e.g. the
394 arginine-to-cysteine substitutions). Vitreoretinal abnormalities including the occurrence
395 of a retinal tear or detachment were statistically more frequent in Stickler syndrome
396 patients with a *COL2A1* mutation compared to those without a mutation. Together with
397 cleft palate and a positive family history these characteristics were shown to be good
398 indicators for a type II collagen defect (in contrast to severe sensorineural hearing loss).
399 Finally, binary regression analysis allowed us to develop a scoring system that
400 highlighted the importance of a thorough vitreoretinal assessment when evaluating
401 individuals suspected with Stickler syndrome type 1.

ACKNOWLEDGEMENTS

We are grateful to the patients and their families for their cooperation. We would like to thank the following clinicians for the referral of samples: M. Ausems, M. Baumgartner, K. Becker, S. Bertok, F. Betis, A.M. Bisgaard, K. Bouman, H. Brunner, O. Calabrese, K. Chandler, S. De Almeida, T. De Ravel, K. Devriendt, M. Drolenga, I. Feenstra, J.P. Fryns, H. Fryssira, F. Goodman, B.C.J. Hamel, J. M. Hertz, T. Homfray, J. Hurst, S. Janssens, D. Johnson, J. Kamphoven, W.S. Kerstjens-Frederikse, K. Keymolen, I. Liebaers, M. Maas, F. Malfait, H. Malmgren, S. Mancini, S. Mansour, I. Mathijssen, T. McDevitt, E.J. Meijers, F. Meire, A. Mendicino, N. Mignone, A. Muellner-Eidenbock, R. Newbury-Ecob, A. Nordgren, C. Postma, E.M. Ruiter, P. Schmidt, C. Schrandt-Stumpel, F. Stanzial, A. Superti-Furga, K. Ten Berg, P. Terhal, S. Tinschert, A. Tzschach, D. van den Boogaard, I. Van Der Burgt, P. Van Kerrebroeck, L. Van Maldergem, N. Van Regemorter, J. Vigneron, A.M.C. Vos, M. Wright, A. Zankl.

This work was made possible by grants G.0331.03 from the Research Foundation-Flanders (FWO) and GOA-grant 12051203 from the Ghent University. Geert Mortier is senior clinical investigator at the Research Foundation – Flanders (FWO).

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Journal of Medical Genetics and any other BMJPG products to exploit all subsidiary rights, as set out in our licence

Supplementary information is available at the European Journal of Human Genetics website
(<http://www.nature.com/ejhg/index.html>)

REFERENCES

- 1 Stickler GB, Belau PG, Farrell FJ, *et al.* Hereditary Progressive Arthro-Ophthalmopathy. *Mayo Clin Proc* 1965;**40**:433-55.
- 2 Stickler GB, Hughes W, Houchin P. Clinical features of hereditary progressive arthro-ophthalmopathy (Stickler syndrome): a survey. *Genet Med* 2001;**3**(3):192-6.
- 3 Majava M, Hoornaert KP, Bartholdi D, *et al.* A report on 10 new patients with heterozygous mutations in the COL11A1 gene and a review of genotype-phenotype correlations in type XI collagenopathies. *Am J Med Genet A* 2007;**143**(3):258-64.
- 4 Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. *Journal of medical genetics* 1999;**36**(5):353-9.
- 5 Richards AJ, Laidlaw M, Whittaker J, *et al.* 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**(7):696-704.
- 6 Ahmad NN, Ala-Kokko L, Knowlton RG, *et al.* Stop codon in the procollagen II gene (COL2A1) in a family with the Stickler syndrome (arthro-ophthalmopathy). *Proc Natl Acad Sci U S A* 1991;**88**(15):6624-7.
- 7 Richards AJ, Yates JR, Williams R, *et al.* A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in alpha 1 (XI) collagen. *Human molecular genetics* 1996;**5**(9):1339-43.
- 8 Vikkula M, Mariman EC, Lui VC, *et al.* Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. *Cell* 1995;**80**(3):431-7.
- 9 Van Camp G, Snoeckx RL, Hilgert N, *et al.* A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. *Am J Hum Genet* 2006;**79**(3):449-57.

- 10 Spranger J, Winterpacht A, Zabel B. The type II collagenopathies: a spectrum of chondrodysplasias. *Eur J Pediatr* 1994;**153**(2):56-65.
- 11 Zankl A, Neumann L, Ignatius J, *et al.* Dominant negative mutations in the C-propeptide of COL2A1 cause platyspondylic lethal skeletal dysplasia, torrance type, and define a novel subfamily within the type 2 collagenopathies. *American journal of medical genetics* 2005;**133**(1):61-7.
- 12 Ganguly A, Rock MJ, Prockop DJ. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proceedings of the National Academy of Sciences of the United States of America* 1993;**90**(21):10325-9.
- 13 Orita M, Iwahana H, Kanazawa H, *et al.* Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceedings of the National Academy of Sciences of the United States of America* 1989;**86**(8):2766-70.
- 14 Xiao W, Oefner PJ. Denaturing high-performance liquid chromatography: A review. *Hum Mutat* 2001;**17**(6):439-74.
- 15 Schouten JP, McElgunn CJ, Waaijer R, *et al.* Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic acids research* 2002;**30**(12):e57.
- 16 Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;**15**(4):361-87.
- 17 Laupacis A, Sekar N, Stiell IG. Clinical prediction rules. A review and suggested modifications of methodological standards. *JAMA* 1997;**277**(6):488-94.

- 18 Spiegelhalter DJ. Probabilistic prediction in patient management and clinical trials. *Stat Med* 1986;**5**(5):421-33.
- 19 Van Der Hout AH, Verlind E, Beemer FA, *et al.* 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**(3):236.
- 20 Hoornaert KP, Dewinter C, Vereecke I, *et al.* The phenotypic spectrum in patients with arginine to cysteine mutations in the COL2A1 gene. *J Med Genet* 2006;**43**(5):406-13.
- 21 Donoso LA, Edwards AO, Frost AT, *et al.* Clinical variability of Stickler syndrome: role of exon 2 of the collagen COL2A1 gene. *Surv Ophthalmol* 2003;**48**(2):191-203.
- 22 McAlinden A, Majava M, Bishop PN, *et al.* Missense and nonsense mutations in the alternatively-spliced exon 2 of COL2A1 cause the ocular variant of Stickler syndrome. *Hum Mutat* 2008;**29**(1):83-90.
- 23 Richards AJ, Martin S, Yates JR, *et al.* COL2A1 exon 2 mutations: relevance to the Stickler and Wagner syndromes. *Br J Ophthalmol* 2000;**84**(4):364-71.
- 24 Kielty C, Grant M. The collagen family: Structure, Assembly, and Organization in the Extracellular Matrix. In: Royce PM, Steinmann B, eds. *Connective Tissue and its Heritable Disorders*: Wiley Liss Inc 2002:159-222.
- 25 Schell T, Kulozik AE, Hentze MW. Integration of splicing, transport and translation to achieve mRNA quality control by the nonsense-mediated decay pathway. *Genome Biol* 2002;**3**(3):REVIEWS1006.
- 26 Richards AJ, Laidlaw M, Meredith SP, *et al.* Missense and silent mutations in COL2A1 result in Stickler syndrome but via different molecular mechanisms. *Human mutation* 2007;**28**(6):639.

- 27 Takahara K, Schwarze U, Imamura Y, *et al.* Order of intron removal influences multiple splice outcomes, including a two-exon skip, in a COL5A1 acceptor-site mutation that results in abnormal pro-alpha1(V) N-propeptides and Ehlers-Danlos syndrome type I. *American journal of human genetics* 2002;**71**(3):451-65.
- 28 Schwarze U, Starman BJ, Byers PH. Redefinition of exon 7 in the COL1A1 gene of type I collagen by an intron 8 splice-donor-site mutation in a form of osteogenesis imperfecta: influence of intron splice order on outcome of splice-site mutation. *Am J Hum Genet* 1999;**65**(2):336-44.
- 29 Wilkin DJ, Bogaert R, Lachman RS, *et al.* A single amino acid substitution (G103D) in the type II collagen triple helix produces Kniest dysplasia. *Hum Mol Genet* 1994;**3**(11):1999-2003.
- 30 Tiller GE, Polumbo PA, Weis MA, *et al.* Dominant mutations in the type II collagen gene, COL2A1, produce spondyloepimetaphyseal dysplasia, Strudwick type. *Nat Genet* 1995;**11**(1):87-9.
- 31 Richards AJ, Baguley DM, Yates JR, *et al.* Variation in the vitreous phenotype of Stickler syndrome can be caused by different amino acid substitutions in the X position of the type II collagen Gly-X-Y triple helix. *American journal of human genetics* 2000;**67**(5):1083-94.
- 32 Zechi-Ceide RM, Jesus Oliveira NA, Guion-Almeida ML, *et al.* Clinical evaluation and COL2A1 gene analysis in 21 Brazilian families with Stickler syndrome: Identification of novel mutations, further genotype/phenotype correlation, and its implications for the diagnosis. *Eur J Med Genet* 2008.

Table 1: Summary of 77 different *COL2A1* mutations identified in a series of 100 affected individuals

Patient ID	Age	Score	Exon/Intron	cDNA	Protein	Mutation type	Mutation effect
1	46	15		del COL2A1	del COL2A1	large deletion	deletion[19]
2	54	13	02	c.211_233dup	p.Glu79ThrfsX2	duplication	frameshift
3	58	17	02	c.264_276del	p.Cys89SerfsX24	deletion	frameshift
4	4	6	IVS 04	c.342+1G>A	p.Asp114_Ile115insIleSerAlaAsnTyr-SerHisProValLeuGlnLeuLeuX14	RNA processing	insertion with premature stop codon
5	42	17	IVS 06	c.430-1G>C	p.Gly144ValfsX54; p.Gln125_Gly126insArgGluGlyGlu-AsnLeuPheLeuArgProPheLeuAlaAla-GlnValThrAspLeuX20; p.Lys143_Asn178delExon7**	RNA processing	frameshift; insertion with premature stop codon; exon deletion
6	6	6	07	c.492delT	p.Gly165ValfsX34	deletion	frameshift
7	3	6	09	c.625C>T	p.Arg209X	nonsense	premature stop codon
8	6	11	09	c.625C>T	p.Arg209X	nonsense	premature stop codon
9	19	13	09	c.625C>T	p.Arg209X	nonsense	premature stop codon
10	12	14	09	c.625C>T	p.Arg209X	nonsense	premature stop codon
11	22	15	09	c.647G>A	p.Gly216Asp	missense	glycine substitution
12	8	8	IVS 09	c.654+1G>A	ND	RNA processing	ND
13	34	10	10	c.655G>C	p.Gly219Arg	missense	glycine substitution
14	27	14	10	c.665G>T	p.Gly222Val	missense	glycine substitution
15	24	11	11	c.744delT	p.Gly249GluX59	deletion	frameshift
16	45	18	12	c.793delG	p.Glu265fsX43	deletion	frameshift
17	37	14	IVS 13	c.870+5 G> A	ND	RNA processing	ND
18	30	9	IVS 14	c.925-1G>A	p.Lys308_Gly309insGluPheAlaGly-GlyGlnGluTrpGlyProArgHisX13	RNA processing	insertion with premature stop codon
19	67	12	17	c.1030C>T	p.Arg344X	nonsense	premature stop codon
20	9	9	17	c.1030C>T	p.Arg344X	nonsense	premature stop codon
21	62	21	IVS 18	c.1123-1G>A	p.Gly375ValfsX253	RNA processing	frameshift
22	6	7	19	c.1172delC	p.Pro391LeufsX238	deletion	frameshift
23	11	11	IVS 19	c.1221+1G>A	ND	RNA processing	ND
24	43	12	21	c.1311_1313delinsCA	p.Gly438ThrfsX191	deletion/insertion	frameshift
25	33	12	23	c.1428_1429insTGGC	p.Gly477TrpfsX12	insertion	frameshift
26	13	8	23	c.1475G>A	p.Gly492Asp	missense	glycine substitution
27	40	13	25	c.1597C>T	p.Arg533X	nonsense	premature stop codon
28	10	10	25	c.1597C>T	p.Arg533X	nonsense	premature stop codon
29	12	15	IVS 25	c.1680+2delGTinsAA	ND	RNA processing	ND
30	24	10	26	c.1693C>T	p.Arg565Cys	missense	arginine-to-cysteine substitution[20]
31	20	8	26	c.1693C>T	p.Arg565Cys	missense	arginine-to-cysteine substitution[20]
32	9	7	26	c.1693C>T	p.Arg565Cys	missense	arginine-to-cysteine substitution[20]
33	11	19	27	c.1777C>T	p.Gln593X	nonsense	premature stop codon
34	14	14	27	c.1828delG	p.Ala610ProfsX19	deletion	frameshift
35	11	10	IVS 27	c.1833+1G>A	ND	RNA processing	ND

Patient ID	Age	Score	Exon/Intron	cDNA	Protein	Mutation type	Mutation effect
36	36	12	IVS 27	<i>c.1833+1G>A</i>	<i>p.Gly609GlyfsX1</i>	<i>RNA processing</i>	<i>frameshift</i>
37	40	14	IVS 27	<i>c.1833+1G>A</i>	ND	<i>RNA processing</i>	ND
38	17	17	IVS 27	<i>c.1833+1 G>A</i>	<i>p.Gly609GlyfsX1</i>	<i>RNA processing</i>	<i>frameshift</i>
39	14	10	IVS 28	<i>c.1888-2A>G</i>	<i>p.Gly630MetfsX53</i>	<i>RNA processing</i>	<i>frameshift</i>
40	13	6	29	<i>c.1931delC</i>	<i>p.Pro644LeufsX144</i>	deletion	<i>frameshift</i>
41	40	19	30	<i>c.1957C>T</i>	<i>p.Arg653X</i>	<i>nonsense</i>	<i>premature stop codon</i>
42	41	12	30	<i>c.1957C>T</i>	<i>p.Arg653X</i>	<i>nonsense</i>	<i>premature stop codon</i>
43	4	6	IVS 32	<i>c.2094+1G>A</i>	ND	<i>RNA processing</i>	ND
44	35	9	IVS 32	<i>c.2095-1G>A</i>	ND	<i>RNA processing</i>	ND
45	40	8	33	<i>c.2101C>T</i>	<i>p.Arg701X</i>	<i>nonsense</i>	<i>premature stop codon</i>
46	31	10	33	<i>c.2101C>T</i>	<i>p.Arg701X</i>	<i>nonsense</i>	<i>premature stop codon</i>
47	8	11	IVS 33	<i>c.2193+2T>C</i>	ND	<i>RNA processing</i>	ND
48	43	14	34	<i>c.2257_2264delGGCGAGAG</i>	<i>p.Glu754SerfsX13</i>	deletion	<i>frameshift</i>
49	5	5	34	<i>c.2263_2264delAG</i>	<i>p.Arg755GlyfsX14</i>	deletion	<i>frameshift</i>
50	9	11	35	<i>c.2353C>T</i>	<i>p.Arg785X</i>	<i>nonsense</i>	<i>premature stop codon</i>
51	14	10	35	<i>c.2353C>T</i>	<i>p.Arg785X</i>	<i>nonsense</i>	<i>premature stop codon</i>
52	37	7	35	<i>c.2353C>T</i>	<i>p.Arg785X</i>	<i>nonsense</i>	<i>premature stop codon</i>
53	33	8	IVS 35	<i>c.2355+5G>A</i>	ND	<i>RNA processing</i>	ND
54	38	13	IVS 35	<i>c.2355+5G>A</i>	<i>p.Arg785_Gly786insValAsnGluCys-GlyLeuLeuAspCysTrpAlaPheGlySerX15</i>	<i>RNA processing</i>	<i>insertion with premature stop codon</i>
55	11	11	36	<i>c.2381dupC</i>	<i>p.Gly795TrpfsX6</i>	duplication	<i>frameshift</i>
56	5	10	36	<i>c.2382delT</i>	<i>p.Gly795Alafs86</i>	deletion	<i>frameshift</i>
57	41	12	36	<i>c.2382delT</i>	<i>p.Gly795Alafs86</i>	deletion	<i>frameshift</i>
58	14	9	38	<i>c.2467G>T</i>	<i>p.Glu823X</i>	<i>nonsense</i>	<i>premature stop codon</i>
59	44	9	38	<i>c.2493dupA</i>	<i>p.Pro832ThrfsX11</i>	duplication	<i>frameshift</i>
60	66	13	IVS 38	<i>c.2517+2T>G</i>	ND	<i>RNA processing</i>	ND
61	24	15	IVS 38	<i>c.2518-1 G>A</i>	<i>p.Gly840ValfsX41</i>	<i>RNA processing</i>	<i>frameshift</i>
62	41	17	39	<i>c.2588-2604delCTGG TCCTCAGGGCCCC</i>	<i>p.Pro863LeufsX16</i>	deletion	<i>frameshift</i>
63	39	17	40	<i>c.2659C>T</i>	<i>p.Arg887X</i>	<i>nonsense</i>	<i>premature stop codon</i>
64	12	12	40	<i>c.2673dupC</i>	<i>p.Ala895SerfsX49</i>	duplication	<i>frameshift</i>
65	33	9	40	<i>c.2673delC</i>	<i>p.Pro893ArgfsX135</i>	deletion	<i>frameshift</i>
66	9	14	41	<i>c.2710C>T</i>	<i>p.Arg904Cys</i>	<i>missense</i>	<i>arginine-to-cysteine substitution[20]</i>
67	18 (8 at exam)	0	41	<i>c.2710C>T</i>	<i>p.Arg904Cys</i>	<i>missense</i>	<i>arginine-to-cysteine substitution[20]</i>
68	70	9	41	<i>c.2715dupT</i>	<i>p.Gly906TrpfsX38</i>	duplication	<i>frameshift</i>
69	40	17	41	<i>c.2719dupC</i>	<i>p.Gly909ArgfsX35</i>	duplication	<i>frameshift</i>
70	44	9	42	<i>c.2813delC</i>	<i>p.Pro938LeufsX90</i>	deletion	<i>frameshift</i>
71	58	9	42	<i>c.2839C>T</i>	<i>p.Gln947X</i>	<i>nonsense</i>	<i>premature stop codon</i>
72	12	10	42	<i>c.2862C>T</i>	<i>p.Gly954Gly*</i>	synonymous	<i>frameshift</i>
73	11	8	IVS 43	<i>c.3003+1G>A</i>	ND	<i>RNA processing</i>	ND
74	20	13	IVS 43	<i>c.3003+5G>A</i>	<i>p.Gly966_Ser1001del;</i> <i>p.Gly990GlyfsX1**</i>	<i>RNA processing</i>	<i>deletion;</i> <i>frameshift</i>

Patient ID	Age	Score	Exon/Intron	cDNA	Protein	Mutation type	Mutation effect
75	32	16	44	c.3081_3087delGACGGT insCCTGG	p.Thr1028LeufsX100	deletion/insertion	frameshift
76	18	17	44	<i>c.3106C>T</i>	<i>p.Arg1036X</i>	<i>nonsense</i>	<i>premature stop codon</i>
77	39	14	44	<i>c.3106C>T</i>	<i>p.Arg1036X</i>	<i>nonsense</i>	<i>premature stop codon</i>
78	10	11	44	<i>c.3106C>T</i>	<i>p.Arg1036X</i>	<i>nonsense</i>	<i>premature stop codon</i>
79	47	13	44	<i>c.3106C>T</i>	<i>p.Arg1036X</i>	<i>nonsense</i>	<i>premature stop codon</i>
80	45	10	44	<i>c.3106C>T</i>	<i>p.Arg1036X</i>	<i>nonsense</i>	<i>premature stop codon</i>
81	49	13	IVS 44	c.3111+1G>T	p.Glu1033LysfsX4	RNA processing	frameshift
82	8	11	IVS 44	c.3112-1G>A	p.Gly1038GlufsX92	RNA processing	frameshift
83	29	12	45	c.3137delC	p.Pro1046LeufsX84	deletion	frameshift
84	8	12	45	c.3137dupC	p.Gly1047TrpfsX11	duplication	frameshift
85	17	13	45	c.3138delT	p.Gly1047AlafsX83	deletion	frameshift
86	20	12	46	c.3228delT	p.Gly1077AlafsX53	deletion	frameshift
87	35	18	46	c.3258_3261delAGAC	p.Asp1087GlufsX42	deletion	frameshift
88	42	9	47	c.3325delC	p.Gln1109ArgfsX21	deletion	frameshift
89	18	11	48	c.3392G>C	p.Gly1131Ala	missense	glycine substitution
90	8	11	50	<i>c.3574C>T</i>	<i>p.Arg1192X</i>	<i>nonsense</i>	<i>premature stop codon</i>
91	47	16	50	<i>c.3574C>T</i>	<i>p.Arg1192X</i>	<i>nonsense</i>	<i>premature stop codon</i>
92	40	18	50	<i>c.3574C>T</i>	<i>p.Arg1192X</i>	<i>nonsense</i>	<i>premature stop codon</i>
93	42	11	51	c.3623delC	p.Pro1208LeufsX19	deletion	frameshift
94	33	12	51	c.3641dupC	p.Gly1215TrpfsX38	duplication	frameshift
95	41	10	51	c.3864-3865delCT	p.Cys1289ProfsX3	deletion	frameshift
96	11	10	51	c.3878G>A	p.Trp1293X	nonsense	premature stop codon
97	55	12	52	c.3891_3898dupCTACTGGA	p.Ile1300ThrfsX15	duplication	frameshift
98	53	12	52	c.3903delC	p.Asn1303ThrfsX9	deletion	frameshift
99	52	17	IVS 52	c.4074+1 G>T	p.Gln1238_Leu1411del; p.Trp1348CysfsX17**	RNA processing	deletion; frameshift
100	8	10	IVS 53	c.4317+2T>C	ND	RNA processing	ND
indication N-propeptide (p.26-181) - triple helical domain (p.201-1214) - C-propeptide (p.1242-1487)							
IVS: intervening sequence							
ND: not determined							
Items in italic are recurrent mutations							
Exons are numbered 1-54							
cDNA mutations are numbered starting from the first base of the							
Amino acid mutations were numbered from the first							
* Synonymous mutation: see Supplementary Figure 2							
** Splice site mutations with multiple isoforms: see Supplementary Figure 1							
Score as calculated by the proposed scoring system in Table 2							

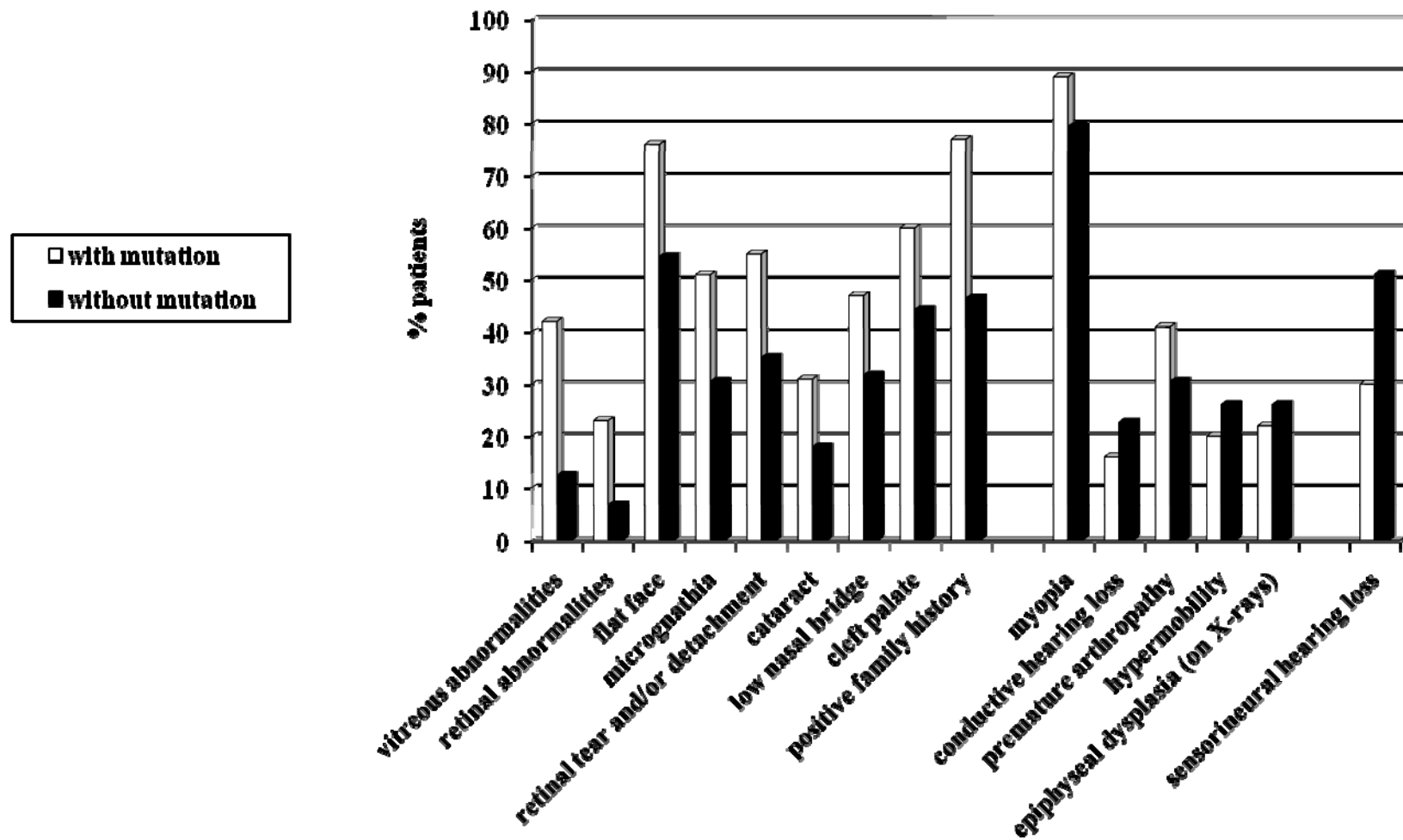


Figure 1 –Frequency of clinical and radiographic characteristics in patients with a COL2A1 mutation (white bars) and patients without a COL2A1 mutation (black bars). From left to right: the first 9 characteristics have a p-value ≤ 0.05 , the following 5 characteristics are not statistically significant, the remaining characteristic (sensorineural hearing loss) shows reverse significance with p-value < 0.005

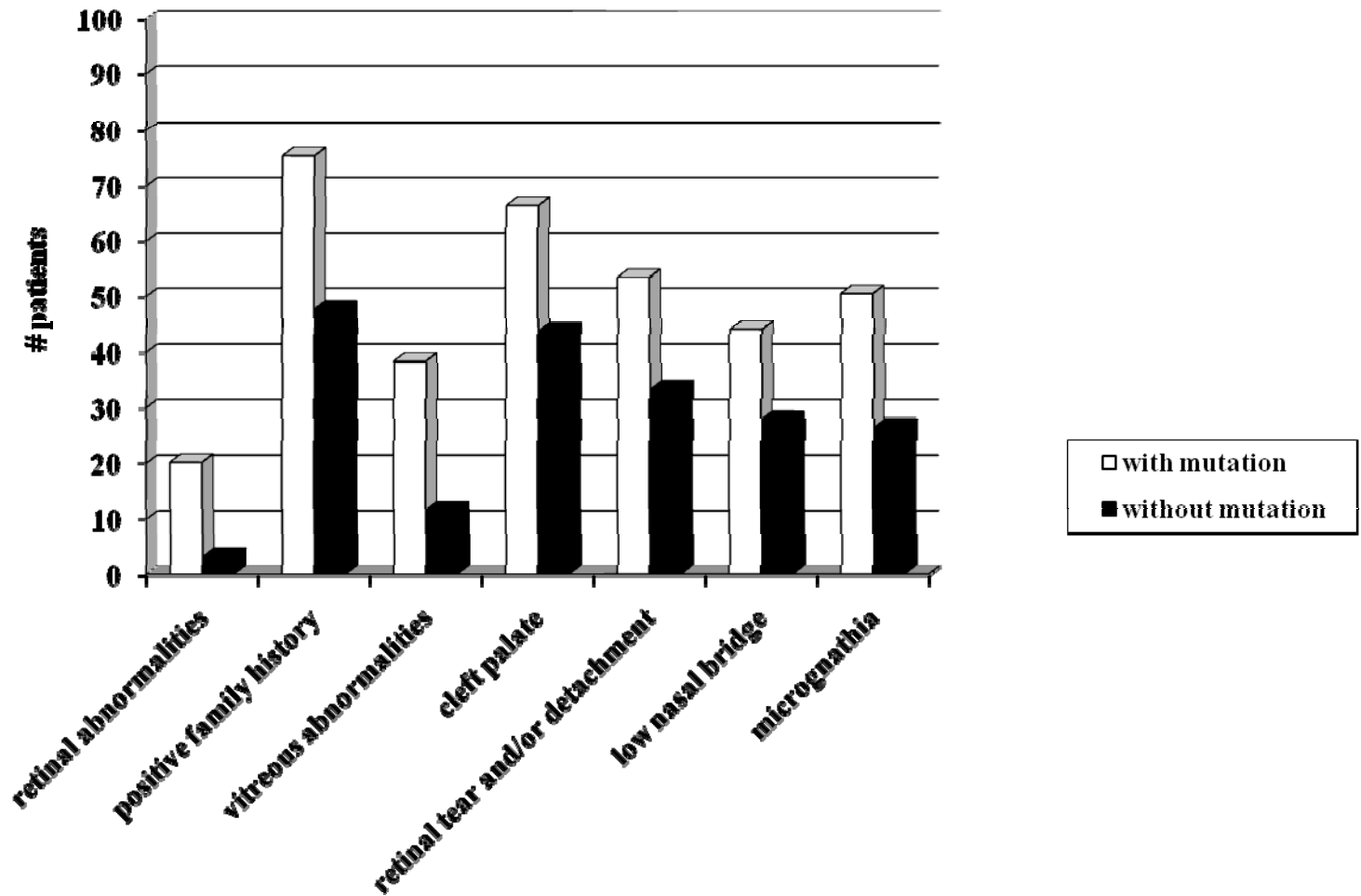


Figure 2 – Frequency of the 7 most distinguishing characteristics in both the mutation negative and mutation positive group

Table 2: Proposed scoring system

<u>Characteristics</u>	<u>Score</u>
- retinal abnormalities	5
- positive family history	5
- vitreous abnormalities	4
- cleft palate	4
- retinal tear and/or detachment	3
- low nasal bridge	1
- micrognathia	1
Total score	23

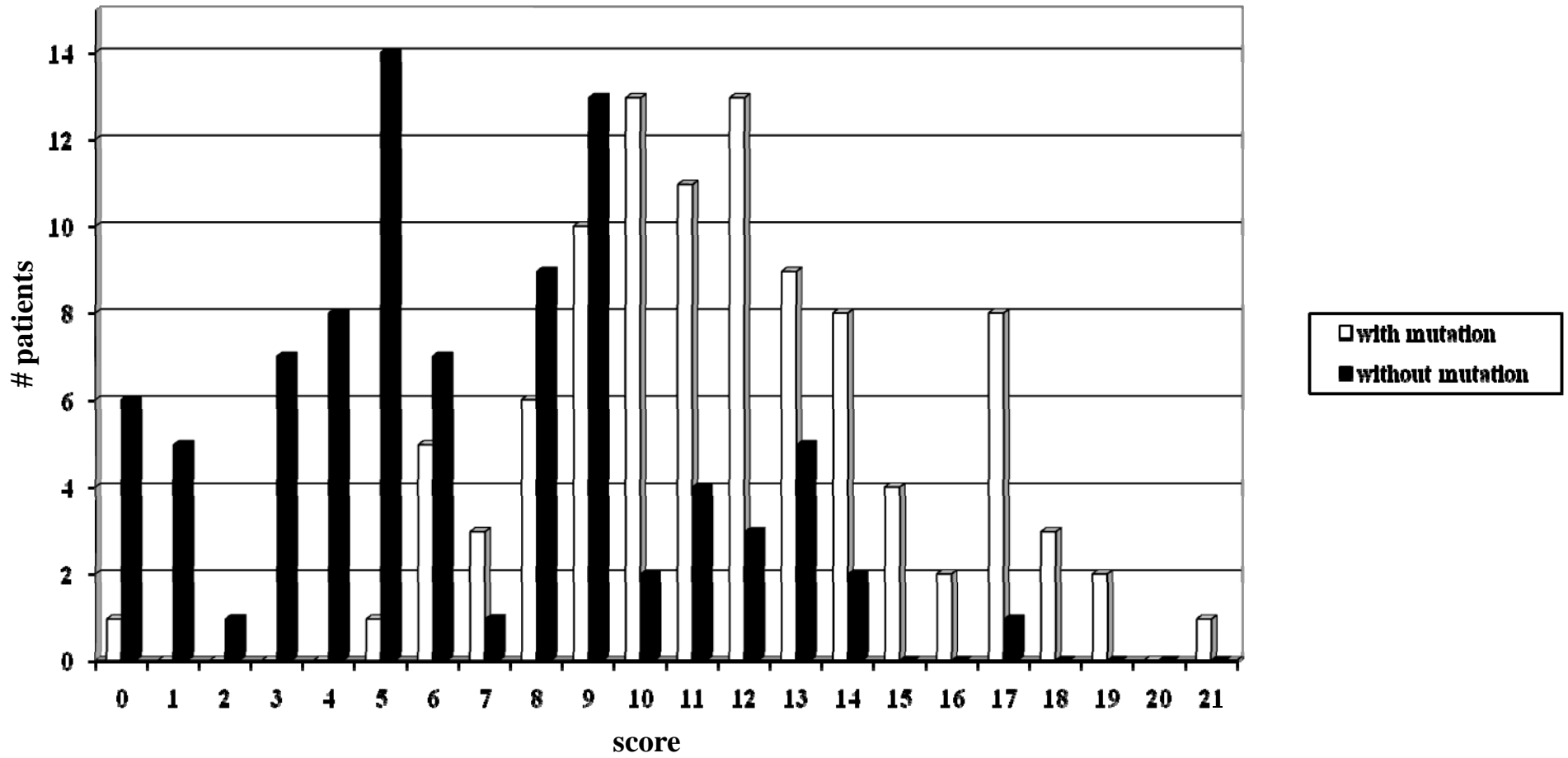


Figure 3 – Overlap in total score between mutation positive and mutation negative group of patients.

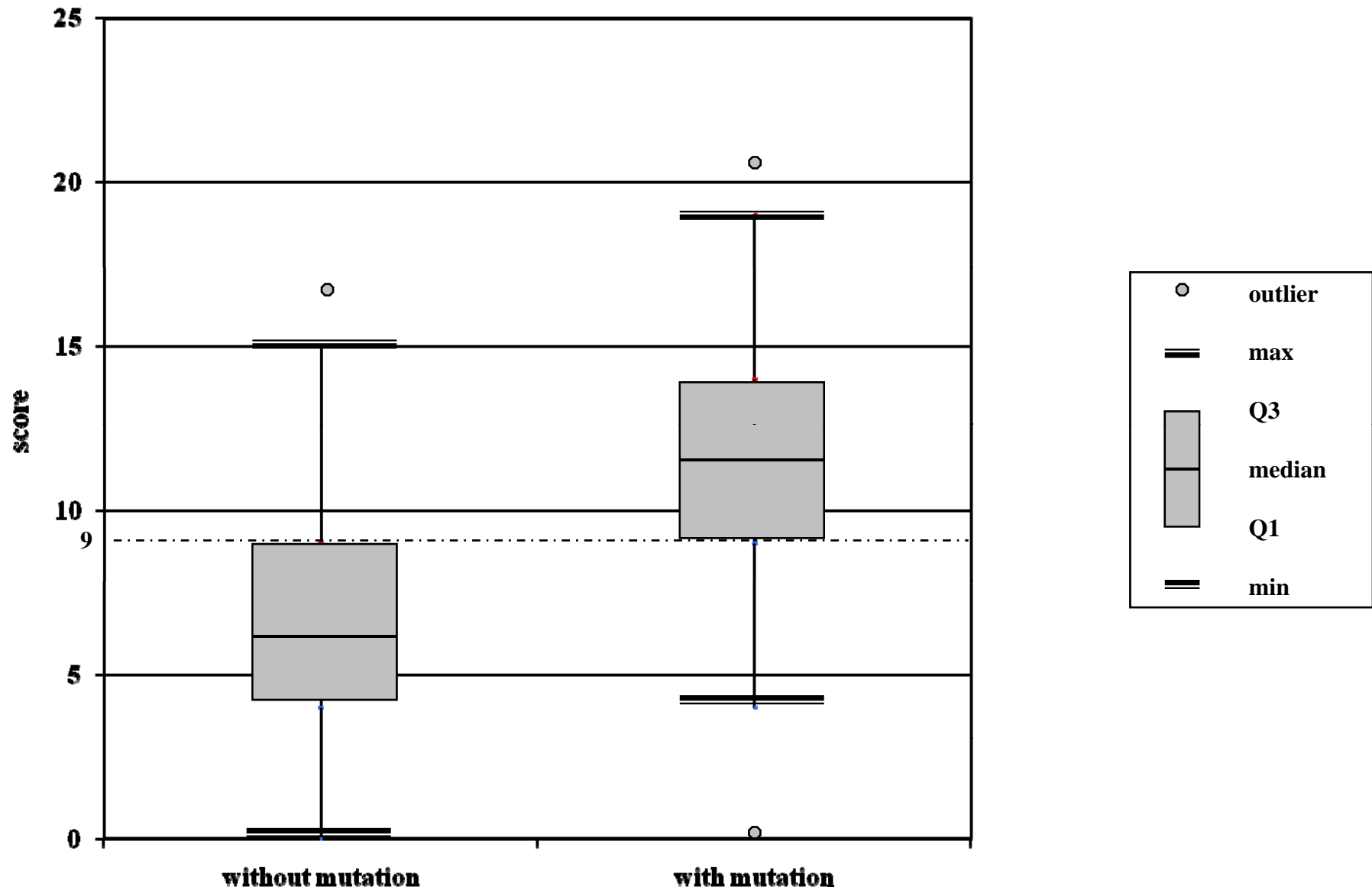


Figure 4 – Box plot presentation of the total scores in both patient groups with Q1 representing the first quartile or 25th centile and Q3 representing the third quartile or 75th centile. Max indicates the maximum score, and min the minimum score, that is not an outlier or that is within 1.5 times the interquartile range (Q1-Q3). 75% of the patients with a *COL2A1* mutation had a total score ≥ 9