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1 **Autosomal recessive osteogenesis imperfecta caused by a novel homozygous *COL1A2***
2 **mutation**

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23

24 **Running title:** Homozygous *COL1A2* mutations in OI

25

26 Supplemental data are included in the submission.

27 **ABSTRACT**

28 Osteogenesis imperfecta (OI) is a skeletal dysplasia characterized by brittle bones and
29 extra-skeletal manifestations. The disease phenotype varies greatly. Most commonly, OI
30 arises from monoallelic mutations in one of the two genes encoding type I collagen,
31 *COL1A1* and *COL1A2* and is inherited as an autosomal dominant trait.

32 Here we describe a consanguineous family with autosomal recessive OI caused by a novel
33 homozygous glycine substitution in *COL1A2*, NM_000089.3: c.604G>A, p.(Gly202Ser),
34 detected by whole-genome sequencing. The index patient is a 31-year-old Greek woman
35 with severe skeletal fragility. She had mild short stature, low bone mineral density of the
36 lumbar spine and blue sclerae. She had sustained multiple long bone and vertebral
37 fractures since childhood and had been treated with bisphosphonates for several years. She
38 also had an affected sister with similar clinical manifestations. Interestingly, the parents and
39 one sister, all carriers of the *COL1A2* glycine mutation, did not have manifestations of OI.

40 In summary, we report on autosomal recessive OI caused by a homozygous glycine to
41 serine substitution in *COL1A2*, leading to severe skeletal fragility. The mutation carriers
42 lacked OI manifestations. This family further expands the complex genetic spectrum of OI
43 and underscores the importance of genetic evaluation for correct genetic counselling.

44

45 **Key words:** autosomal recessive; type I collagen; osteogenesis imperfecta; BMD; fracture

1. INTRODUCTION

Osteogenesis imperfecta (OI) is a skeletal dysplasia characterized by bone fragility and low bone mineral density (BMD). In 9 out of 10 cases, OI is caused by dominantly inherited heterozygous mutations in one of the two genes encoding the $\alpha 1$ and $\alpha 2$ chains of type I collagen (*COL1A1* and *COL1A2*, respectively). The remaining 10% of cases are due to autosomal recessive and X-linked mutations in one of the several other recently identified OI genes that are involved in post-translational modification of type I collagen, in osteoblast function, or in other aspects of bone metabolism [1, 2].

Biallelic mutations in *COL1A2* have been described in a few consanguineous families, in which OI is inherited as an autosomal recessive trait [3-5]. Depending on the location of the mutation, homozygous mutations in *COL1A2* can also give rise to Ehlers-Danlos syndrome [6-8].

Here we report on a novel biallelic mutation in *COL1A2* causing moderately severe OI in two Greek women born to consanguineous parents.

2. SUBJECTS AND METHODS

2.1 Subjects

As part of an ongoing research project on genetic causes of inherited skeletal diseases we recruited a Greek-Cypriot family with skeletal fragility. The index patient had traits suspicious of OI and her consanguineous parents (2nd cousins) were healthy. The patient had three sisters of whom one also had a history of multiple fractures.

2.2 Methods

Clinical data were collected from hospital records. In order to investigate the genetic cause of the skeletal disease in our index patient we performed whole-genome sequencing (WGS)

70 on all family members (index patient, her parents and her three sisters). Detailed
71 information about this method can be found in Supplemental Appendix S1.

72 Variant validation was performed using Sanger sequencing.

73

74 **3. RESULTS**

75 **3.1 Clinical report**

76 A Greek-Cypriot woman, born in 1986, was first evaluated for potential OI when she was 27
77 years old. She was born to healthy consanguineous parents (2nd cousins) at term and had
78 no antenatal or perinatal history of note. Apart from her mild short stature, she had blue
79 sclerae, while dentition was normal (Table 1).

80 At age 8-11 yrs, she had suffered multiple low-energy fractures (bilateral Colle's, forearm,
81 clavicle, ankle, pubic bone and at least three vertebral fractures). Thereafter, she had no
82 further skeletal complications until age 21 yrs, when she sustained a new radius fracture.

83 After bisphosphonate treatment (oral risedronate for 1 year and thereafter 3 yearly courses
84 of 5 mg intravenous zoledronic acid), at age 25 yrs bone mineral density (BMD) at the
85 lumbar spine (LS) was compatible with osteoporosis (Table 1). Two years later, during a
86 respiratory tract infection, she suffered multiple rib fractures and a comminuted right
87 acetabular fracture after a fall from standing height (Fig. 1). She underwent internal
88 fixation, complicated by pulmonary embolism. At that time, she received one dose (60 mg)
89 of denosumab s.c. Three months later, at 28 yrs, she suffered a fracture of the left femoral
90 neck. At this time some spinal changes were also detected and confirmed recently by
91 vertebral fracture assessment (VFA) (Fig.1). Due to this stormy clinical course, nine months
92 later, treatment with zoledronic acid was resumed and continued until the age of 29 yrs. At

93 most recent evaluation at 31 yrs, she had not experienced any further fractures and BMD
94 measurements were within normal limits (Table 1).

95 The patient's family history was suggestive of a recessive form of primary osteoporosis. Her
96 parents, who were 2nd cousins, were free of skeletal fragility. The mother had osteopenia on
97 the DXA scan but her fracture history was negative. The father's BMD was normal. The
98 index patient had three older sisters, two free from skeletal manifestations and one
99 affected by excessive bone fragility and OI features, including blue sclerae and
100 dentinogenesis imperfecta (Fig. 2A; Table 1). This affected sister had sustained a tibial
101 fracture and multiple rib fractures between 11 and 12 yrs of age and a scaphoid fracture of
102 the wrist at the age of 15 yrs. She also had three healthy children.

103 **3.2 Genetic investigations**

104 WGS in the six family members identified two autosomal recessive variants with potentially
105 damaging effect: 1) a novel single nucleotide variant in exon 13 of *COL1A2*, NM_000089.3:
106 c.604G>A, p.(Gly202Ser) (Fig. 2B) and 2) a 4-nucleotides intronic indel, TAAA, in *RP11-*
107 *682N22.1*, which encodes a long intergenic non-coding RNA (lincRNA). Both variants were
108 identified in homozygosity in the index patient and her affected sister and in heterozygosity
109 in the parents and one healthy sister. Surprisingly, all the mutation carriers were healthy.
110 The other healthy sister was not a mutation carrier.

111 The molecular function of the lincRNA is unknown. However, monoallelic mutations
112 affecting the Gly-Xaa-Yaa repeats in the triple helix of type I collagen are the most common
113 cause of OI. For this reason, we regarded the *COL1A2* variant, inherited with an atypical
114 autosomal recessive pattern of inheritance, as the cause of the disease in our index patient
115 and her affected sister. This variant is also determined as pathogenic according to the
116 ACMG guidelines and was confirmed by Sanger sequencing (Fig. 2C) [9].

117

118 **4. DISCUSSION**

119 In this report we describe a novel autosomal recessive *COL1A2* mutation, c.604G>A,
120 p.(Gly202Ser), identified in a Greek consanguineous family. The clinical features of the
121 index patient and her affected sister were compatible with moderately severe OI whereas
122 the mutation carriers lacked OI manifestations.

123 While biallelic mutations of the $\alpha 1(I)$ chain are probably lethal, homozygous mutations of
124 the $\alpha 2(I)$ chain have been described in a handful of families [3-5, 10-12]. Similar to the case
125 reported by De Paepe et al, our index patient harbours a homozygous mutation leading to
126 glycine to serine substitution in the $\alpha 2(I)$ chain [4]. She presents with several OI hallmarks
127 including mild short stature, blue sclerae, low BMD and recurrent fractures since childhood.
128 Interestingly, her fracture pattern is unusual, since it includes pelvis and acetabular
129 fractures, which are uncommon in OI. Similarly, the affected sister had sustained multiple
130 rib fractures and a scaphoid fracture of the wrist, both of which are not typical OI fractures.
131 Although there is no evidence of a similar fracture pattern in other patients with biallelic
132 *COL1A2* mutations, this finding may reflect atypical bone brittleness due to a complete lack
133 of normal type I collagen. Considering that type I collagen is the most abundant component
134 in bone and cartilage, it is likely that complete depletion of the wild-type protein causes a
135 severe skeletal phenotype with a fracture pattern that differs from the common autosomal
136 dominant type I collagen-related OI. On the other hand, despite the autosomal recessive
137 inheritance pattern, these clinical features were in line with type I collagen-related OI
138 rather than the several autosomal recessive forms of OI [13].

139 In contrast to the mutation carriers described by De Paepe et al. who had mild skeletal
140 manifestations, the mutation carriers reported here lack signs and symptoms of skeletal

141 fragility or other OI manifestations [4]. One of them (the mother) has osteopenia but other
142 carriers have normal BMD and all have a negative fracture history. It is surprising that a
143 heterozygous glycine substitution within the triple-helical region does not result in any
144 impaired skeletal phenotype [1, 3, 5, 6]. This suggests that the severity of the OI phenotype
145 depends on location of the glycine substitution within the helical region. In fact, other
146 patients with a glycine to serine substitutions in the $\alpha 2(I)$ chain have been described as
147 having mild to moderate OI [4, 12]. However, the mutation we identified locates closer to
148 the N-terminal $\alpha 2(I)$ chain than the other reported cases. In fact, it affects glycine 202 and
149 mutations in the first 200 N-terminal amino acids only slightly influence the triple helix
150 stability [1, 14]. This could explain why our carriers do not show any OI manifestation.
151 Furthermore, it is possible that a more detailed bone tissue assessment with histology and
152 histomorphometry could have detected mild abnormalities in the heterozygotes, but in any
153 case there are no clinically obvious manifestations of OI.

154 In summary, we diagnosed a challenging case of a patient with autosomal recessive OI due
155 to a novel homozygous *COL1A2* mutation, NM_000089.3:c.604G>A, p.(Gly202Ser).
156 Interestingly, this mutation leads to OI features only in homozygosity. Our finding
157 underscores the complexity of OI and the variability in its genetic causes and inheritance
158 pattern. Further, our study emphasizes the importance of a genetic diagnosis in all patients
159 with OI to ensure correct genetic counselling.

160

161 **ACKNOWLEDGMENTS**

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163 Science for Life Laboratory (SciLifeLab) for supplying bioinformatics support.

164

165 **COMPLIANCE WITH ETHICAL STANDARDS**

166 All authors declare no conflict of interest.

167 The study protocol was approved by the Institutional Ethics Committee and the
168 experiments were performed in accordance to the Declaration of Helsinki.

169 A written informed consent was obtained from each participant before inclusion into the
170 study.

171

172 **CONTRIBUTIONS**

173 Study design: AC, AD, OM. Study conduct: AC, ST, AD. Data collection: AC, ST, AD, OM. Data
174 analysis: AC. Data interpretation: AC, ST, AK, NUA, FT, AD, OM. Drafting manuscript: AC, ST,
175 AD, OM. Revision of manuscript content: all authors. Approval of final version of
176 manuscript: all authors. Responsibility for the integrity of the data: all authors.

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227 **Table 1. Clinical features of the family members; the index patient and her affected sister**
228 **are marked with red text. M= male; F= female; NA= not available; += present; -= absent**
229
230
231

	age (y/o)	sex	height (cm)	weight (kg)	# peripheral fractures	vertebral fractures	BMD lumbar spine	blue sclerae	dentinogenesis imperfecta	bisphosphonates	other clinical manifestations
father (I-1)	68	M	172	100	0	0	-1.0 (T-score)	-	-	-	hypertension
mother (I-2)	59	F	158	90	0	0	-2.1 (T-score)	-	-	-	type 2 diabetes, arterial hypertension
index (II-4)	31	F	154	48	>10	3	-2.2 (Z-score)	+	-	+	-
sibling1 (II-1)	42	F	157	58	0	0	-0.2 (Z-score)	-	-	-	-
sibling 2 (II-2)	41	F	163	98	0	0	1.2 (Z-score)	-	-	-	Graves' disease
sibling 3 (II-3)	38	F	155	80	>4	NA	-1.3 (Z-score)	+	+	-	-

232 **Figure legends**

233

234 **Figure 1**

235 Lateral radiograph of the thoracolumbar spine showing A) A mild biconcave deformity of L1
236 vertebra. The vertebral fractures sustained during childhood are not clearly evident in the
237 X-ray and VFA, probably due to vertebral body reshaping during growth. B) Internal fixation
238 of the pelvis. C) VFA of the same patient; mild biconcave deformity of L1 vertebra. D)
239 Anteroposterior radiograph of the pelvis showing internal fixation of a right acetabular
240 fracture.

241

242 **Figure 2**

243 A) Pedigree of the family. B) Screenshot from the Integrative Genomic Visualization (IGV)
244 program showing the WGS reads mapped to reference genome GRCh37 at the COL1A2
245 locus where the c.604G>A, p.(Gly202Ser) mutation locates. Roman numerals refer to the
246 pedigree in Fig. 2A. C) Sanger sequencing validation. Arrows indicate the mutation.

247

248 **Figures**

249 **Figure 1**

250

251

252 **Figure 2**

253