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

2   **mutation**

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

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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 α1 and α2 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 2[nd]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].

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4. DISCUSSION

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

While biallelic mutations of the α1(I) chain are probably lethal, homozygous mutations of the α2(I) chain have been described in a handful of families [3-5, 10-12]. Similar to the case reported by De Paepe et al, our index patient harbours a homozygous mutation leading to glycine to serine substitution in the α2(I) chain [4]. She presents with several OI hallmarks including mild short stature, blue sclerae, low BMD and recurrent fractures since childhood. Interestingly, her fracture pattern is unusual, since it includes pelvis and acetabular fractures, which are uncommon in OI. Similarly, the affected sister had sustained multiple rib fractures and a scaphoid fracture of the wrist, both of which are not typical OI fractures. Although there is no evidence of a similar fracture pattern in other patients with biallelic *COL1A2* mutations, this finding may reflect atypical bone brittleness due to a complete lack of normal type I collagen. Considering that type I collagen is the most abundant component in bone and cartilage, it is likely that complete depletion of the wild-type protein causes a severe skeletal phenotype with a fracture pattern that differs from the common autosomal dominant type I collagen-related OI. On the other hand, despite the autosomal recessive inheritance pattern, these clinical features were in line with type I collagen-related OI rather than the several autosomal recessive forms of OI [13].

In contrast to the mutation carriers described by De Paepe et al. who had mild skeletal 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 α2(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 α2(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.

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165 **COMPILANCE 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**
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	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
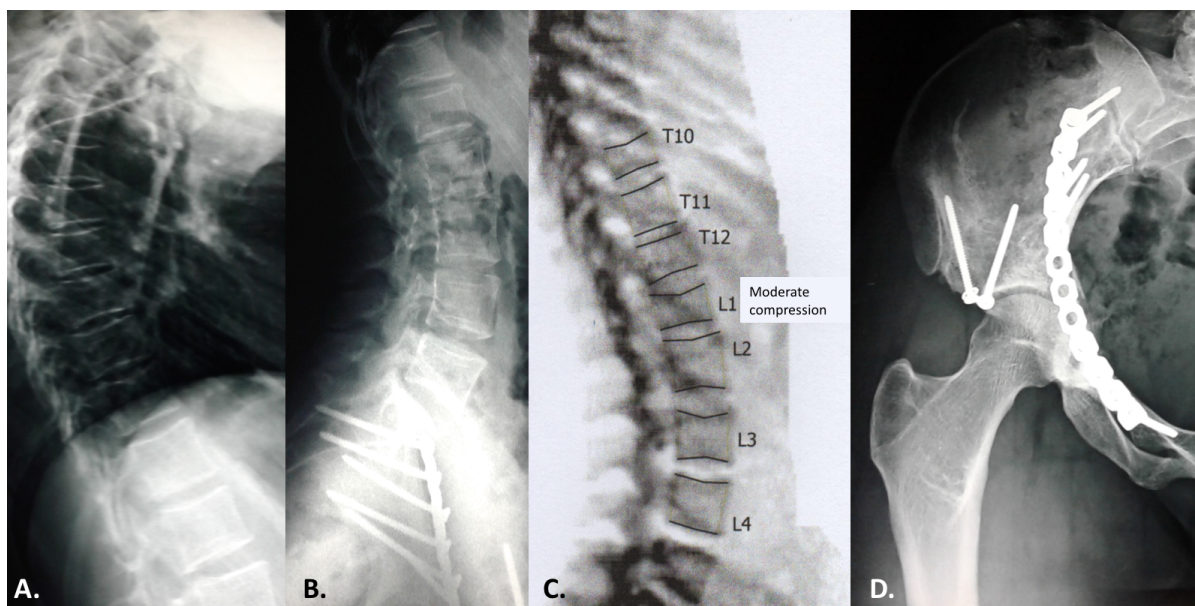
240 fracture.

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

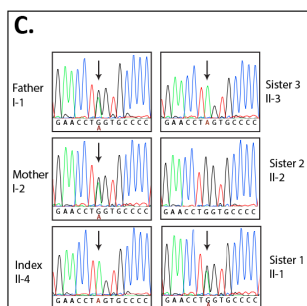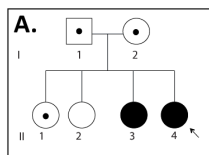246 pedigree in Fig. 2A. C) Sanger sequencing validation. Arrows indicate the mutation.

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Figures

249   **Figure 1**

250

251

252   **Figure 2**

253