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Exome sequencing reveals a novel *COL2A1* mutation implicated in multiple epiphyseal dysplasia

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Abstract

Mutations in the *COMP*, *COL9A1*, *COL9A2*, *COL9A3*, *MATN3* and *SLC26A2* genes cause approximately 70% of multiple epiphyseal dysplasia (MED) cases. The genetic changes involved in the etiology of the remaining cases are still unknown, suggesting that other genes contribute to MED development. Our goal was to identify a mutation causing an autosomal dominant form of MED in a large multigenerational family. Initially, we excluded all genes associated with autosomal dominant MED using microsatellite and SNP markers. Follow-up with whole-exome sequencing analysis revealed a mutation c.2032G>A (p.Gly678Arg) in the *COL2A1* gene (NCBI Reference Sequence: NM_001844.4), which co-segregated with the disease phenotype in this family, manifested by severe hip dysplasia and osteoarthritis. One of the affected family members had a double-layered patella, which is frequently seen in patients with autosomal recessive MED caused by *DTDST* mutations and sporadically in the dominant form of MED caused by *COL9A2* defect.

Keywords

multiple epiphyseal dysplasia; novel mutation in *COL2A1*; double-layered patella

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Conflict of interest

All authors declare that they have no conflict of interest.

Introduction

Irregular ossification of epiphyses and early-onset osteoarthritis (OA) are typical radiological findings in multiple epiphyseal dysplasia (MED), a relatively common chondrodysplasia. Doubled-layered patella, a type of bipartite patella with a coronal septum that divides the patella into anterior and posterior segments, is recognized as a specific radiological feature of the recessive form of MED [Scheffield EG; 1998]; however, it was also seen in a patient with an autosomal dominant form caused by *COL9A2* mutation [Nakashima et al., 2005]. Initial MED symptoms include pain and stiffness in the weight-bearing joints, waddling gait and restriction of joint mobility. Progression of the disease eventually causes severe joint pain in the majority of MED patients, and they usually undergo early joint replacement surgery at the age of 45–55.

The *COL2A1* gene encodes the alpha-1 chain of collagen type II, a homotrimeric protein that is the main constituent of cartilage. Mutations in *COL2A1* cause a spectrum of disorders classified as collagenopathies type II; however, MED is not classified as one of these [Bonafe et al., 2015; Barat-Touitou et al., 2015].

Patients and Methods

Patients and control group

We recruited a large multigenerational Caucasian family with MED phenotype from the Polish population with autosomal dominant form of MED (Fig. 1A) under approval of the Bioethical Committee from the Poznan University of Medical Sciences and IRB approval from Tulane University. Informed consent was obtained from each analyzed family member. The proband (Fig. 2) and most of his family members (Fig. 1A) underwent detailed clinical and radiological examinations. Blood samples were obtained from fourteen family members for genomic DNA isolation and molecular analysis. Additionally, we included in this study data collected from *COL2A1* analysis of 100 unaffected individuals from the same population for comparison. This control group was evaluated clinically and radiologically and includes adults at the age of 25–40 without any features of bone dysplasias.

Exome sequencing and exome data analysis

A TargetSeq Exome enrichment approach on Ion Torrent next-generation sequencing platform was taken to identify genetic changes causing MED in the reported multigenerational family. Trio analysis included individuals: III:3, III:4, and IV:4 (Fig.1A). Exomes were sequenced on the Ion Proton next-generation sequencing system with Ion TargetSeq Exome Kit for the Ion Proton System (ThermoFisher/Life Technologies, Grand Island, New York, US) according to the manufacturer's protocol (MAN0006730). Genomic DNA (1 µg), was purified using Agencourt Ampure XP beads (Beckman Coulter, Brea, California, US), then fragmented enzymatically. Barcoded adapters were ligated and nick repaired using the Ion Xpress Barcode Kit and the Ion TargetSeq Exome kit (ThermoFisher/Life Technologies). Barcoded fragments were size selected for ~285 bp on an E-Gel Size Select agarose gel (ThermoFisher/Invitrogen, Carlsbad, California, US), then amplified in ten PCR cycles. Amplified libraries were analyzed on the Agilent Bioanalyzer hsDNA chip

(Agilent Technologies, Santa Clara, California, US), then exome-enriched and amplified again using the Ion TargetSeq Exome kit (Thermofisher/Life Technologies). The final barcoded exome libraries were analyzed on the Agilent Bioanalyzer hsDNA chip. A template preparation was performed with Ion PI Hi-Q OT2 200 Kit (Thermofisher/Life Technologies) according to the manufacturer's protocol (https://tools.thermofisher.com/content/sfs/manuals/MAN0010857_Ion_PI_HiQ_OT2_200_Kit_UG.pdf). In this step, each library template was clonally amplified on Ion Sphere Particles (ISPs) using the Ion OneTouch system. After amplification, the template-positive ISPs were recovered and enriched with the Ion OneTouch ES system. The enrichment efficiency was estimated with Qubit (Thermofisher/Life Technologies). Sequencing on Ion Proton PI chip was completed with Ion PI Sequencing 200 Kit (Thermofisher/Life Technologies) according to the manufacturer's protocol (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0010948_Ion_PI_HiQ-Sequencing_200_Kit_QR.pdf).

We used Torrent Suite Software to map reads to hg19 and to perform variant calling and coverage analysis of exomes. Analysis of BAM files from exome-sequencing was completed in the trio model with Ion Reporter ver. 4.4 software (Ion Torrent/Life Technologies). This software predicts consequences of sequence changes based on algorithms PolyPhen, PhyloP, and SIFT.

PCR and Sanger sequencing

Analysis of exon 31 in the *COL2A1* gene (NM_001844.4) with detected change via exome sequencing was continued by sequencing of PCR products in the remaining family members (Fig. 1) and in the 100 unaffected individuals from the control group. Primers F2Col (5'-CTG TCA CTG CTG CTG CTT C) and R1Col (5'-GTG TCA TCT GTG GAG GCT G) were designed for PCR amplification, which included an initial denaturation for 10 min at 96°C, 34 cycles at 96°C for 30 s, at 60°C for 30s, and at 72°C for 30 s, and one cycle at 72°C for 10 min. We sequenced PCR products with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermofisher, California, USA) on an ABI 3130xl Genetic Analyzer (Thermofisher, California, USA), after prior treatment with ExoSAP-IT (Affymetrix, Santa Clara, California, USA).

Modeling of mutated COL2A1 and homology with wild type

To understand the structural consequences of the glycine-to-arginine substitution at amino acid position 678 in collagen type II, we used the COL2A1 sequences

(wild-type, ⁶⁶⁶GLPGPPGPPGEGGKPGDQGVP⁶⁸⁶ and the G678R mutant, ⁶⁶⁶GLPGPPGPPGEGRKPGDQGVP⁶⁸⁶) and built their homology models using a rat type I collagen structure (PDB code 3HQV) as a template. The type I collagen structure consists of a heterotrimer of two collagen alpha-1(I) chains encoded by *COL1A1* gene and one collagen alpha-2(I) chain encoded by *COL1A2* gene. Using the best-matched sequence of rat collagen alpha-1(I) chain (⁴⁸²GLPGPAGPPGEAGKPGEQGV⁵⁰²), we built a homotrimer for wild-type human collagen type II encoded by the *COL2A1* gene. Since the rat collagen alpha-1(I) chain contained the C α positions of amino acids, we constructed the rest of the amino acid atom positions for the human wild-type collagen type II sequence using

software COOT [Emsley and Cowtan, 2004]. Then, the homotrimer was subjected to model refinement using software GalaxyRefineComplex [Heo et al., 2016]. Similarly, we built the homotrimer of the COL2A1 G678R mutant by replacing glycine at position 678 with arginine using COOT, followed by the refinement using GalaxyRefineComplex.

Results

Exome sequencing and Sanger sequencing results

Exome sequencing was performed among three direct relatives (proband III:3, his wife III:4 and one of his daughters IV:4, Fig. 1A) within the reported Caucasian family with the autosomal dominant form of MED (Fig. 1A). Exome trio analysis revealed a mutation at cDNA position c.2032G>A in the exon 31 of the *COL2A1* gene in the affected father and his affected daughter (Fig. 1B). This mutation changes the codon for glycine to arginine (GGA to AGA) at the protein level (p.Gly 678Arg) and was not detected in the unaffected mother (Fig. 1B). The following scores were obtained for algorithms predicting severe consequences of glycine to arginine change at position 678: 0.907 for PolyPhen-2 (range 0 – 1), 3.995 for PhyloP (range –14 to +6) and 1 for PhastCons (range 0–1). PolyPhen-2 algorithm estimated the effect of the detected change as possibly damaging and PhyloP and PhastCons indicated that this is a disease-causing substitution of highly conserved glycine. Following the whole-exome sequencing in the selected trio from the multigenerational family with MED, we analyzed additional eleven family members by sequencing of PCR products covering the region of mutation c.2032G>A located in the exon 31 of the *COL2A1* gene. The analysis revealed that detected mutation co-segregates with disease phenotype in all affected individuals and is not present in all unaffected family members (Fig. 1). The control group of 100 individuals was negative for the mutation.

Clinical and radiological findings

Proband from analyzed family (III:3, Fig. 1A and Fig.2) is a 59-year-old man with normal height (174 cm) and normal weight (85 kg). He is heterozygous for c.2032G>A mutation (p.Gly678Arg) in the *COL2A1* gene. He was born as a second child of unrelated Caucasian parents with Polish origin after normal pregnancy and delivery. Proband's mother (II:1, Fig. 1A), who died at the age of 67, and sister (III:2, Fig. 1A) were unaffected. His father (II:2, Fig. 1A) has c.2032G>A mutation and severe hip pain since childhood, which progressed throughout his life. He underwent bilateral hip replacement surgery at the age of 60. Proband father's age of onset and dysplasia features were similar to those of his son. Molecular analysis of proband's unaffected sister (III:2, Fig. 1A) did not reveal the presence of mutation.

Proband (III:3, Fig. 1A and Fig. 2) was developing normally as a child until the age of 13 when he started to have pain in the right hip after long walks. At the age of 17 he had hip pain with slightly restricted motion mobility in hips and was slightly limping on the right lower extremity. At that time, his clinical and radiological evaluation at the orthopedic hospital concluded with a MED diagnosis and, since that time, he underwent several courses of physical therapy. At the age of 19, proband had pain in both hips, which gradually

increased and was reported as severe at the age of 41. Total hip replacement surgery was conducted at 45 and 46 years of age.

Radiographs of proband's skeleton at the age of 17 show that his hips were most severely affected, with visible coxa vara, short femoral necks and flat femoral heads (Fig. 3A). Lumbar spine was slightly abnormal (Fig. 3D); however, cervical spine as well as knees and ankles had normal radiological appearance (Fig. 3B & C). At the age of 40 years, he had mild flattening of the thoracic vertebral bodies (Fig. 3E). Radiographs of hands and elbows obtained at 58 years of age documented features of osteoarthritis, i.e., a narrowing of intra-articular spaces of metacarpophalangeal and interphalangeal joints, flattened condyles of the humeri and osteophytes at the joint margins (Fig. 3F & G). Proband has two daughters, who are 36 (patient 2, IV:4, Fig. 1A) and 37 (IV:3, Fig. 1A) years old, and both inherited from their father an autosomal dominant mutation c.2032G>A in *COL2A1* (p.Gly678Arg). The older daughter (IV:3) was born with cerebral palsy and was only evaluated clinically and radiologically.

Patient 2 is proband's younger daughter (IV:4, Fig. 1A), who inherited mutation c.2032G>A from her father. Her height is normal (158 cm) and her weight is 56 kg. She had no MED symptoms when she was investigated by an orthopedist and geneticist at the age of 22 years. She has two sons, who are 9 and 2 years old and, thus far, do not have joint problems. Since the age of 34 she has had knee pain. A radiological examination of patient 2 at the age of 35 showed a milder degree of coxa vara compared to her father and shortening of the femoral necks with almost normal femoral heads. Analysis of a thoracic spine radiograph revealed scoliosis and signs of osteochondrosis (Fig. 4.).

Patient 3 is proband's uncle (patient 3, II:5, Fig. 1A), who is 69 years old and heterozygous for c.2032G>A (p.Gly678Arg) mutation in the *COL2A1*. He is a brother of proband's father (II:2). They both inherited *COL2A1* from their mother (I:2, Fig. 1A), who was 92 years old at the time of evaluation. Her height was 160 cm and weight was 65 kg, and she was the oldest family member. She had severe hip pain and waddling gait. We also evaluated her daughter (II:6, Fig. 1A), who is unaffected and has no *COL2A1* mutation.

Patient 3's height is 170 cm and his weight is 85 kg. He reported having knee pain and waddling gait since he was 30 years old. At the age of 55 he had severe pain in the hips and knees. Radiological examination of his skeleton at the age of 55 documented slight thoracolumbar platyspondyly and features of osteochondrosis (Fig. 5A). Short femoral necks and flattening of capital femoral epiphyses with superimposed degenerative changes were noted in his hips (Fig. 5B). Radiographs of knees showed mildly flattened tibial epiphyses and double-layered patella (Fig. 5C & D).

Patient 3 has three children: two affected sons and one unaffected daughter (III:5, III:6 and III:7, Fig. 1A). Both sons (III:5 and III:7) inherited the mutation from their father. The mutation was not detected in his unaffected daughter (III:6) or wife (II:4, Fig. 1A).

Patient 4 is the older son of patient 3 (III:5, Fig. 1A) and inherited the *COL2A1* mutation c.2032G>A from his father. He complained at the age of 21 of pain in both hips, especially after physical exercise. Clinical examination of patient 4 revealed limitation of motion and

flexion contractures in both hips. His height at the age of 21 was 170 cm and his weight was 60kg. Radiographs of hips taken at the age of 17 indicate short femoral necks with slight flattening of the femoral heads (Fig. 5E).

Patient 5 is the younger nine-year-old son (III:7, Fig. 1A) of patient 3, who also inherited the c.2032G>A mutation in *COL2A1* from his father, and at this age he started to feel hip pain after exercises. His height is 128 cm (5th centile) and his weight is 26.5 kg (25 centile). Clinical examination of patient 5 did not reveal any abnormalities in hips. He was not evaluated radiologically at that time.

None of the affected family members has hearing or vision impairment except of proband's grandmother, who was 92 years old and developed it late in life due to aging. Radiological findings in all affected family members are summarized in Supplementary Table 1.

Modeling of collagen type II

When the two predicted structures of the mutated and wild type collagen type II were compared, p.Gly678Arg mutation caused conformational changes near the mutation site, resulting in unwinding of the triple helix. This unwinding is due in part to the steric repulsion from the side chain of arginine at position 678, and in part to the disruption of the network of side chain salt bridges and side chain - backbone hydrogen bonds that stabilize the proline-poor region of the protein. In addition, the mutation to arginine introduces three uncompensated (+) charges, creating a region of strongly positive surface potential (Fig. 6).

Discussion

Mutations in *COL2A1* result in a broad spectrum of skeletal dysplasias ranging from mild to lethal forms [Kannu et al., 2010; Nishimura et al., 2005; Terhal et al., 2015]. Nishimura et al. [2005] analyzed *COL2A1* mutations in 56 families (77 patients) with SED spectrum phenotypes; however, none of the previously analyzed patients resembled the phenotype of family reported in this publication. Terhal et al. [2015] analyzed clinical features of 93 patients with *COL2A1* mutations and confirmed SEDC or a related phenotype. Patients reported by Terhal [2015] had mostly affected wrists, spine, hips and knees. Nine patients from this group had a milder phenotype, consistent with premature osteoarthritis caused by mutation in the *COL2A1* p.Arg719Cys or mild SEDC, which resembled MED/Perthes at younger age and was a result of p.Gly744Ser and the p.Gly945Ser substitutions. Contradictory to patients reported by Terhal et. al. [2015] the family that we report shows the phenotype consistent with MED, also at the advanced age, with dominance of premature osteoarthritis in all family members. Interestingly, in another publication of Terhal et al. [2012] focused on growth charts for SEDC and related dysplasias from the *COL2A1* spectrum, the authors reported a patient with unspecified diagnosis and p.Gly684Arg substitution. His height was 158 cm, however; his affected father's height was only 136 cm, which excludes MED diagnosis in this family. Mutated codon 684 is located close to the codon 678 changed by mutation in our patients (p.Gly678Arg) who have consistent MED phenotype and normal height. Unfortunately, no other clinical data of these two patients were available for comparison. Although these two mutations are located near each other and result in an identical amino acid substitution (glycine to arginine), the two groups of

patients had very different heights. This supports previous observations that the effects of *COL2A1* mutations on growth are much more complex than expected from its relative position within the triple-helical domain of collagen type II [Terhal et al., 2012]. Proposed in the past, a severity gradient [Byers P.H., 2001] of glycine substitutions, indicating N-terminal to C-terminal direction, with more severe mutations located close to C-terminus due to more significant interference with collagen folding, does not explain the diversity of phenotypes in the codons located very close to each other. Recent studies indicate the importance of the glycine substitutions in changing stability of the triple helix and interactions of collagen with other proteins of extracellular matrix like fibronectin etc. [Ito et al., 2005; Chhum et al., 2016]. Mutations of *COL2A1* may result in conditions other than skeletal dysplasias [Kannu et al., 2009]. Kannu et al. [2010] presented two families affected by precocious arthritis with no features of either skeletal dysplasia or extra-skeletal manifestation, e.g., hearing or vision impairment. Individuals from one of these families with p.Gly393Ser substitution had normal height, and only their hips were affected.

COL2A1 mutations were also found in patients with a familial form of Legg-Calve-Perthes (MIM150600) disease and avascular necrosis of a femoral head (MIM 608805), [Al-Omran & Sadat-Ali, 2013; Andersen et al., 1988; Griffiths & Witherow, 1977; Li et al., 2014]. Liu et al. [2005] reported three families with avascular necrosis of a femoral head caused by p.Gly717Ser and p.Gly1170Ser substitutions. All of these patients had normal height and spine development with no abnormalities in the ocular and auditory systems. Su et al. [2008] reported p.Gly1170Ser change in *COL2A1* that affected five generations in a Chinese family. Sixteen members of this family were affected, and authors noted three distinct disease phenotypes among them, which were associated with age of onset. Family members with age of onset between 6–14 years were diagnosed with Legg-Calve-Perthes disease, avascular necrosis of femoral head occurred in individuals with age of onset between 15–18 years, and hip osteoarthritis in individuals whose age of onset was 21–34 years. A variety of these phenotypes in one family was explained by progressive closure of the femoral head epiphysis [Su et al., 2008]. Unlike our patients, members of the family reported by Su et al. [2008] did not suffer from OA changes in other joints.

The reported family (Fig. 1A) with point mutation causing p.Gly678Arg substitution (Fig. 1B) has severe hip dysplasia and OA. Significant OA changes were also detected in the spine and to a milder degree in other parts of the skeleton. We had no clinical/radiological evidence in this family of Legg-Calve-Perthes disease, a condition that usually resolves on its own at earlier ages [Kannu et al., 2011]. By contrast, affected individuals with p.Gly678Arg change had severe hip osteoarthritis notable at older age. In conclusion, the phenotype observed in this family resembles multiple epiphyseal dysplasia, which is a heterogeneous disease due to several genes involved in its etiology (*COMP*, *DTDST*, *MATN3*, *COL9A1*, *COL9A2* and *COL9A3*), (Bonafe et al., 2015).

A high number of MED patients without detected mutations indicates the presence of one or more unknown genes associated with this disease [Czarny-Ratajczak et al., 2001; Jakkula et al., 2005; Lachman et al., 2005; Unger et al., 2008]. The MED phenotype of patients with autosomal dominant mutations in *COL9A1* (EDM6, MIM 614135), *COL9A2* (EDM2, MIM 600204) and *COL9A3* (EDM3, MIM 600969) genes is relatively mild and characterized by

normal stature, predominant knee dysplasia and mild osteoarthritis [Mabuchi et al. 2004]. Patients with autosomal dominant *MATN3* mutations (EDM5, MIM 607078) have predominantly affected knees and hips, and their phenotype shows more intermediate severity. The most severe form of MED, whose features include advanced osteoarthritis, mild shortening of stature, and severe dysplasia of hips, is caused by autosomal dominant mutations in the *COMP* (EDM1, MIM 132400) and autosomal recessive mutations in *DTDST* (EDM4, MIM 226900), [Mabuchi et al. 2004, Unger et al., 2008].

Makitie et al. (2004) described 12 patients from seven families with mild MED and detected mutations in the *MATN3* gene encoding matrilin-3. In all of these patients, hips were most severely affected by osteoarthritis, epiphyseal changes were observed in knees and hips, and irregularities of the endplates in the thoracic vertebral bodies were present. Also, valgus deformity was noted in knees of these patients. In our patients, degenerative changes in hips were similar to the above; however, the spine was only mildly affected with OA. This correlates with the MED spectrum where the spine is usually slightly affected with mild vertebral flattening, irregularities of the endplates and occasionally with Schmorl's nodules [Chapman et al., 2003]. Mabuchi et al. [2004] reported four MED patients with *MATN3* mutations; of whom three had mild endplate irregularities and one had mild platyspondyly. They had normal height.

Although knees were only slightly affected in the reported family, double-layered patella was noted in patient 3 (Fig.5D). Double-layered patella has not been observed so far in patients with collagenopathies type II. At the time of radiographic survey, the patella had only slight features of partition possibly due to the fusion of layers that commonly occurs with age (Fig.5D), [Sheffield 1998]. Double-layered patella and hip dysplasia are the most common radiographic findings in a recessive form of MED caused by *DTDST* mutations. On the other hand, this is not an exclusive feature of *DTDST* defect considering that Nakashima et al. [2005] observed it in a 12-year-old boy with an autosomal dominant form of MED caused by mutation in the *COL9A2* gene.

In conclusion, the above evidence suggests that the family presented in this report suffers from a medium severity MED caused by *COL2A1* mutation, with predominant premature osteoarthritis affecting mostly hips and less the spine. The phenotype severity of the reported family seems milder than MED caused by *MATN3*, *COMP*, and *DTDST* mutations, and more severe than in patients with mutations in genes encoding collagen type IX. The reported MED phenotype could be another entity in the spectrum of collagenopathies type II, which includes relatively mild osteoarthritis, Legg-Calve-Perthes disease, Stickler syndrome type I (MIM 108300), different forms of spondyloepiphyseal dysplasia, and severe forms like achondrogenesis type II/hypochondrogenesis (MIM 200610). Identification of double-layered patella in one of the family members reinforces the notion that this MED feature seems to be, not only exclusive to the autosomal recessive form, but is also observed in patients with autosomal dominant forms of MED.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A. Family with autosomal dominant form of multiple epiphyseal dysplasia and early-onset OA. Exome analysis included individuals III:3, III:4, and IV:4. B. Substitution G to A causes (p.Gly678Arg) change and co-segregates with affected phenotype in all eight analyzed family members. This mutation was not detected in six unaffected individuals from this family.

Figure 2. Photographs of 40-year-old proband from the MED family (individual III:3, Fig. 1A) with detected mutation c.2032G>A (p.Gly678Arg) in the *COL2A1* gene (Fig. 1B). His height is 174 cm.

Figure 3.

Proband (III:3, Fig. 1A and Fig. 2) at age 17 years (A-D). A. Coxa vara; very short femoral necks; capital femoral epiphyses are flat. B. Normal appearance of the knees. C. Normal cervical spine D. Slight flattening of the lumbar vertebral bodies with regular endplates. E. Proband at the age of 40 years with mild thoracic platyspondyly and mild progression of degenerative changes. F-G. Proband at the age of 58 years. F. Narrowing of the metacarpophalangeal and interphalangeal joint spaces; the articular interphalangeal surfaces are flat; lucent areas at the distal ends of the proximal phalanges. G. Elbow with flattened condyles of the humeri; osteophytes at the joint margins.

Figure 4.

Patient 2 (IV:4, Fig. 1A) at age 35 years (A-C). A. Normal shoulder joints. B. Coxa vara with short femoral necks; prominent greater trochanters; slight flattening of the left femoral head. C. Knees with prominent femoral condyles. D. Mild thoracic scoliosis; narrowed intervertebral disc spaces; small osteophytes at the vertebral margins.

Figure 5.

Patient 3 (II:5, Fig. 1A) at age 55 years (A-C). A. Mild thoracolumbar platyspondyly; vertebral bodies with osteophytes at the margins; endplate irregularities; deformed L1 vertebral body. B. Right hip with short femoral neck; flat femoral head; osteoarthritis with narrowing of the articular space and osteophytes. C. Mild flattening of proximal tibial epiphysis. D. Lateral knee in 49-year-old patient 3 with double-layered patella. E. Patient 4 (III:5, Fig. 1A) at age of 21 years. Short femoral necks with slight flattening of the femoral heads.

Figure 6.

Modeling studies of COL2A1. The left panel shows a stick representation of the wild-type triple helix structure (carbon, yellow) and the right panel shows that of the G678R mutated structure (carbon, cyan). In the middle panel, an overlay of these two structures reveals differences in conformation near the mutation site (carbon, purple). The zoomed-in boxes show surface potential, more positive for the G678R mutant than wild-type.