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ARTICLE

Mutations in *COL27A1* cause Steel syndrome and suggest a founder mutation effect in the Puerto Rican population

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Osteochondrodysplasias represent a large group of developmental structural disorders that can be caused by mutations in a variety of genes responsible for chondrocyte development, differentiation, mineralization and early ossification. The application of whole-exome sequencing to disorders apparently segregating as Mendelian traits has proven to be an effective approach to disease gene identification for conditions with unknown molecular etiology. We identified a homozygous missense variant p.(Gly697Arg) in *COL27A1*, in a family with Steel syndrome and no consanguinity. Interestingly, the identified variant seems to have arisen as a founder mutation in the Puerto Rican population.
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INTRODUCTION

Steel syndrome (MIM %615155) was first described in 1993[1] as a novel orthopedic syndrome observed in 23 Hispanic children from Puerto Rico. The main clinical features are bilateral hip and radial head dislocations, short stature, scoliosis, carpal coalitions and *pes cavus*. Later, these and additional patients were further characterized and retrospectively evaluated. Some patients were also described as mildly dysmorphic, with a long oval face and prominent forehead, hypertelorism, and a broad nasal bridge.[2] Standard treatment for the congenital hip dislocation is surgical intervention, which generally has a poor outcome. A retrospective review of surgical outcomes revealed subluxation of the hips with acetabular dysplasia and pain in the majority of the patients.[2]

To date, the gene responsible for Steel syndrome had not been identified, although autosomal recessive inheritance was suspected based on the occurrence of affected siblings among the reported cases.[1,2] Interestingly, the majority of reported cases have been observed in patients of Puerto Rican origin.

MATERIALS AND METHODS

Subjects

The proband (Patient II-1, Figure 1a) was a 2 11/12 years Hispanic male of Puerto Rican ethnicity with bilateral congenital hip dysplasia and coxa vara at the time of first evaluation. He was the product of a 34-week uncomplicated pregnancy to a 26-year-old female and 30-year-old male. The neonatal course was unremarkable and the patient was discharged from the hospital at 7 days. At presentation, he had six previous unsuccessful surgical procedures to correct the hip dislocation. The physical findings on examination are summarized in Table 1. Radiographs of the lower extremities showed dislocated femoral heads, under ossification of the capital femoral epiphysis and coxa vara deformity with surgical hardware in place (Figure 2a1). The cervical spine was significant for odontoid hypoplasia.

The younger female sibling (Patient II-2, Figure 1b) was first evaluated at the age of 22 months and had a history of bilateral hip dislocation not diagnosed until she walked at 13 months. Prenatal history was significant for gestational diabetes type A2. Both neonatal and postnatal histories were uncomplicated and she had no surgeries. The physical findings are listed in Table 1. Similar to her sibling, radiographs of the lower extremities showed bilateral hip dislocations, poorly ossified femoral heads and shallow bilateral acetabula (Figure 2a2).

Follow-up examinations of patients II-1 and II-2 at 14 and 12 years of age, respectively, showed short stature (<5%), mild midface hypoplasia with slightly anteverted nares, bilateral fifth finger clinodactyly, decreased adduction of the hips bilaterally and *pes planus*. Patient 1 had decreased elbow extension bilaterally and thoracic levoscoliosis. Patient 2 had lumbar lordosis and mild thoracic scoliosis. Both had bilateral capitate and hamate bone coalitions (Figures 2b1 and b2). Patient II-1 had unilateral radial head dislocation. Both had normal intelligence. Family history was positive for a maternal female first cousin (Figure 3, individual II-3) who was born in Puerto Rico with dislocated hips and leg length discrepancy. Based on the clinical and radiographic findings in all three children, a clinical diagnosis of Steel syndrome was made.

Genomic sequencing and analysis

The family composed of the two described siblings (patients II-1 and II-2, Figure 3) affected with Steel syndrome and both unaffected parents (Figure 3, individuals I-1 and I-2) were recruited for genomic sequencing studies through the Baylor-Hopkins Center for Mendelian Genomics research initiative. The family's consent to the study was obtained through an Institutional Review Board (IRB)-approved research protocol of Baylor College of Medicine. Exome sequencing of the two affected siblings was performed as previously described.[3] Genomic DNA (1 μg) from each individual was fragmented by sonication. Whole-exome targeted capture was performed in solution using the BCM-HGSC Core design followed by sequencing on the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA, USA). An average of 8.7 Gb of raw sequencing data were generated per sample; the produced sequence reads were mapped and aligned to the human genome reference assembly GRCh37/hg19

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using the BCM-HGSC Mercury pipeline.[4] An average depth of coverage of 107× with >90% of the target bases covered at a minimum of 20× coverage was obtained. Variant calling was performed using Atlas2[5] and SAMtools.[6]

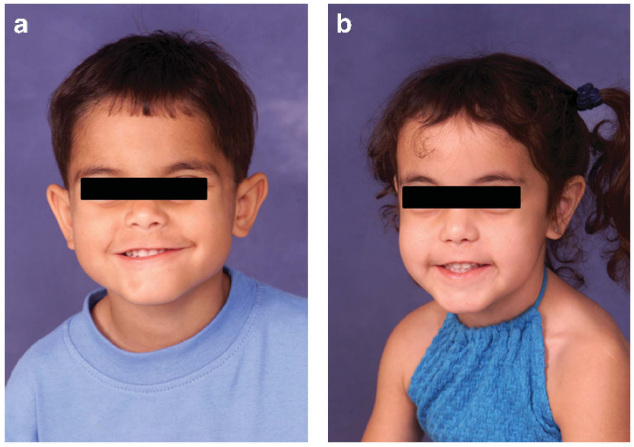

Figure 1 Photographs of index patient (**a**: patient II-1) and sister (**b**: patient II-2). Oval-shaped face with prominent forehead and mild frontal bossing and broad nasal bridge in both patients.

An in-house developed annotation pipeline[7] that is based on ANNOVAR[8] and custom scripts was used for variant annotation to inform on the identified variants.

Exons containing the variants of interest were PCR amplified using specific primers. The variants were orthogonally verified using Sanger dideoxynucleotide sequencing.

We performed high-resolution SNP array genotyping on the Illumina OmniExpress platform in all members of the extended family according to the manufacturer's protocol.

Variant data generated will be released and deposited into the database of Genotypes and Phenotypes (dbGaP: http://www.ncbi.nlm.nih.gov/gap) of the National Center for Biotechnology Information (NCBI) as part of the Centers for Mendelian Genomics research initiative.

RESULTS

A total of 24 250 coding variants in II-1 and 23 915 in II-2 were called. Based on the previous observations that Steel syndrome followed a recessive mode of inheritance, we compared the exomes of the two affected sequenced individuals in search for rare (<1% minor allele frequency) shared homozygous or compound heterozygous variants. Of the total coding variants identified in these two siblings, they shared homozygous variants in three genes (*COL27A1*, *B3GALNT2*, and *MKI67*) and compound heterozygous variants in two additional genes (*PSMD6* and *ZBBX*). Combined scores of conservation and

Table 1 Phenotypic findings

Steel Syndrome Characteristics (Flynn *et al*.[2])	Short Stature (100%)	Scoliosis (53%)	Carpal Coalition (73%)*	Dislocated Hips (100%)	Radial head dislocation (91%)	Pes cavus (34%)
Patient 1 (Male)	+	+	+	+	+	-
Patient 2 (Female)	+	+	+	+	-	-

[a]Capitate–hamate coalition most common. Percentages are given based on the cases reviewed by Flynn *et al*.[2]

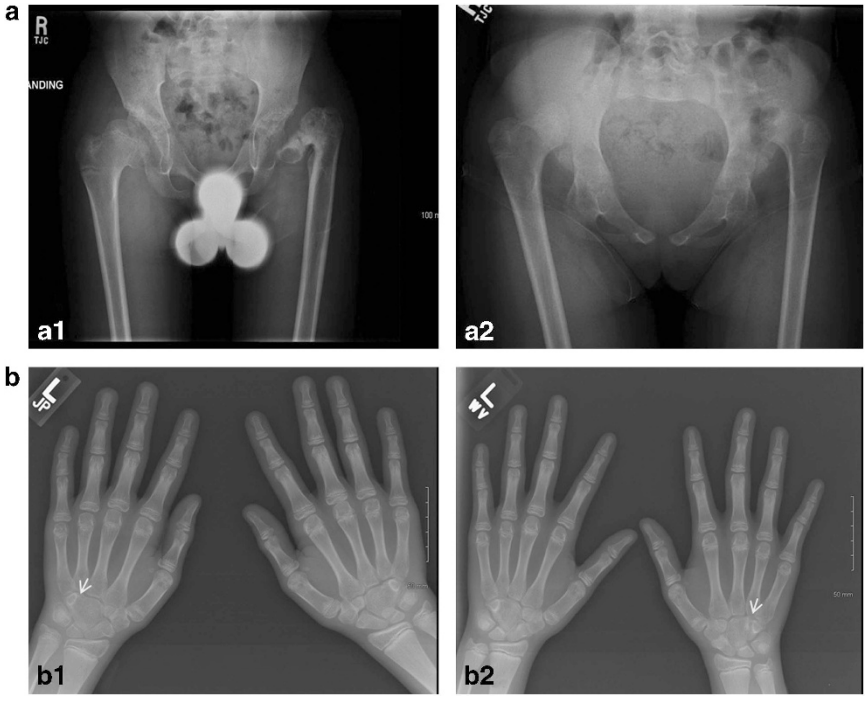

Figure 2 (**a**) Bilateral hip dysplasia in a sibling pair. Patient II-1 at 13 years of age (a1) and patient II-2 at 12 years of age (a2). (**b**) Bilateral capitate–hamate coalition in patient II-1 at 13 years of age (b1) and patient II-2 at 12 years of age (b2).

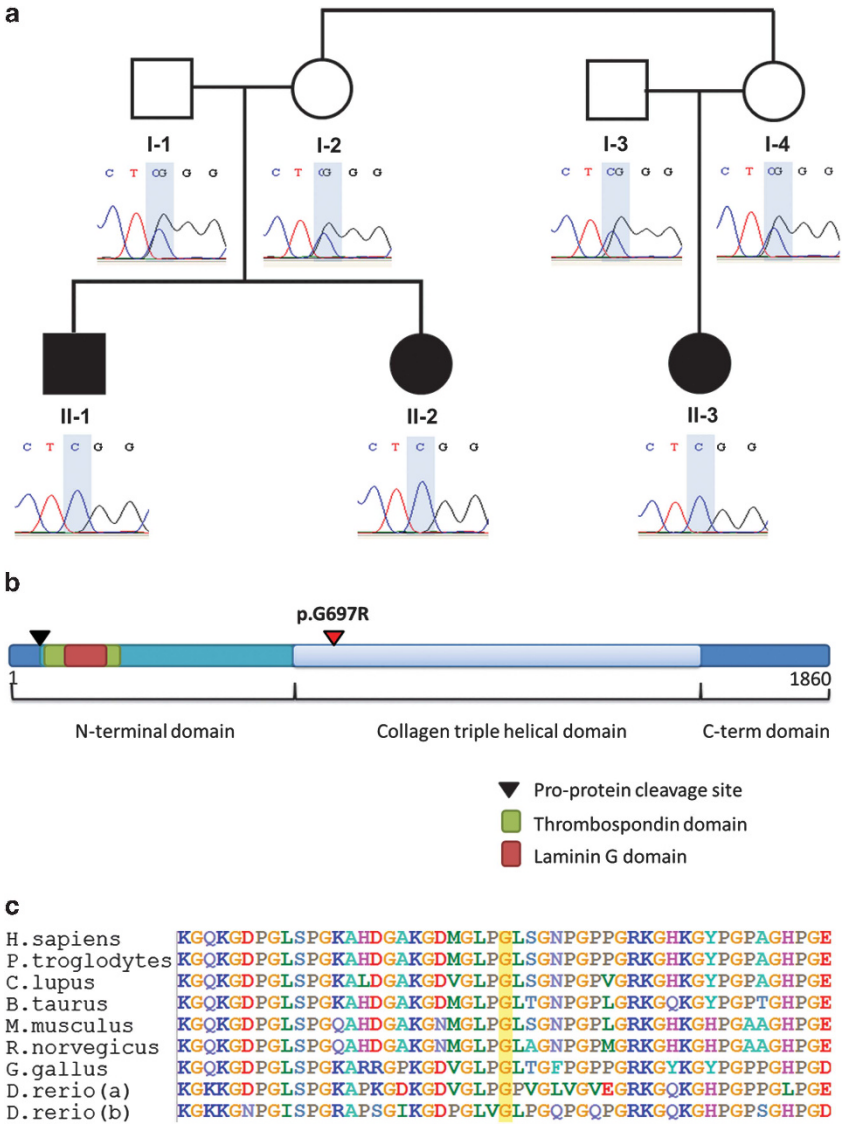

Figure 3 (**a**) Pedigree showing family with Steel syndrome. Affected individuals are denoted as black filled symbols and are homozygous for the p.(Gly69Arg) variant. All unaffected parents are heterozygous carriers for the variant. (**b**) Domain structure of the COL27A1 protein is shown. It is a 1860-amino-acid propeptide with an N-terminal cleavage site and overlapping laminin G and thrombospondin domains in the N-terminus, followed by the triple helical domain characteristic of collagen genes. (**c**) Alignment and conservation of amino-acid residues surrounding the identified p.(Gly697Arg) variant. The variant changes a highly conserved Glycine residue that is part of the Gly–Xaa–Yaa repeat motif characteristic of collagen proteins' triple helical domain.

bioinformatic prediction algorithms highlighted two of the three homozygous variants as highly conserved and likely pathogenic. We performed confirmation and segregation of the variants of interest in the affected siblings and both parents. Additionally, we were able to recruit the affected cousin of the sequenced individuals (Figure 3, individual II-3) and her two unaffected parents (Figure 3, individuals I-3 and I-4) for segregation studies. After confirmation and segregation of variants in these additional related samples, only one variant co-segregated with the disease phenotype. This was a shared homozygous rare variant (chr9.hg19:g.116958257G>C; c.2089G>C; p.(Gly697Arg)) in the collagen type XXVII, alpha 1 gene, *COL27A1* (NM_032888.1), in all three affected individuals of this extended family. All four unaffected parents were heterozygous carriers (Figure 3a) fulfilling Mendelian expectations for a recessive disorder. In addition, we performed high-resolution SNP array genotyping in all members of the family and observed no large regions of homozygosity in any of the affected children, except for the region surrounding the *COL27A1* gene in chromosome 9, consistent with self-reported non-consanguinity of the parents (Figure 4). The occurrence of this variant in the homozygous state in all the affected individuals in the context of no evidence for parental consanguinity suggests that the variant arose as a founder mutation event that is segregating in the Puerto Rican population. The haplotype in which the mutation arose and appears to be segregating spans 5.7 Mb; however, it is broken by recombination in subject II-2, resulting in a smaller shared haplotype of 129.1 kb encompassing the 5′ half of *COL27A1*.

DISCUSSION

There are more than 40 collagen genes identified in vertebrates that comprise the extracellular matrices of bone, cartilage, and other connective tissues providing support and strength. Collagen proteins

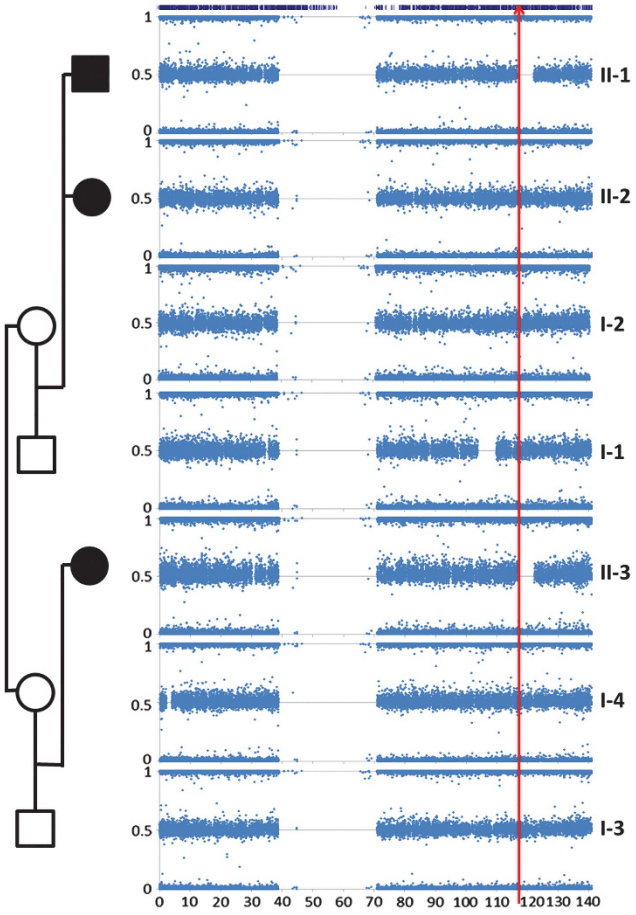

Figure 4 View of complete chromosome 9 on high-resolution SNP arrays for all the family members. The red line indicates the chromosomal location of *COL27A1*. No large regions of homozygosity were observed in the three affected individuals besides the regions surrounding *COL27A1*.

can be subdivided into different families, but all share a similar structure characterized by triple helical domains of the repeating triple amino acids (Gly–Xaa–Yaa).[9,10] Many fibrillar collagen genes have been implicated in a variety of human diseases; mutations in collagen type I are responsible for osteogenesis imperfecta (OI, MIM #166200), while mutations in genes for collagen type II, IX, X and XI can lead to a variety of chondrodysplasias.[11,12]

COL27A1 encodes the pro-alpha chain of fibrillar collagen type XXVII. The gene is 156 kb in length, located on chromosome 9q32; it is formed by 61 exons and encodes a 1860-amino acid pro-peptide. The protein has the characteristic structure of collagens (Figure 3b); however, its triple helical domain is shorter (990 aa) as compared to other proalpha collagen proteins (1012 aa). The encoded protein differs additionally in a few other minor features from its counterparts, such as the lack of a minor triple helix domain and some sequence differences in the triple-amino-acid repeat motif of collagen proteins.[9]

Expression of human *COL27A1* is regulated by the transcription factor SOX9, an important regulator of chondrogenesis in vertebrates.[13] The first intron of *COL27A1* contains conserved enhancers for binding of SOX9 dimers that can activate transcription of the gene in chondrocytes, similar to other collagen genes involved in chondrogenesis.[13] In addition, it can be transcriptionally upregulated in combination with SOX9 by the long form of the basic leucine zipper transcription factor, Lc-Maf, in chondrocytes,[14] which is an important factor in endochondral bone development and chondrocyte differentiation.[15]

COL27A1 is developmentally regulated and is highly expressed in the developing cartilage and to a lesser extent in other tissues.[9,16] Protein expression of human COL27A1 accounts for ~1.1% of all collagen transcripts in early human epiphyseal cartilage development.[17] It is most abundant in prehypertrophic proliferating chondrocytes[14,18] and its expression also overlaps with type X collagen, albeit at higher levels. It is expressed at the primary ossification centers during the transition from cartilage to bone in the process of cartilage calcification in skeletogenesis.[18] Based on this expression pattern, it is hypothesized that COL27A1 may play an important role during cartilage mineralization, providing a scaffold for the entry of other cell types and invasion of blood vessels in order to form bone structures.

In order to better characterize the role of COL27A1 in vertebrate development *in vivo*, animal models have been utilized to observe the effects of potential loss of function of this gene. In zebrafish, two orthologues of the gene exist, *col27a1a* and *col27a1b*. Morpholino knockdown in zebrafish embryos of *col27a1a* alone and in conjunction with its paralogue *col27a1b* resulted in delayed and decreased vertebral mineralization, morphologically abnormal and curved vertebrae, and scoliosis.[19] In addition, deletion and mutation knock-in mice have been generated.[20] Mice heterozygous for a mutation that changed a conserved residue in the collagen domain were phenotypically normal, while the homozygous offspring displayed a slight disruption of the growth plate architecture. Moreover, mice that carried a heterozygous deletion of 87 amino acids were normal, but their homozygous counterparts displayed severe chondrodysplasia with midline defects and died shortly after birth due to respiratory problems.[20] When the deletion allele was targeted in chondrocytes only, homozygous mice survived after birth but were significantly smaller, with short long bones, mild thoracic kyphosis, distortion of the pelvis and thoracic cage, and craniofacial defects such as a shorter snout and a domed skull. Similar to the point mutation mice, the homozygous cartilage-specific deletion mice had abnormal growth plate architecture, with severe flattening of the chondrocytes and disorganization of the collagen fibers in the pericellular matrix.[20]

To date, there are no human disease phenotypes associated with mutations in *COL27A1*. However, based on its important role in chondrogenesis and cartilage to bone transition and its high conservation in vertebrates, several groups have hypothesized a role for COL27A1 in human disease and looked for potential disorders linked to mutations in *COL27A1* in patients with various molecularly undefined chondrodysplasias.[9,18] Here, we report the first Mendelian disorder associated with a likely founder mutation in *COL27A1* that results in the described Steel syndrome, a chondrodysplasia characterized by a myriad of skeletal findings consistent with the thoroughly investigated role of COL27A1 in chondrogenesis and early skeletogenesis. The identified p.(Gly697Arg) variant changes a highly conserved glycine residue in one of the Gly–Xaa–Yaa motifs of the collagen triple helical domain (Figure 3c). Similar to what was observed in the fish and murine animal models of *COL27A1*, the patients reported here present poor ossification/mineralization, short stature, mild midface hypoplasia, scoliosis and other skeletal abnormalities. This p.(Gly697Arg) variant most likely leads to a hypomorphic allele, since all carrier individuals are phenotypically normal. Moreover, the presence of this variant in the homozygous state in all the affected individuals born to unrelated carrier parents and the observation that the majority of patients with Steel syndrome are of Puerto Rican ancestry strongly suggests that it arose as a founder

mutation in the personal genome of an individual from this population and it has been maintained at a low carrier frequency. This variant, albeit extremely rare in the general population, is not novel, corresponding to dbSNP identifier rs140950220, as it has been observed previously just once in the heterozygous state in one individual, most likely of Puerto Rican ancestry, in the NHLBI Exome Sequencing Project (Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA, http://evs.gs.washington.edu/EVS/). In addition, we searched for this specific variant in the available data from the 1000 Genomes Project (http://www.1000genomes.org) for 105 individuals of Puerto Rican origin and it was not present in these data. Screening of a larger number of Puerto Rican individuals will help to further investigate our founder effect hypothesis and to determine the carrier frequency of this specific allele and the disease frequency in that population.

The identification of *COL27A1* associated with Steel syndrome will enable molecular testing for individuals with the clinical presentation characteristic of Steel syndrome, as well as genetic counseling for carrier individuals. Other patients with Steel syndrome or similar clinical presentations should be screened for variants in *COL27A1* to determine the contribution of variants in this gene to bone disease and assess allele frequencies in other world populations.

CONFLICT OF INTEREST

JRL has stock ownership in 23andMe, and Ion Torrent Systems, Inc. and holds multiple US and European patents for DNA diagnostics. RAG is an advisor to GE Healthcare/Clarient and the Allen Institute for Brain Science. Some of the authors are based in the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from genetic laboratory testing through the Medical Genetics Laboratories and the Whole Genome Laboratory. The remaining authors declare no conflict of interest to declare.

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