A novel de novo COL1A1 mutation in a Thai boy with osteogenesis imperfecta born to consanguineous parents

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Abstract

Osteogenesis imperfecta (OI) is genetically heterogeneous. Mutations in COL1A1 and COL1A2 are responsible for at least 90% of the cases, which are transmitted in an autosomal dominant manner or are de novo events. We identified a Thai boy with OI whose parents were first cousins. Because the proband was the product of a consanguineous marriage, we hypothesized that he might be homozygous for a mutation in a known gene causing a recessive form of OI. Using whole exome sequencing (WES), we did not find any pathogenic mutations in any known gene responsible for an autosomal recessive form of OI. Instead, we identified a COL1A1 frameshift mutation, c.1290delG (p.Gly431Valfs*110) in heterozygosis. By Sanger sequencing, the mutation was confirmed in the proband, and not detected in his parents, indicating that it was a de novo mutation. These findings had implication for genetic counseling. In conclusion, we expanded the mutational spectrum of COL1A1 and provided another example of a de novo pathogenic mutation in heterozygosis in a patient born to consanguineous parents.

Keywords: osteogenesis imperfect, COL1A1, exome sequencing, next generation sequencing, Thai.

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Osteogenesis imperfecta (OI), or brittle bone disease, is a disorder characterized by low bone mass, bone fragility and often short stature. Extraskeletal manifestations may include blue sclerae, hearing loss and dentinogenesis imperfecta. The phenotypic severity of OI ranges from a very mild form without fractures to intrauterine fractures and perinatal lethality. The prevalence of OI is estimated to be about 7 per 100,000 births (Lindahl et al., 2015; Stevenson et al., 2012).

OI is genetically heterogeneous. About 90% of OI patients carry mutations in the COL1A1 or COL1A2 genes, which are de novo events or transmitted in an autosomal dominant manner due to parental mosaicism. Other inherited forms are rare and can be caused by mutations in different genes, including one gene responsible for the dominant form, IFITM5, 14 genes for the recessive form (BMP1, CRTAP, CREB3L1, FKBP10, LEPRE1, PLOD2, PP1B, SEC24D, SERPINF1, SERPINH1, SP7, SPARC, TMEM38B and WNT1 (Forlino and Marini, 2016; Garbes et al., 2015; Mendoza-Londono et al., 2015), and one gene for the X-linked form, MBTPS2 (Lindert et al., 2016). These genes play an important role in the process of collagen type I synthesis or in the control of osteoblast differentiation or function. Notably, the causative mutation in some patients with OI remains unidentified. Identification of mutations is important for genetic counseling, risk assessment, and facilitation of prenatal or preimplantation diagnosis.

Since OI can be caused by mutations in at least 18 genes, Sanger sequencing of all these genes would be time-consuming and prohibitively expensive. In 2010, whole exome sequencing (WES) was used to successfully
discover a new gene for a genetic disease for the first time (Ng et al., 2010). Several novel causative genes were sub-
sequently reported by next generation sequencing (NGS),
including the most recently identified X-linked gene for OI,
MBTPS2 (Lindert et al., 2016). NGS can analyze multiple
regions in one reaction. It is therefore, a suitable tool for
studying diseases with genetic heterogeneity, such as OI.

This study reports the clinical features and mutation
analysis of a child with OI. He is the fourth child born to a
healthy couple of first cousins. Their three older brothers
are healthy, and there is no family history of OI or other
bone diseases. He was born at full term and delivered by ce-
sarean section, because of breech presentation. His birth
weight was 2,950 g (25th centile) and his birth length was 47
cm (3rd centile). At birth, he had a closed fracture of the
right femoral shaft, and was treated with a long leg cast for
two weeks. His second fracture occurred at the age of one
month at the left proximal femoral shaft. Serum calcium
and alkaline phosphatase were within normal limit. DEXA
scan showed markedly decreased bone density (lumbar
spine 0.159 g/cm², left hip 0.163 g/cm², whole body 0.262
g/cm²). OI was then diagnosed clinically. Pamidronate was
started at the age of five months.

From birth to the age of three years, his height was be-
tween the 3rd and 10th centiles, weight below the 3rd centile,
and head circumference around the 75th centile, consistent
with relative macrocephaly. By the age of four years, even
under medication, he had six lower limb fractures caused
by minor traumas. Bone deformities of lower extremities
became notable. His cranium radiographs revealed Wor-
mian bones, an important characteristic of OI. No clinical
or radiographic evidence of spinal or upper extremity frac-
ture was noted. From the age of four to eight years, he was
free from fractures. However, at the age of eight years, he
had a fracture of the right femoral shaft after a simple fall.
His mental development was appropriate. At the last follow
up, when he was 11 years old, his height was 123 cm (Z
score of -3) and his weight was 27 kg (Z score of -1). He
also had blue sclerae and pectus carinatum. Dentinogenesis
imperfecta was not observed (Figure 1A). He had a limping
gait due to leg length discrepancy. The standing antero-
posterior radiograph of the pelvis and femora revealed tilt-
ing of pelvis and malunion at the right femoral shaft caus-
ing varus deformity (Figure 1B). There was a hypertro-
phic nonunion of the shaft of the right femur (Figure 1B,C).
Multiple dense metaphyseal lines caused by bisphospho-
nate therapy were notable in both distal femoral and proxi-
mal tibia metaphyses. He did not show platyspondyly or
scoliosis. Based on clinical findings and follow-up, as well
as radiological findings, the diagnosis of OI type IV was
given to the patient.

After informed consent was obtained, genomic DNA
was isolated from peripheral blood leukocytes using a
Puregene Blood kit (Qiagen, Hilden, Germany). The geno-
mic DNA was sent to Macrogen Inc. (Seoul, South Korea),
for whole-exome sequencing (WES). DNA was captured
using the TruSeq DNA Sample Prep kit (Agilent Technol-
ogies, Santa Clara, CA) and sequenced on a HiSeq2000 in-
strument. Base calling was performed and quality scores
analyzed using Real Time Analysis software version 1.7.
Sequence reads were aligned against the University of Cali-
ifornia Santa Cruz human genome assembly hg19 using
Burrows-Wheeler Alignment software (BWA). Single-nu-
cleotide variants (SNVs) and insertions/deletions (Indels)
detected by Sequence Alignment/Map tools (SAMtools) and annotated against dbSNP & the 1000 Genomes Project. After quality filtering, we looked for
variants located in the coding regions of known skeletal
dysplasia genes for all potential pathogenic SNVs and
Indels. Variant calling exclusion criteria were: (a) coverage
< 10x, (b) quality score < 20, (c) minor allele frequency
≥1% in the 1000 Genomes Project, and (d) non-coding vari-
ants and synonymous exonic variants. The remaining vari-
ants were subsequently filtered out if they were present in
our in-house database of 200 unrelated Thai exomes. Ex-
isting SNVs or known pathogenic mutations were filtered
out using the Human Gene Mutation Database (HGMD)
and the Exome Aggregation Consortium database (ExAC).
The pathogenic variant identified was confirmed by Sanger
sequencing. DNA samples from the unaffected parents
and one of the unaffected brother of the patient were also Sanger
sequenced to search for the mutation.

With a history of parental consanguinity, we hypothe-
sized that our patient could be homozygous for a mutation
in one of the 14 genes responsible for an autosomal rece-
sive form of OI. A caveat is that he manifested blue sclerae.
Patients with recessive forms of OI generally do not have
blue sclerae or other secondary features. However, this is
not absolute, as there have been reports of patients with re-

Figure 1 - Clinical and radiographic features of the patient with osteo-
genesis imperfecta. (A) Dentinogenesis imperfecta was not present. (B) Standing anteroposterior radiograph of the pelvis and femora showing til-
ing of the pelvis and varus deformity of both femora. (C) Radiograph of
his right femur showing a hypertrophic malunion of the femoral shaft.
cessive forms of OI who had grayish sclerae (Becker et al., 2011). Nonetheless, no mutations in any one of the known genes responsible for an autosomal recessive form of OI was identified in our patient.

We then looked for a heterozygous mutation for the autosomal dominant form of OI, and found a c.1290delG mutation in exon 19 of the COL1A1 gene. The deletion leads to a frameshift, resulting in a premature termination codon (PTC) at residue 540 (p.Gly431Valfs*110). The gene product, the alpha 1 chain of type I collagen, is expected to be shortened from 1464 to 539 amino acid residues. Sanger sequencing confirmed the presence of the COL1A1 c.1290delG mutation in heterozygosis (Figure 2). It was not present in neither of his parents nor the unaffected brother, indicating a de novo mutation. This mutation was not present in the HGMD, the Osteogenesis Imperfecta Variant Database, or the in-house 200 Thai Exome Database.

Dentinogenesis imperfecta was not present in our patient. It has been suggested that collagen plays non-identical roles in bone and dentin, since individuals with COL1A1 mutations have a wide variety of dentin and bone defects (O’Connell and Marini, 1999; Rauch et al., 2010).

Mutations in the COL1A1 gene can lead to OI by either haploinsufficiency or dominant negative effects. Haploinsufficiency is usually the consequence of splice site mutations, nonsense mutations, deletions or insertions. These mutations usually create a premature termination codon. These aberrant RNAs would usually be degraded by nonsense-mediated mRNA decay (NMD). With normal alpha chains of type I collagen being produced from the wild-type allele, haploinsufficiency generally leads to a mild OI phenotype (Marini et al., 2007). On the other hand, dominant negative effects result from missense mutations or from premature termination codon mutations that avoid NMD; the mutated alpha chain binding to normal alpha chains produced by the wild-type allele results in abnormal collagen type I. Classically known OI mutations with dominant-negative effect are the substitution of an amino acid for one of the obligatory glycine residues occurring in ev-

![Figure 2 - Electropherogram showing the COL1A1 c.1290delG mutation in heterozygosis in the proband compared to a normal control.](image)
The severe phenotype of our patient suggested that the COL1A1 c.1290delG mutation led to a truncated alpha chain able to bind to the normal alpha chains, resulting in the production of defective collagen type I. The more severe phenotype of the patient reported could be explained by a possible abnormality in NMD efficiency. Although the mutation found in our patient is located in the NMD sensitive zone, 50-55 nucleotides upstream of the last exon-exon junction, the mutant RNA might still escape NMD. In 2014 the evidence pointing to the inter-individual variability in NMD efficiency and its correlation with clinical presentations was reviewed, and it was proposed that it was a common phenomenon in human populations (Nguyen et al., 2014). Therefore, it is possible that the mutant RNA escapes NMD, at least partially, in our patient. There were previous reports of patients with severe OI who had premature truncation mutations. A Korean patient with OI type IV had an eight-base pair deletion in exon 46 (Lee et al., 2006). Vietnamese patients with OI type III or IV were reported to have frameshift mutations located in the NMD sensitive zone, similar to our patient (Ho Duy et al., 2016). Taken together, we hypothesize that variable NMD efficiency may be a cause of variable OI phenotypes in different patients with similar out-of-frame mutations.

Identification of this pathogenic mutation allowed more accurate genetic counseling, ruling out recessive inheritance. Although the recurrence risk in this case is likely to be very low, the probability of parental mosaicism had to be considered, since no other tissue than blood was tested. An empirical recurrence risk of 27% for the perinatal lethal form of OI (type II) in the offspring of parents carrying mosaicism for collagen I mutations was reported (Pyott et al., 2011). With mutation identification, prenatal or pre-implantation diagnosis can be provided.

In this study, using WES, we successfully identified a novel de novo mutation in the COL1A1 gene in a patient with OI, expanding its mutational spectrum. In addition, we provided another example of identification of a heterozygous de novo mutation as the cause of a disease in a patient born to consanguineous parents, which allowed more accurate genetic counseling.

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**Internet Resources**

Burrows-Wheeler Alignment (BWA), bio-bwa.sourceforge.net/ (accessed April 2015).

Sequence Alignment/Map tools (SAMtools), samtools.sourceforge.net/ (accessed April 2015).

The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff (HGMD), http://www.hgmd.cf.ac.uk/ac/index.php (accessed February 2016).

Exome Aggregation Consortium database (ExAC), exac.broadinstitute.org (accessed February 2016).

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