CLINICAL QUIZ (p182) ANSWER

The baby suffered from pathological fractures of the left femur and right radius with evidence of callus formation. Skeletal survey showed generalised decrease in bone density. Shortening and broadening of bilateral humeri, radii, ulnar, femurs, tibias and fibulas were seen. Bony bowing was found at the right femur, tibia and fibula. Widened metaphyseal plates of both femurs and humeri were also observed. No wormian bones were seen. Serum calcium, phosphate and alkaline phosphatase (ALP) levels were normal. The provisional clinical diagnosis is osteogenesis imperfecta (OI) type III.

Gene sequence analysis of *COL1A1* and *COL1A2* was performed for our patient. A single nucleotide substitution occurred on exon 37 of chromosome 17 (c.2461G>A, GGC>AGC), resulting in a dominant *COL1A1* gene mutation of serine substitution for glycine at protein position 821, or the equivalent position 643 of the triple helix; *COL1A2* sequence result was normal. Such mutation in *COL1A1* have been reported previously in individuals all having a clinical picture in the OI type III to OI type III-IV range, consistent with our patient's presentation; it is usually de novo; however there is a 2-5% chance of recurrence in siblings as a result of parental gemline mosaicism for the mutation.

OI is an inherited disorder of connective tissue with a prevalence of 1 in 10000 characterised by altered production of type I collagen resulting in bone fragility and fractures; other clinical features include blue sclerae, dentinogenesis imperfecta, hearing loss and joint laxity.[1] Disease severity ranges from lethality in the perinatal period to occasional fracture.

All known genetic causes of OI affect the biosynthesis of type I collagen in bones and connective tissues. Most patients have mutations in one of the two genes, *COL1A1* and *COL1A2*, with the majority inherited with autosomal dominant trait. Autosomal recessive pattern is rare. *COL1A1*, located on chromosome 17q21.33, encodes pro-α 1(I) chains of type I collagen while *COL1A*2, located on chromosome 7q22.1, encodes pro-α 2(I) chains. Figure 5 shows the subsequent synthesis pathway of type I collagen.

Four major types of OI were delineated on clinical, genetic and radiological grounds in 1979 – the Sillence classification (Table 1). Genotype-phenotype correlation is significant, with the clearest distinction in phenotype being between those mutations that decrease the production of type I procollagen and those that produce abnormal molecules.[1] The former result in the mild OI type I phenotype, whereas the latter produce the more severe and lethal varieties, type

Table 1 The original sillence classification of types of osteogenesis imperfecta

Type	Clinical manifestations & severity	Inheritance	Common molecular defects
Type I (Mild)	• Mild • Predominantly blue sclerae • Normal stature	AD	*COL1A1 Null*
Type II (Perinatal lethal)	• Severe to perinatal lethal • Dark blue sclerae • Severely short stature	AD/AR	*COL1A1* and *COL1A2* Substitutions for glycine Exon skipping Partial gene deletion
Type III (Deforming)	• Moderate – mild • Blue/grey/white sclerae • Very short stature	AD/AR	*COL1A1* and *COL1A2* Substitutions for glycine Exon skipping
Type IV (Mild deforming)	• Moderate • Normal – grey • Variable short stature	AD/AR	*COL1A1* and *COL1A2* Substitutions for glycine Exon skipping Partial gene deletion

Expansion of the classification with OI type V-VIII was proposed by Rauch (2004) and Cabral (2007) after new discoveries of gene mutations. Modified from Byers PH, Cole WG. Osteogenesis Imperfecta, in Connective Tissue and Its Heritable Disorders: Molecular, Genetic, and Medical Aspects, Second Edition (eds Royce PM and Steinmann B). John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2003. doi: 10.1002/0471221929.ch8;[1] and van Dijk FS, Cobben JM, Kariminejad A, et al. Osteogenesis Imperfecta: A Review with Clinical Examples. Mol Syndromol 2011;2:1-20.[6]

II, III and IV. OI type I is mostly characterised by a 50% reduction of the amount of type I collagen, usually resulting from variants in one *COL1A1* allele (frameshift, nonsense, and splice-site alterations) that lead to mRNA instability and haploinsufficiency.[2] OI types II, III and IV are characterised by intertwining of mutated and normal procollagen chains resulting in production of abnormal type I collagen (Figure 6).

Historically, the laboratory confirmation of the diagnosis OI rested on cultured dermal fibroblasts to identify decreased or abnormal production of abnormal type I (pro)collagen molecules, measured by gel electrophoresis.[3] However, in the context that genomic sequencing has a less invasive way and shorter time to diagnosis as well as more precise determination of recurrence risk nowadays, a new diagnostic approach is adopted (Figure 7).

Multidisciplinary approach is adopted in the management of OI, which involves paediatricians, clinical geneticists, orthopaedic surgeons, endocrinologists, physical and occupational therapists. Management is mainly supportive, including pain relief, splints for fractures and physical therapy. Bisphosphonates are synthetic analogues of pyrophosphates which inactivate osteoclasts. This antiresorptive property is a potential mechanism in OI treatment in children, with pamidronate already evaluated in several open-ended non-randomised studies.[4] The use of bisphosphonates in infants with OI aged below three, however, is still being studied.[5] Orthopaedic intervention including

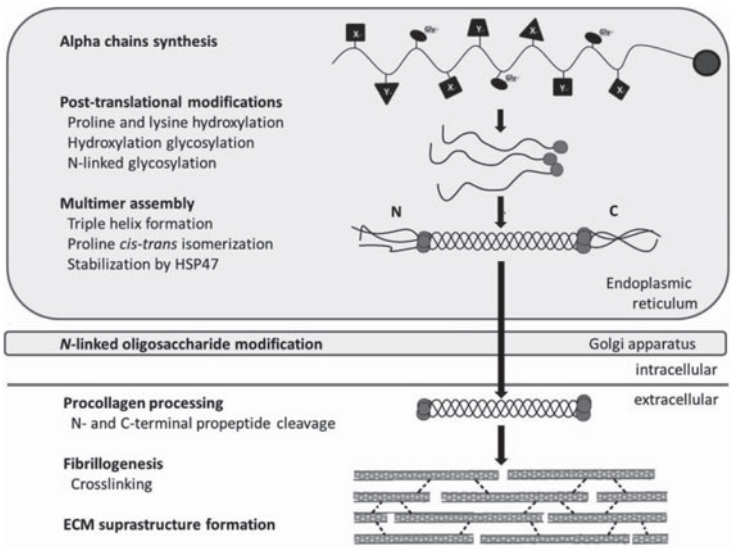

Figure 5 Synthesis pathway of type I collagen. After translation, pro-α 1(I) and pro-α 2(I) chains undergoes hydroxylation, glycosylation, disulfide bond formation and triple helix formation, first in rough endoplasmic reticulum and then Golgi apparatus. Extracellular modifications include cleavage of N- and C-propeptides, self-assembly into fibrils and then fibers. Some of these processes are prone to be affected by mutations. Gly=glycine; X=frequently proline; Y=frequently hydroxyproline; N=N-propeptide; C=C-propeptide.

Figure 6 Dominant negative effect, which is defined by a mutation whose gene product adversely affects the normal, wild-type gene product within the same cell, usually by dimerizing (combining) with it. Usually, due to causative variants in either *COL1A1* or *COL1A2*, substitutions for glycine occur and lead to synthesis of mutated procollagen chains. Intertwining of mutated and normal procollagen chains result in production of abnormal collagen type I, posttranslational overmodification and poses a deleterious effect on extracellular matrix (ECM) stability or function.

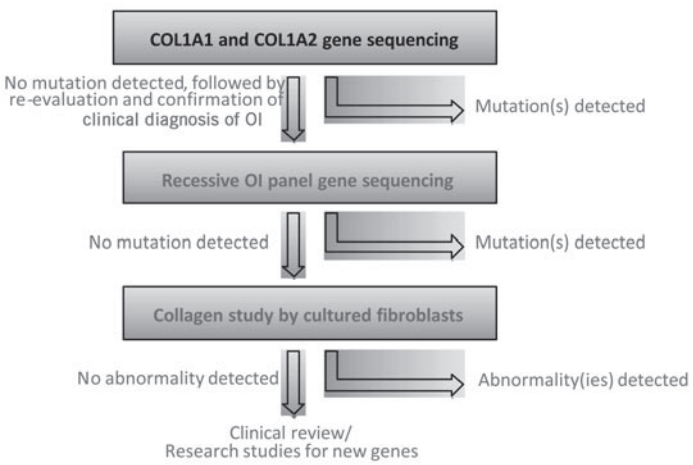

Figure 7 Simplified diagram of the new approach of genetic diagnosis of osteogenesis imperfect (OI).

insertion of intramedullary rods may be indicated in selected cases.[6] Bone marrow transplantation or mesenchymal stem cells transplantation has been tried and pre-liminary results showed improvement in phenotypes though engraftment of mesenchymal stem cells remained suboptimal.[7-10]

Despite the above, the prognosis for infants with OI type III is usually poor because of severe bone fragility with progressive deformities of the long bones and spine, muscle weakness, and joint contractures. Progressive scoliosis is usual and most of the children are wheelchair-bound. Life-threatening cardiopulmonary decompensation may occur.

Another differential diagnosis considered in our patient is the infantile form of hypophosphatasia, a rare metabolic bone disease characterised by rickets and craniosynostosis in the first 6 months of life. Premature shedding of deciduous teeth and death within the first year of life is common.[11] Mutations in the tissue non-specific alkaline phosphatase (TNSALP) gene cause a subnormal serum alkaline phosphatase activity, affecting mineralisation of bones and teeth. Reduced enzyme activity results in accumulation of its substrates including phosphoethanolamine, pyridoxal 5'-phosphate and inorganic pyrophosphate, which can be detected in serum and urine. Hypophosphatasia is considered in our patient because of bone undermineralisation, an inappropriately 'normal' ALP level after fracture and elevated levels of pyridoxal-5'-phosphate and phosphoethanolamine. Sequencing of the TNSALP gene in our patient was negative for pathogenic mutations. Retrospective analysis revealed that continuous prescription of multivitamin drop during measurement of pyridoxal-5'-phosphate caused the falsely elevated level, as the drop contained vitamin B6, a precursor of pyridoxal-5'-phosphate.

In infants with multiple atraumatic fractures, child abuse should always be considered apart from OI.[12] The nature of the fractures, the developmental capabilities of the patient and the given history are crucial in the evaluation. Worrisome injuries that might be inflicted by physical abuse include rib fracture, metaphyseal fracture, subdural hemorrhage and shear-type brain injury, which often are results of violent shaking and squeezing. Careful clinical and radiological examinations would be valuable in differentiation between OI and physical abuse.

Acknowledgement

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