Two novel COL1A1 mutations in patients with osteogenesis imperfecta (OI) affect the stability of the collagen type I triple-helix

Joanna Witecka1,3, Aleksandra M. Auguściak-Duma1,3, Anna Kruczek2, Anna Szydło1,3, Marta Lesiak1,3, Maria Krzak1,3, Jacek J. Pietrzyk2, Minna Männikkö4, Aleksander L. Sieroń1,3

1Department of General and Molecular Biology and Genetics, Medical University of Silesia, Katowice, Poland
2Department of Genetics, Polish-American Children’s Hospital, Collegium Medicum, Jagiellonian University, Krakow, Poland
3Center of Excellence for Research and Teaching of Matrix Biology and Nanotechnology, Network of CoE BioMedTech Silesia, Poland
4Collagen Research Unit, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland

Abstract. Osteogenesis imperfecta (OI) is a bone dysplasia caused by mutations in the COL1A1 and COL1A2 genes. Although the condition has been intensely studied for over 25 years and recently over 800 novel mutations have been published, the relation between the location of mutations and clinical manifestation is poorly understood. Here we report missense mutations in COL1A1 of several OI patients. Two novel mutations were found in the D1 period. One caused a substitution of glycine 200 by valine at the N-terminus of D1 in OI type I/IV, lowering collagen stability by 50% at 34°C. The other one was a substitution of valine 349 by phenylalanine at the C-terminus of D1 in OI type I, lowering collagen stability at 37.5°C. Two other mutations, reported before, changed amino residues in D4. One was a lethal substitution changing glycine 866 to serine in genetically identical twins with OI type II. That mutated amino acid was near the border of D3 and D4. The second mutation changed glycine 1040 to serine located at the border of D4 and D0.4, in a proband manifesting OI type III, and lowered collagen stability at 39°C (2°C lower than normal). Our results confirm the hypothesis on a critical role of the D1 and D4 regions in stabilization of the collagen triple-helix. The defect in D1 seemed to produce a milder clinical type of OI, whereas the defect in the C-terminal end of collagen type caused the more severe or lethal types of OI.

Keywords: collagen D-periods, collagen mutations, collagen thermal stability, heteroduplex analysis, osteogenesis imperfecta, surveyor endonuclease.

Introduction

Osteogenesis imperfecta (OI) is a diffused connective tissue disorder with a heterogeneous clinical picture due to changes in the quality or quantity of collagen type I, the protein forming the framework of the bone (Berg and Prockop 1973; Cheah 1985; Prockop and Chu 1993; Byers 1993; Prockop and Kivirikko 1995). The prevalence of all OI types is estimated at 1 per 10,000, and its frequency does not differ depending on ethnicity (Berg and Prockop 1973; Cheah 1985; Prockop and Chu 1993; Byers 1993; Prockop and Kivirikko 1995). For a number of years, OI was divided into 2 types: the Vrolik type (congenital OI) and the Lobstein type (OI tarda) (Sillence et al. 1979). In 1979, Sillence classified the disease basing on the clinical and radiological picture into 4 types (Roughley et al. 2003). This classification is the most useful in clinical practice and can be used as the basis of diagnostic criteria, although recently the classification has been further expanded, up to 7 types (Glorieux et al. 2000, 2002; Ward et al. 2002). Currently, the clinical classifi-
cation is based on the time of fracture detection by ultrasound examination and bone histology (Marini et al. 2007). Because of the very high perinatal mortality, OI type II is considered a lethal condition. Among the patients with longer survival, the most severe deformations and disability are observed in OI type III. The disease is progressive, i.e. the deformations of the extremities and vertebral column are more profound with time.

Originally, physicians linked OI to abnormalities in the metabolism of minerals and they tried to treat the disease with a calcium-rich diet. However, since the 1970s it has become clear that OI results from abnormalities in the main protein of the extracellular bone matrix, collagen type I (Byers et al. 1991; Kuivaniemi et al. 1991; Willing et al. 1994; Slayton et al. 2000; Ward et al. 2002). The abnormalities are caused by mutations in 2 genes encoding 3 polypeptides forming the protein. Collagen type I consists of 2 identical polypeptides called alpha-1 chains, and the third one, which is different and is called alpha-2 chain. Each of the chains consists of over 1000 amino acids. The genes encoding the polypeptides are located on chromosomes 17q21.31-q22 and 7q22. Each of them consists of 52 exons and 51 introns, containing about 18 000 (COL1A1) and about 38 000 (COL1A2) base pairs (Cheah 1985). Currently, over 300 mutations leading to OI (about 200 in COL1A1 and 100 in COL1A2) are deposited in a collagen database (http://www.le.ac.uk/genetics/collagen/) and over 800 novel mutations have been published recently (Marini et al. 2007). Despite the large number of mutations detected in both genes, it is still not possible to link directly a particular mutation to the clinical type of the disease (Willing et al. 1994). For example, the substitution of glycine by arginine at position 154 (Gly154Arg) can be found in OI types I and III. In COL1A2, for example, mutation Gly238Ser can be found in types I, III and IV. Despite the lack of a clear association between the molecular defect and its clinical presentation, molecular diagnostics is important in difficult cases and for genetic counselling.

Point mutations, insertions, deletions, duplications, shifting of the reading frame, abnormalities in posttranscriptional mRNA processing in one of the collagen genes (COL1A1 or COL1A2) lead to structural defects in alpha-1 or alpha-2 chains, or to a lack of collagen expression. The lack of expression causes a 50% reduction of collagen production, while a structural defect can cause changes in collagen quality and quantity, and both of them are manifested as more severe types of OI (Prockop and Kivirikko 1995; Marini et al 2007). In addition to collagen gene mutations, some 10% of OI cases are due to defects of other genes (Colige et al. 1997). The analysis of the correlation between a molecular defect and its clinical presentation leads to an observation that severity of the symptoms depends on the collagen chain affected by the mutation and on the nature and location of the defect. An identical codon change can lead to various clinical consequences depending on the position of the mutation in the alpha chain (Prockop and Kivirikko 1995). For example, C-terminal changes in the alpha-2 chain are usually more severe than changes near the N-terminus. However, this is not obvious for the alpha-1 chains. Also, the clinical symptoms are milder when there is an abnormal quantity of collagen, and more severe when there are changes in collagen quality. Here we have searched for mutations in the sequence of the COL1A1 gene in patients revealing abnormal stability of the collagen triple-helix or those with severe OI type II in order to characterize the type of OI by location of the mutation.

Materials and methods

Patients

The studied children were admitted to the outpatient clinic of the Polish-American Children’s Hospital, Collegium Medicum, Jagiellonian University in Krakow, Poland. The diagnostic criteria were based on Sillence’s (1979) OI classification. All the participants were informed about procedures related to the study, and appropriate agreement from legal guardians of the children was obtained. The procedures were conducted in accordance with Institutional Guidelines and Regulations and with the Guidelines for the Conduct of Research Involving Human Subjects, approved by the appropriate institutional review board (IRB), i.e. the Bioethical Committee of the Jagiellonian University (Permission No. KBET/356/B/2003).

Patient No. 5 was diagnosed with OI type III (Table 1). The child was a boy born from the first pregnancy, in the 38th week of gestation; the delivery was spontaneous. The pregnancy and the delivery were uneventful. His birthweight was 2300 g, length 45 cm, head circumference 35 cm, Apgar score 8. The child presented signs of bone dysplasia and was referred to the outpatient clinic (Department of Genetics). He was examined at the