**MCD Tutorial 4: *Osteogenesis imperfecta*: brittle bones or battered baby?**

**Take home messages**

**Inherited defects in ECM molecules**

There are a number of human diseases in which there are inherited defects in the structure of particular ECM molecules, with consequences for connective tissues. The best understood examples are those affecting collagen.

Collagen is an extracellular matrix protein synthesised by and secreted from a variety of cells, such as fibroblasts, and organised into insoluble fibres. These fibres are a major part of the extracellular matrix surrounding cells and giving mechanical strength and rigidity to tissues and organs. In particular they provide the tensile strength of skeletal tissues including bone, cartilage, tendons and ligaments. There are at least five major types of collagen which occur in different tissues. Although these have distinct properties they all have the same triple helix structure which is the special feature of collagen. Associated with this is the unusual amino acid composition with its high concentration of glycine. Glycine is the smallest of the amino acids and occurs at every third position in collagen where it faces the interior of the helix. Other features are the presence of the modified amino acids hydroxyproline and hydroxylysine.

# Osteogenesis imperfecta

The main feature of this disease is repeated fracture of long bones, and for this reason it can easily be misdiagnosed as child abuse. There are also malformed bones. There is a whole range of genetic disorders which can lead to the disease.

In the example used here, the defect is a point mutation (G→T) in the gene for collagen type I. This results in substitution of the normal glycine at this residue with cysteine. The larger amino acid in the mutant molecule will cause steric hindrance which generates a kink in the normally straight triple helix, with a resulting defect in the assembly into fibres. Most cases of *osteogenesis imperfecta* result from mutations in the glycine residues producing defective structural assembly. In this case the amino acid introduced is cysteine which contains a reactive sulphydryl group in its side chain. Thus not only is formation of the collagen triple helix disrupted but there can be inappropriate disulphide bonds between the two α1(I) chains in the helix. The resulting crosslinked polypeptide chains will migrate much more slowly than the individual chains when examined by gel electrophoresis in the presence of SDS. However in the presence of 2-mercaptoethanol the disulphide bonds will be cleaved allowing the chains to migrate according to their Mr.

The patient in this case is heterozygous, so only some of her α1(I) chains will be abnormal while the other allele is making the normal version. In principle 50% of the chains would be normal and 50% abnormal, though in practice this exact ratio rarely occurs in real genetic diseases. There may be differences in the rates of transcription of the gene, rate of translation, stability of mRNA or stability of the protein which lead to a different ratio. Because the collagen triple helix contains two α1(I) chains and will be disrupted if only one is the mutant form, the majority of the collagen fibres will be affected leading to a dominant phenotype.

The major consequence is in the formation of bone. Bone is formed by laying down hydroxyapatite (a form of calcium phosphate) on an ordered scaffold of collagen-I. The abnormal collagen structure leads to defects in this mineralisation process, so that the patient ends up with skeletal abnormalities and generally weak bones. Other problems also occur commonly in *osteogenesis imperfecta*, especially with the eyes, teeth, skin and ears.

In the case presented, the patient was investigated by direct study of her collagen protein. This would not be a suitable approach for prenatal diagnosis since sampling of collagen from a fetus would be impractical and risky. A better approach would be genetic screening of fetal DNA obtained by chorionic villus sampling or amniocentesis, and amplified by PCR. Specific probes could be used which were complementary to part of the DNA sequence where a mutation was known to occur: under the right conditions of temperature and ionic strength the probe will only hybridise (bind to the DNA) if it has the exact complementary sequence, enabling normal and mutant genes to be distinguished. Alternatively if the mutation altered a restriction enzyme recognition site, that would allow identification of normal and mutated DNA since only one would be cleaved by the enzyme to shorter fragments. Both methods rely on the mutation being one that is already known, but that could be checked on other family members.

**Revision work**

Use this tutorial case as the basis for revising other topics you have already met.

* The structural features of proteins, including α-helix and β-sheet, and the types of bond involved including hydrogen bonds, hydrophobic interactions, ionic interactions and disulphide bonds. Distinguish between an α-helix and the triple helix of collagen.
* The synthesis and secretion of extracellular proteins including: the role of signal sequences; post-translational modifications including glycosylation, hydroxylation, proteolytic processing and cross-linking reactions; the role of different organelles.
* The genetic code and how to read off the amino acid sequence from a given gene sequence; the initiation codon; exons and introns.
* Different types of mutation and the way they give rise to genetic patterns of inheritance.
* Hybridisation between complementary pieces of DNA, and the reason this depends on temperature and ionic strength.
* Ways in which electrophoresis can be used to distinguish protein isoforms.