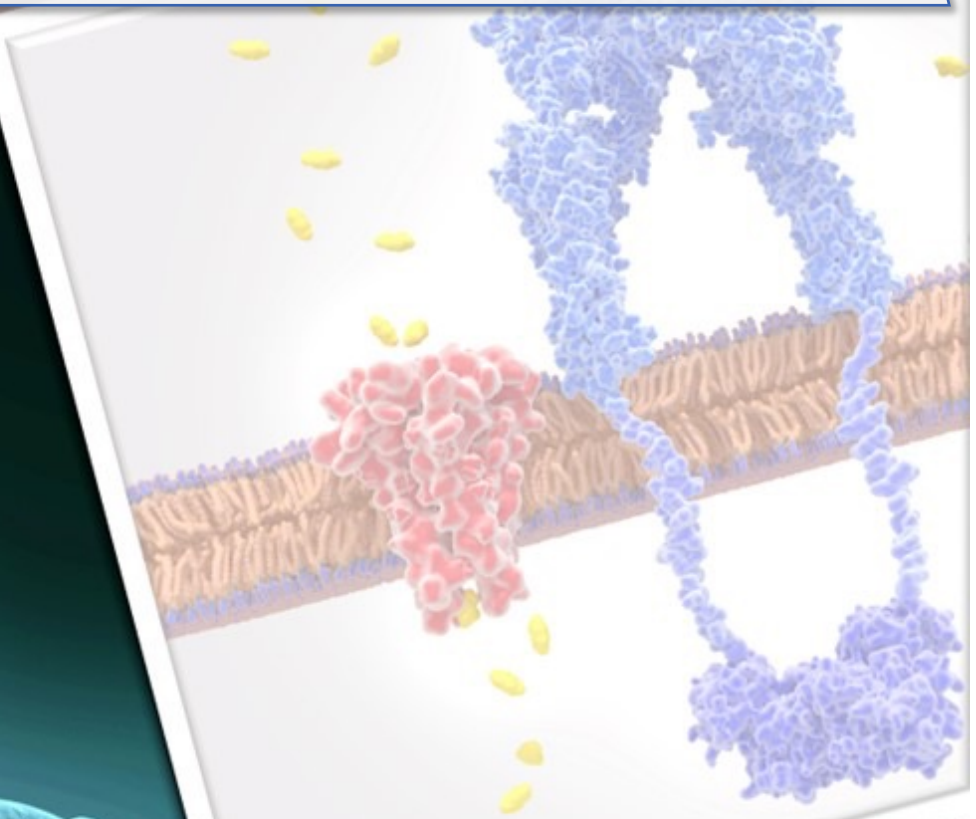
IMPERIAL

Brittle bones - tutorial



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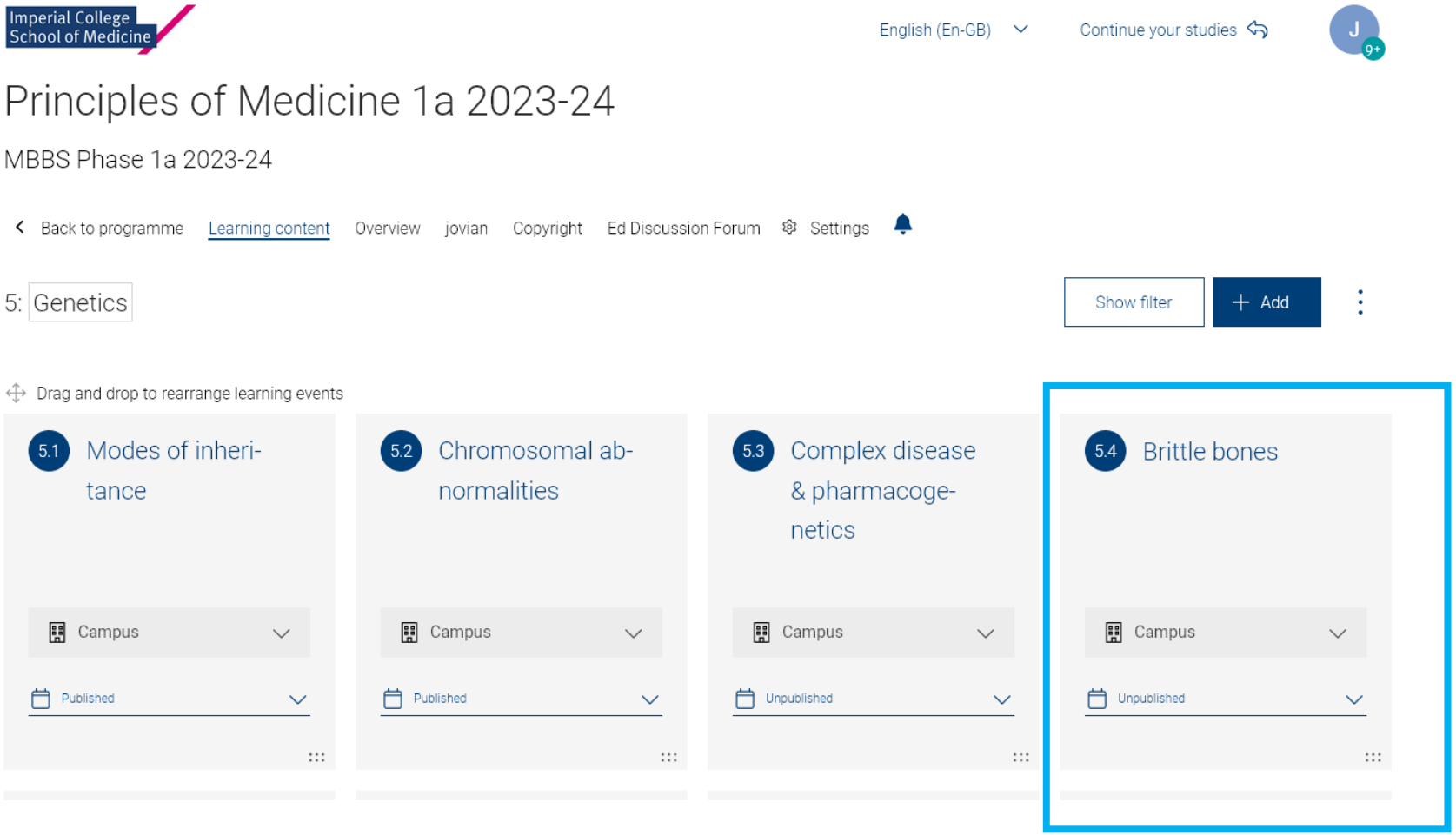
Insendi: Genetics – Brittle Bones



Session Plan

Part 1

Case information - tutor

- Recap Insendi material
- How does it link to what I know?
- How does it link to future PoM material?

Part 2

Questions (Group work)

- Engage with content
- Discuss with each other/staff
- Formulate answers - active learning

Part 3

Consolidation

- Debrief with tutor
- Clarify understanding – ask questions?
- Take home messages

Susan M

- A female child, Susan M, had been born after a normal pregnancy.
- She had a history of frequent long-bone fractures resulting from minor injury and necessitating treatment in the Casualty Department of the local hospital.
- Child abuse was initially suspected.
- As Susan developed, skeletal abnormalities were detected which were confirmed on X-ray.
- At 3 years of age, she could still only crawl and had difficulty standing.
- Susan's type I collagen genes were investigated.

Brittle bones - 1

- Fibroblasts were cultured from a skin biopsy, and the collagen I they synthesised was analysed by gel electrophoresis in the presence of SDS.

- Type I collagen consists of two α_1(I) polypeptide chains and one α_2(I) chain. Some of the patient's α_1(I) polypeptide chains had unusually low electrophoretic mobility in the absence of 2-mercaptoethanol but co-migrated with normal α_1(I) chains in the presence of the reagent

1-POM-1-5

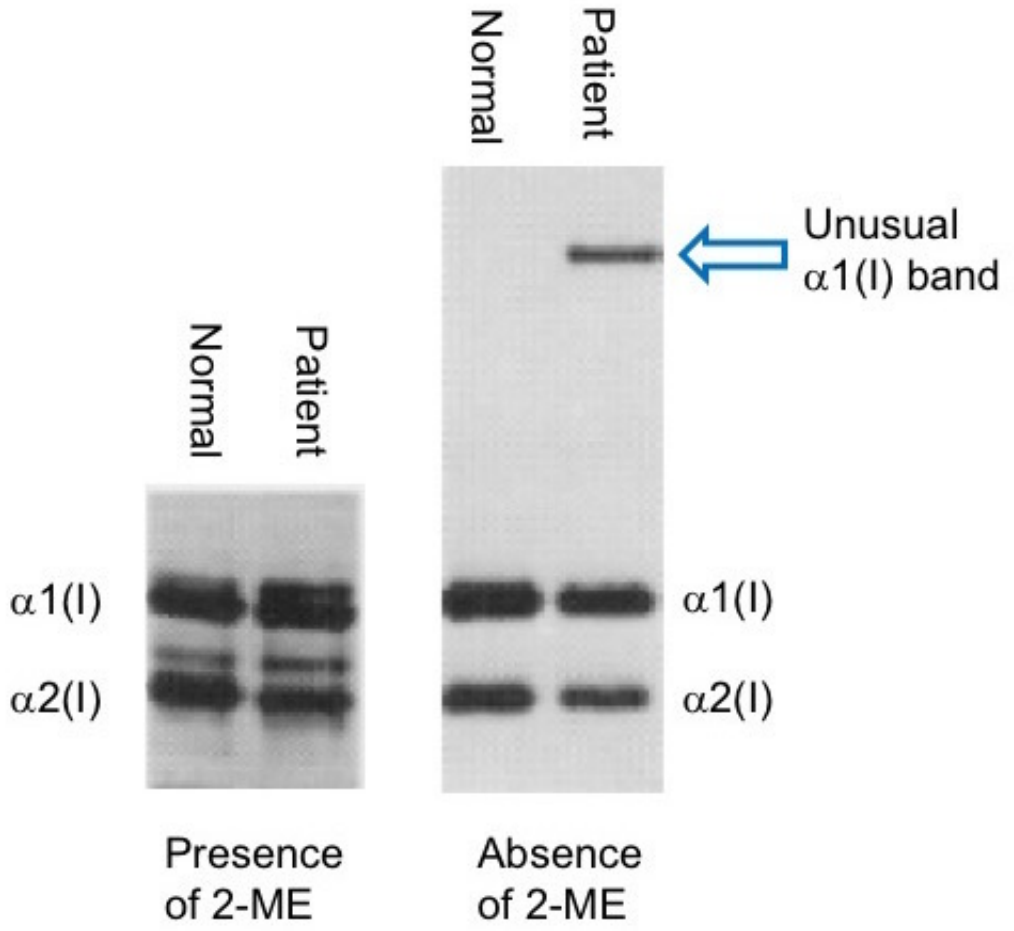


Figure: SDS-poly acrylamide gel electrophoresis of normal and patient samples

1-POM-4-1: Modes of inheritance: Differentiate between recessive and dominant autosomal and X-linked disorders, mitochondrial disorders, and explain their segregation patterns.

1-POM-1-8: Tissues: Summarise the main components of tissues, and outline the organisation of different types of epithelial tissues.

Brittle bones - 2

The child was shown to be heterozygous for a mutation in the COL1A1 gene which encodes the procollagen precursor of α_1(I) collagen. A comparison of part of the nucleotide sequences of the normal and mutant COL1A1 genes is shown below.

Normal COL1A1 sequence

5'…CCT CGG GGT GCC AAT GGT GCG CCT **G**GC AAC GAC GGT GCT AAG GGC GAT GCC GGT…3'

Patient's COL1A1 sequence

5'…CCT CGG GGT GCC AAT GGT GCG CCT **T**GC AAC GAC GGT GCT AAG GGC GAT GCC GGT…3'

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Questions to be addressed (in groups)

- Use the genetic code to predict the effect of the mutation on the protein sequence.
- Explain why this change might cause the altered electrophoresis pattern.
- Why are only some of the α_1(I) collagen chains affected?

Take home messages - 1

- Use the genetic code to predict the effect of the mutation on the protein sequence.

Normal COL1A1 sequence

5'…CCT CGG GGT GCC AAT GGT GCG CCT **G**GC AAC GAC GGT GCT AAG GGC GAT GCC GGT…3'

P R G A N G A P **G** N D G A K G D A G

Patient's COL1A1 sequence

5'…CCT CGG GGT GCC AAT GGT GCG CCT **T**GC AAC GAC GGT GCT AAG GGC GAT GCC GGT…3'

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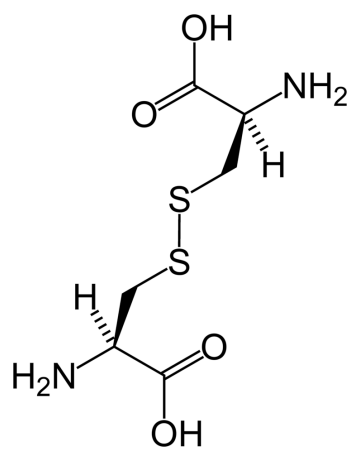
Take home messages - 2

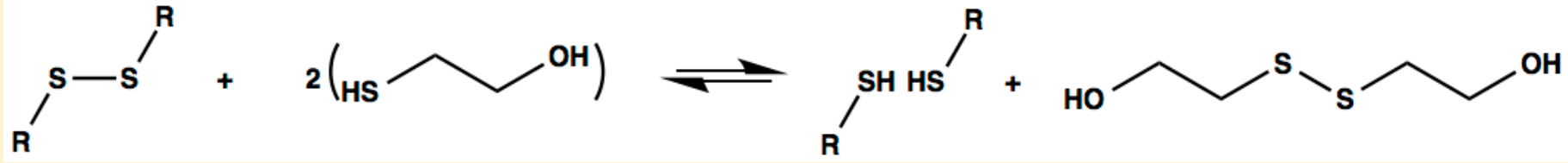
- Explain why this change might cause the altered electrophoresis pattern.

Sequence changes from glycine to cysteine

Cysteines can form disulphide bridges linking two chains together

2-Mercaptoethanol breaks these disulphide bridges





mercaptoethanol

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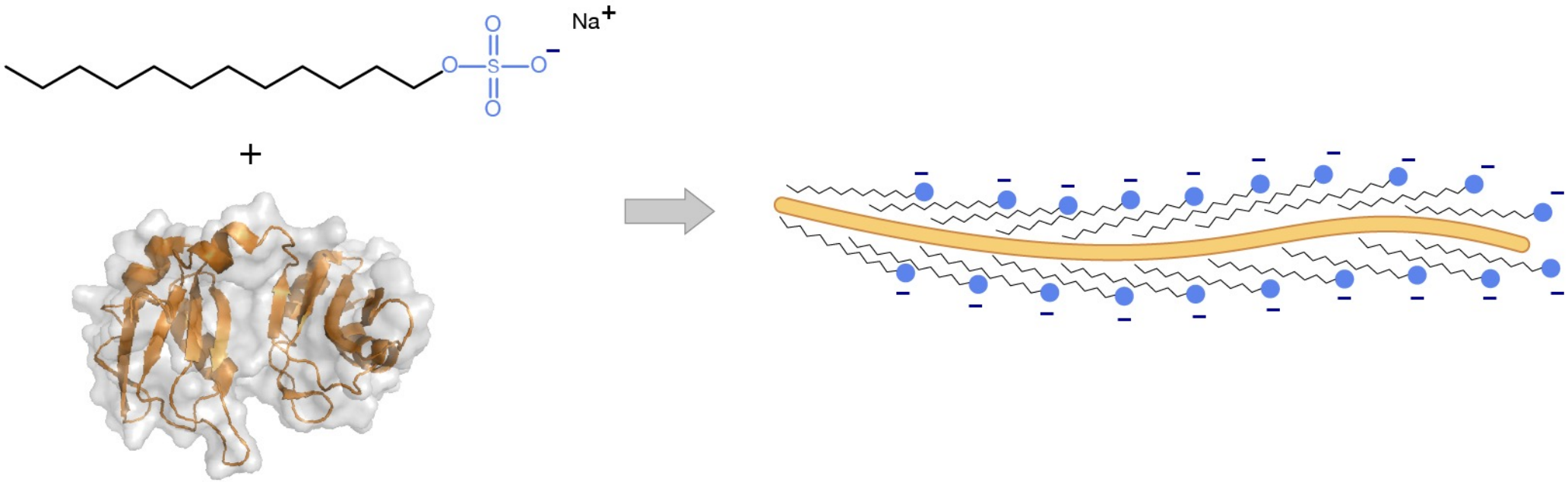
Take home messages - 2

- Explain why this change might cause the altered electrophoresis pattern.

SDS-PAGE sodium dodecyl sulfate poly acrylamide gel electrophoresis (sodium lauryl sulfate)



Take home messages - 2

- Why are only some of the α_1(I) collagen chains affected?

Since only one of the two copies of ColA1 gene are mutated only some collagen molecules carry the mutation
To form complex need two copies of mutated protein to combine

Questions to be addressed (in groups)

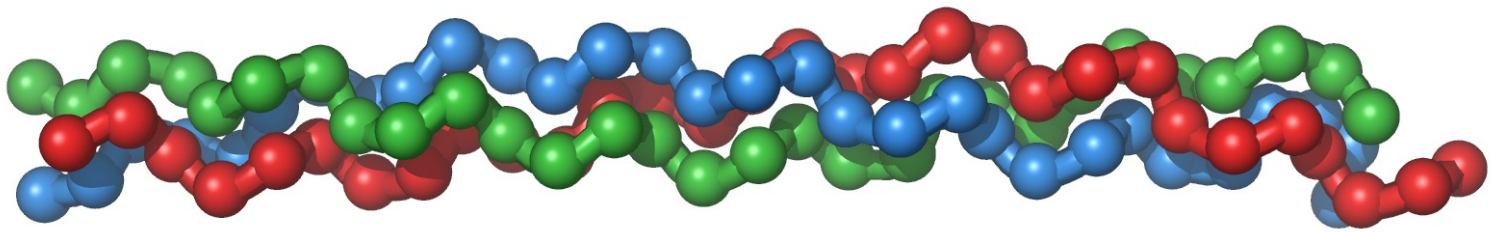
- Predict possible biochemical consequences of the change on the assembly of type I collagen.
- Subsequent investigations showed the disorder had a dominant pattern of mutation in the patient's family. Explain this by reference to the structure of collagen.
- Why might the predicted change cause skeletal abnormalities and brittle bones?

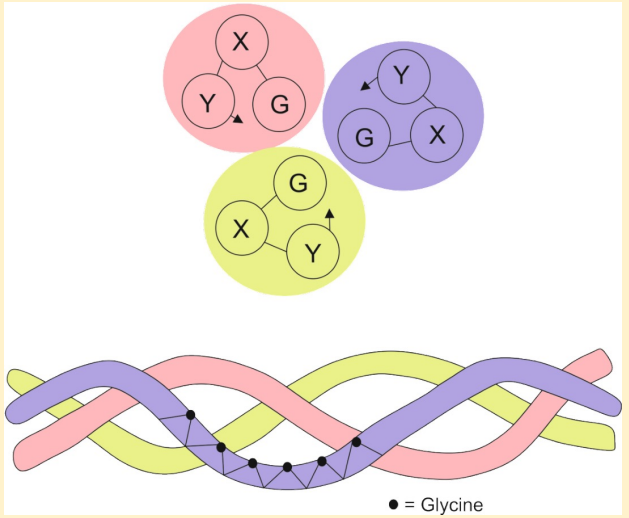
Take home messages - 4

- Predict possible biochemical consequences of the change on the assembly of type I collagen.

-Gly, X, Y-

Glycine is in centre of triple helix. Other AA wont fit





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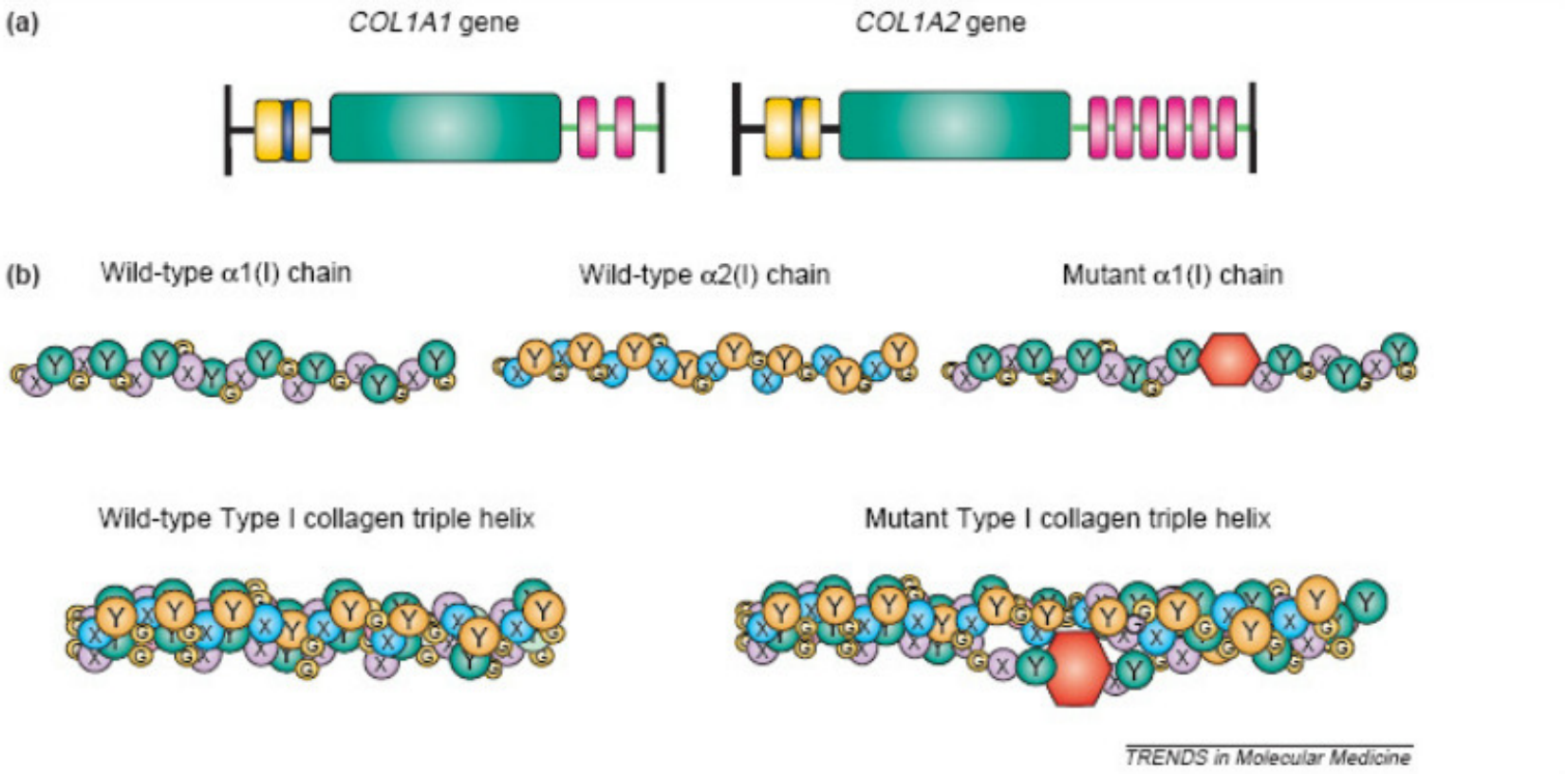
Take home messages - 4

- Predict possible biochemical consequences of the change on the assembly of type I collagen.



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Take home messages - 5

- Subsequent investigations showed the disorder had a dominant pattern of mutation in the patient's family. Explain this by reference to the structure of collagen.

Dominant negative effect or Gain of function mutation

The mutation disrupts the activity of the normal version of ColA1

1-POM-4-1

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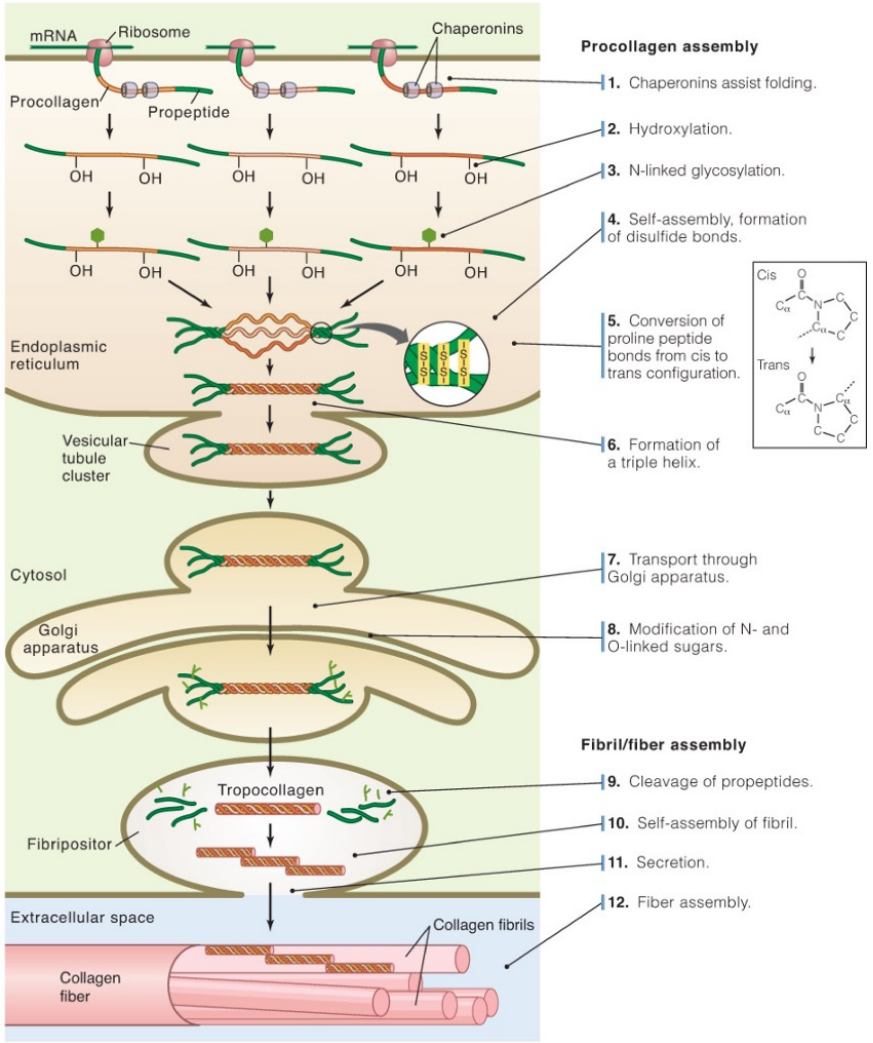
Take home messages - 5



only half of colα1 protein mutated
all fibrils will be affected due to packing

Take home messages - 6

- Why might the predicted change cause skeletal abnormalities and brittle bones?

Initially skeleton laid down as collagen

Later stage mineralisation

If collagen defective then bone defective.

Skeleton is an active tissue whole skeleton turns over every 5-10 years

Questions to be addressed (in groups)

- Suggest a suitable prenatal diagnostic test to identify foetus who will suffer from OI

Take home messages - 7

- Suggest a suitable prenatal diagnostic test to identify foetus who will suffer from OI

1-POM-4-8

Need a sample derived from foetus eg amniocentesis or chorionic villus sampling

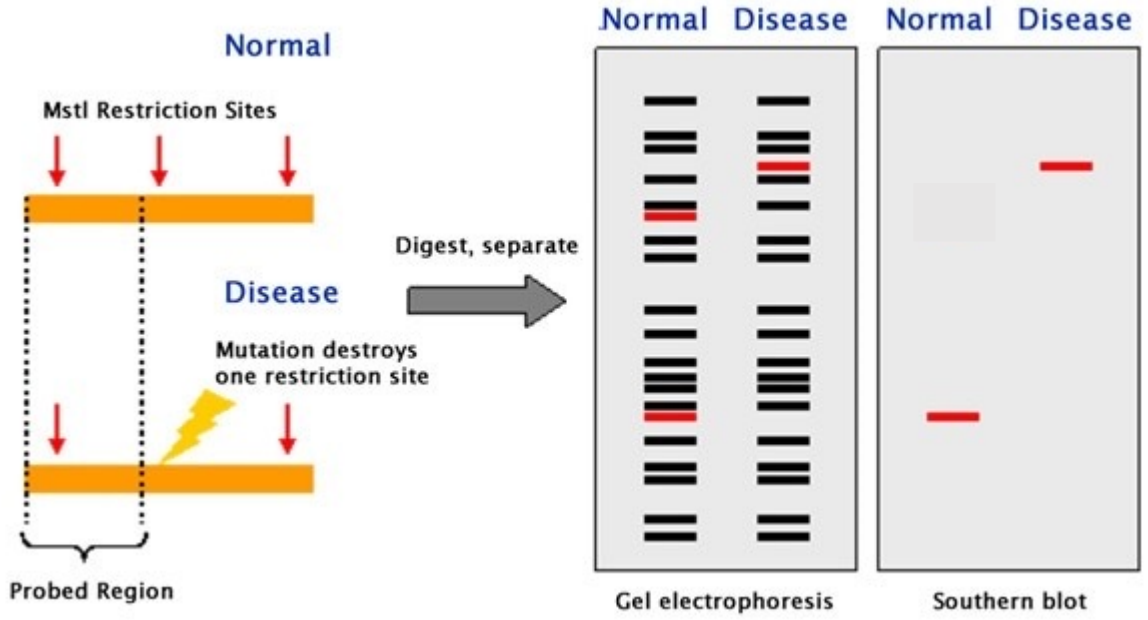
Then can use one of several approaches

Take home messages - 7

- Suggest a suitable prenatal diagnostic test to identify foetus who will suffer from OI

RFLP –restriction fragment length polymorphism

Mutation either removes or creates restriction site
Digest DNA
Gel electrophoresis
Use a probe to region of DNA near mutation



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Take home messages - 7

- Suggest a suitable prenatal diagnostic test to identify foetus who will suffer from OI

PCR –amplify region with mutation
Gel electrophoresis
Use a probe specific for mutation

Sequence PCR product.