Protein secretion

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How do specific amino acid changes alter the function of proteins and cause disease when the protein is secreted from the cell? The authors' studies of human collagen mutations are helping us understand this complex structure–function relationship

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How proteins function is primarily determined by their molecular structure and shape, which is itself dependent on their amino acid sequence. Mutations in a DNA sequence may change the amino acid sequence of a protein and so may affect a protein's function.

Genetic mutations

DNA is the genetic material inside our cells that encodes the instructions for life. Eye and hair colour are well-known examples of human genetic variation. Genetic variation can also underlie characteristics that we cannot immediately see, such as different blood groups. There is genetic variation in our ability to digest lactose, a sugar found in milk. If you have the genetic variant that allows you to digest lactose you are able to drink milk without feeling sick. While DNA variation can result in beneficial things, such as being able to get nutrition from milk, a surprisingly large number of diseases are also caused by changes in DNA — mutations.

DNA mutations can happen during our lives or can be inherited from our parents. Most cancers are examples of the former, and when the mutations are passed down to us from our parents, we call them inherited mutations. One well-known example of a disease caused by an inherited mutation is cystic fibrosis. Inherited diseases are hard to treat because every single cell in the body has the same mutation inside it. However, this also makes them very interesting to study and can teach us a lot about how different proteins are made and function in the body.

In this article we examine inherited genetic diseases that affect a particular class of proteins that are secreted from our cells, and consider how we might treat those diseases.

How do DNA mutations affect secreted proteins?

When a mutation causes the DNA sequence of a gene to change, it can affect the amino acid sequence of the protein it encodes. These changes may be severe

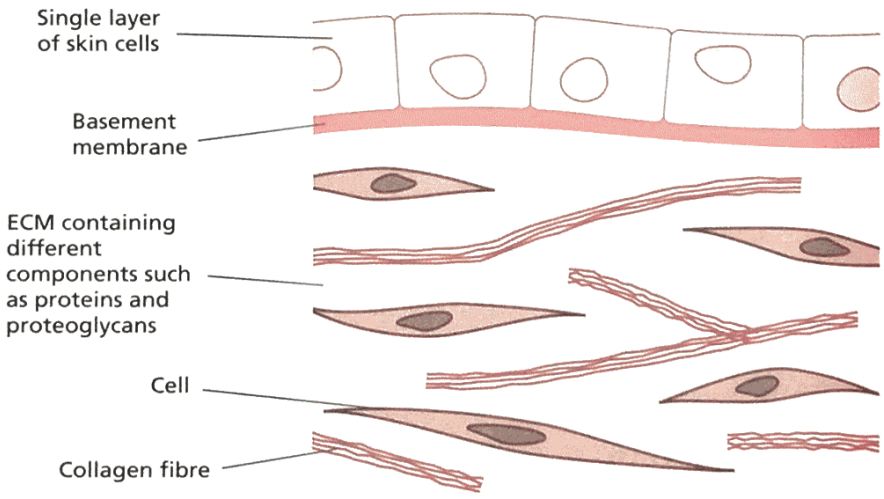
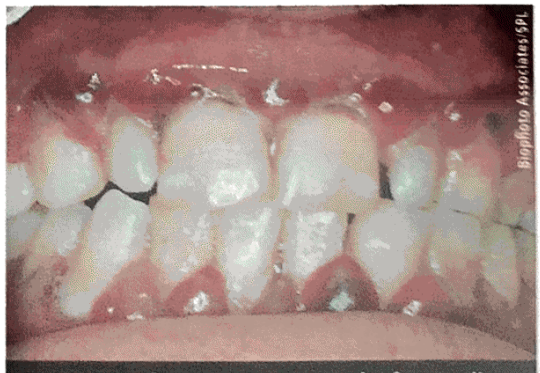


Figure 1 The extracellular matrix (ECM). While cells can be surrounded by an extracellular matrix, other cells form a single layer of cells that sit on top of the extracellular matrix. In this case the extracellular matrix can contain a special structure called the basement membrane. Collagen is a major component of the ECM but the matrix contains many different components.

Box 1 Eat your fruit and veg!

Did you know that vitamin C is essential for effective collagen folding in the ER? But we humans are one of the few mammals unable to make our own vitamin C so we must get it from our diet. Before 1930, sailors on long voyages would go for many months without any vitamin C and as a consequence would suffer a gruesome disease called scurvy, which causes teeth to fall out, gums to bleed and is eventually fatal.



Symptoms of scurvy. Scurvy results from a diet that is devoid of fresh fruit and vegetables

enough to disturb the way the protein folds and modify its physical and chemical characteristics. If these effects alter or destroy protein function, they may cause disease. However, treating genetic diseases effectively can be difficult because they are often poorly understood. If we are to develop better medicines, we need to understand exactly how a mutation in a particular gene causes a specific disease.

Following the journey from DNA (genotype) to disease (phenotype) is especially interesting for proteins that are secreted from cells. This is because they interact with lots of proteins inside the cells when they are being made, as well as with other proteins outside the cells once they are secreted. Any mutation may potentially affect one or several of these interactions and cause a disease. This means that DNA mutations in different parts of the same gene can lead to very different diseases.

Pump it out

We all know that the human body is made up of cells, but it may surprise you to learn that most of the proteins your cells produce are pumped out of the cells into the surrounding space (see Figure 1). The space between cells is filled with proteins, water and other components including modified proteins (such as collagen, see Box 1). These components interact with each other to form the extracellular matrix (ECM). Excluding water, 80% of your body mass comes from the ECM and just 20% from the cells. The ECM is essential for tissue and organ structure and function — without it we would collapse into a big pink blob of cells. So it is important to understand the synthesis and secretion of these critical proteins.

Modifying proteins

Proteins are made in the cytoplasm by the process of translation. This is where the messenger RNA is translated into a long string of amino acids called polypeptides. The polypeptides need to be folded into three-dimensional (3D) structures before they can function. For proteins that function in the cell, this folding happens in the cytoplasm. Proteins that are destined for secretion from the cell or are embedded in the cell membrane are folded in the endoplasmic reticulum (ER), an organelle that is covered with ribosomes (see Figure 2).

Folding in the ER

As the ribosomes on the surface of the ER make polypeptide chains, they feed them into the inside compartment of the ER. Here specific conditions, including pH, temperature and proteins called chaperones, are required for 3D folding. These conditions are controlled by the ER, which also has a proofreading mechanism that should prevent

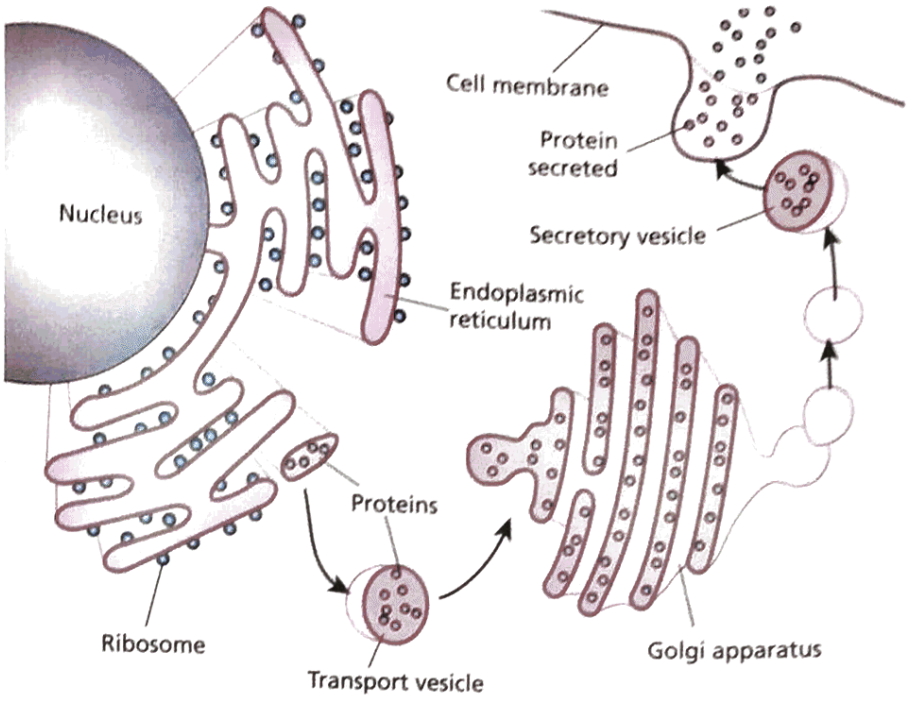
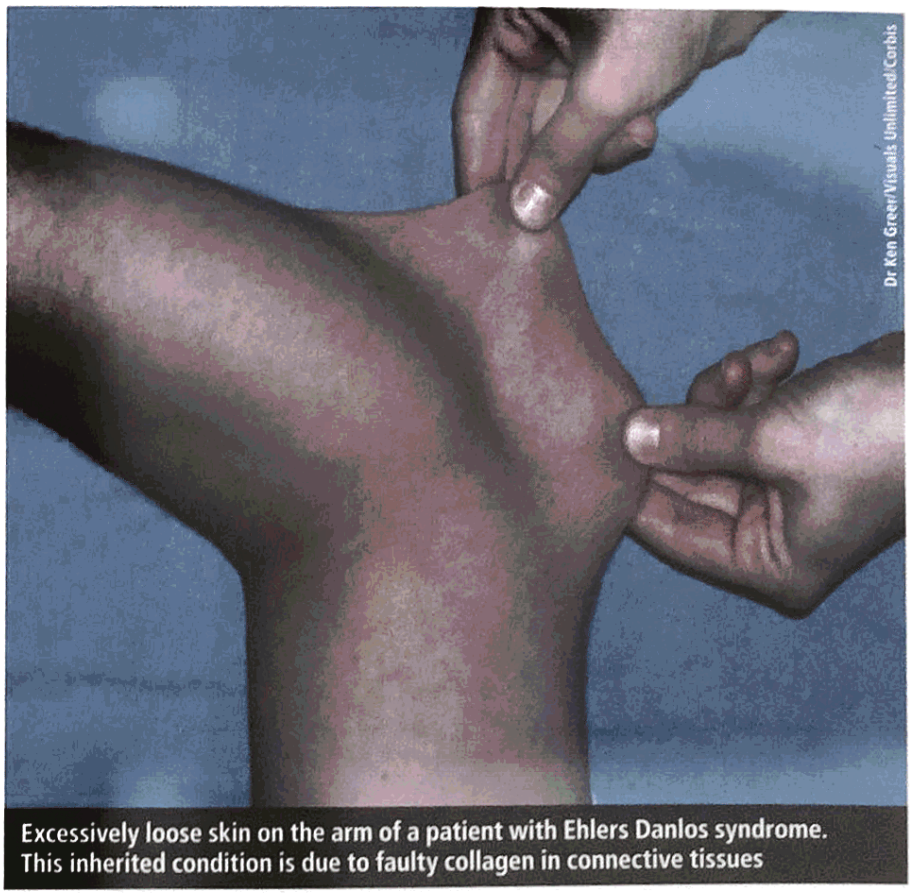


Figure 2 The protein secretion pathway. Secreted proteins are made by ribosomes located on the membrane of the endoplasmic reticulum. These proteins are then folded in the endoplasmic reticulum after which they are transferred to the Golgi complex, which packages the proteins into vesicles. These then travel to the cell membrane and expel the protein

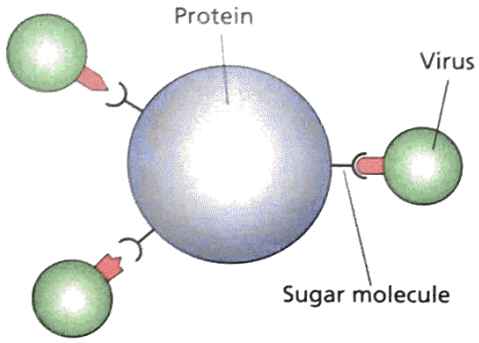
partially or misfolded proteins from leaving. The secretion of misfolded proteins can be bad news for the body and cause diseases. For example, when insulin is not folded correctly it does not function properly and the body is unable to absorb sugar from the blood, which causes diabetes.



Excessively loose skin on the arm of a patient with Ehlers Danlos syndrome. This inherited condition is due to faulty collagen in connective tissues

Box 2 The important final touches

Proteins often need other molecules to be attached to them for them to fulfil their function. For example, adding sugar groups onto certain proteins can help protect them from degradation or enable them to recognise bacteria and viruses. Other kinds of molecules that can be added to proteins after translation are lipids, other proteins and phosphates.



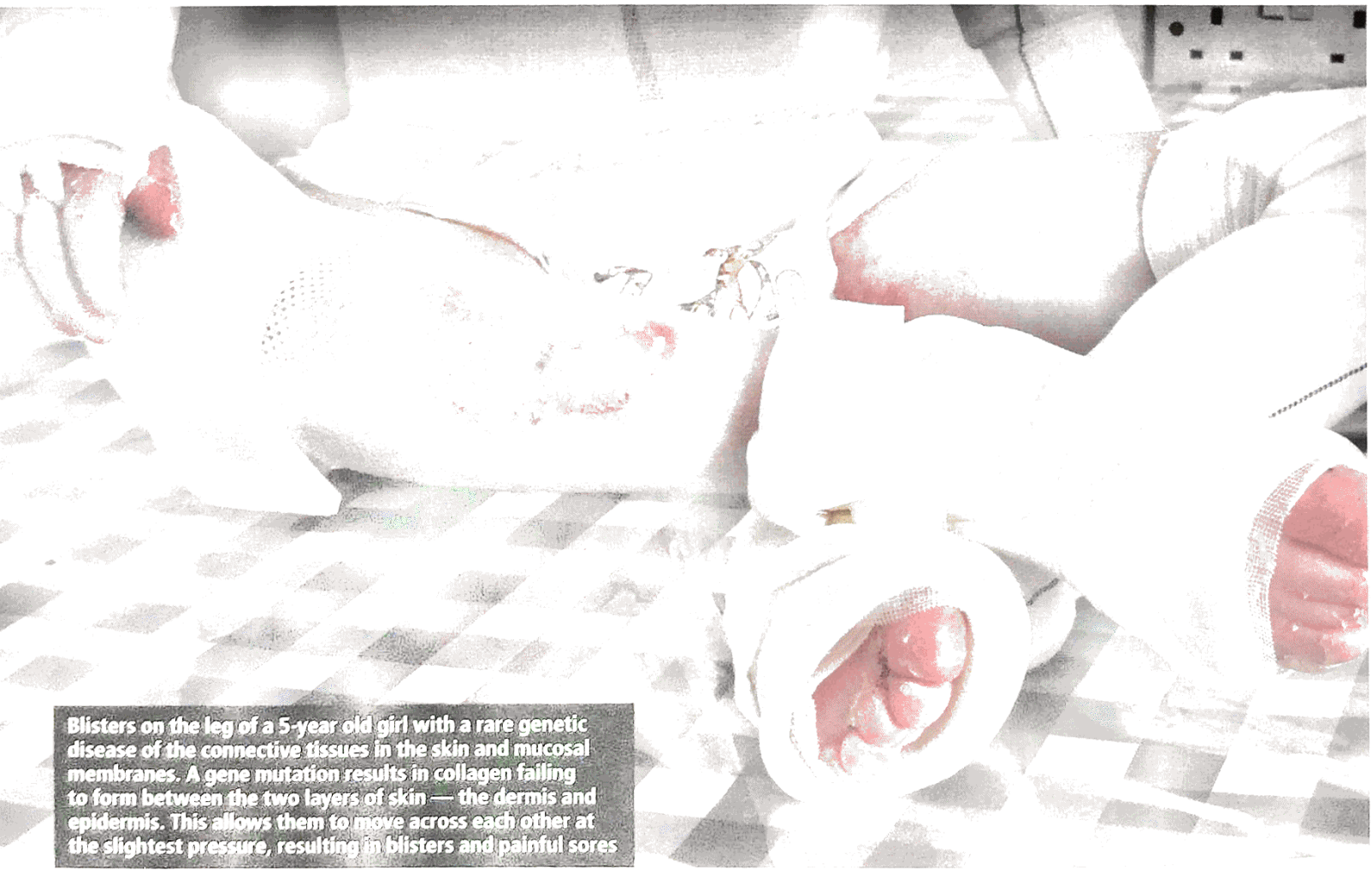
The sugar groups attached to the cell surface proteins allow the cell to recognise dangerous viruses and clear them from the body

When misfolded proteins get trapped in the ER, they may be degraded by the cell, but often the additional time in the ER can result in them eventually becoming correctly folded and being allowed to exit the cell. Once folded, proteins are often further modified by enzymes in the ER (see Box 2), which contribute further to the protein's 3D shape and, consequently, its function. Mutations can also affect any of these important modification steps.

Packaging and sorting in the Golgi apparatus

When the proteins have been appropriately modified and folded into the correct shape, they are transferred out of the ER to the Golgi apparatus. This organelle acts as a packaging and sorting centre. The Golgi apparatus packages the folded proteins into parcels, called vesicles, which transport them to the outer cell membrane. In order to secrete the proteins from the cell, the vesicles merge with the cell membrane and their contents are released (see Figure 2). While some of the proteins are incorporated into cell membranes as receptors and channels, the majority are transported out of the cell. Some of these proteins, such as hormones, travel through the tissues, but many are incorporated into the ECM.

Determining exactly how a mutation in an ECM protein gene can lead to disease means analysing the long journey of the mutant protein from its synthesis and folding to its secretion and incorporation into the ECM. If we can show that mutations are affecting



Blisters on the leg of a 5-year old girl with a rare genetic disease of the connective tissues in the skin and mucosal membranes. A gene mutation results in collagen failing to form between the two layers of skin — the dermis and epidermis. This allows them to move across each other at the slightest pressure, resulting in blisters and painful sores

Maria Platt-Evans/SPL

a step in this pathway, it may allow us to identify the factors that are contributing to a condition. It is only once researchers have determined the mechanism of disease that it is possible to develop treatments and cures.

Collagen

Our laboratory is trying to determine how mutations in one critical component of the ECM — collagen — cause different diseases. In the popular press, collagen is usually associated with cosmetic procedures, but it has important roles in the body. It is the main supportive protein in the ECM and is responsible for bearing the high levels of tension in bone, cartilage and tendon. It is therefore essential for human life.

Collagen interacts with several proteins outside cells to create a variety of structures including fibrils and networks in every single tissue in the body. Consequently, if the 3D structure of a collagen protein is altered, the impact on the body can be widespread and severe. Some mutations in collagen genes can be so catastrophic to the protein function that a fetus with the mutation will be unable to develop to full term. However, even the less severe mutations can still be hugely debilitating, causing a range of diseases such as broken bones, skin blisters, brain bleeds, kidney failure and blindness. So research into how and why collagen mutations lead to disease is of huge clinical importance.

Secreted or not secreted

When researchers discovered that mutations in one type of collagen lead to brittle bone disease, they found that the collagen in the bone ECM of these patients looks very different from that found in healthy people. This is because the mutated protein is still secreted by the cells and disrupts the important ECM structures.

Some mutant collagen genes can damage the protein so much that it can no longer be released from the ER. The ER continues to try to fold the mutant protein but because more and more protein is being pushed inside the ER to be folded, this causes a 'traffic jam' and huge amounts of faulty collagen build up in the ER. When this becomes too much, the stressed cell may commit suicide rather than release the mutant protein. The cell stress caused by a collagen mutation can lead to a type of dwarfism. Although we are making great progress in understanding collagen mutations, with nearly 30 different types of collagen, the mechanism of disease could be different for each mutation (see Figure 3).

Future research

The complexities of the route that all secreted proteins must take to escape from the cell and

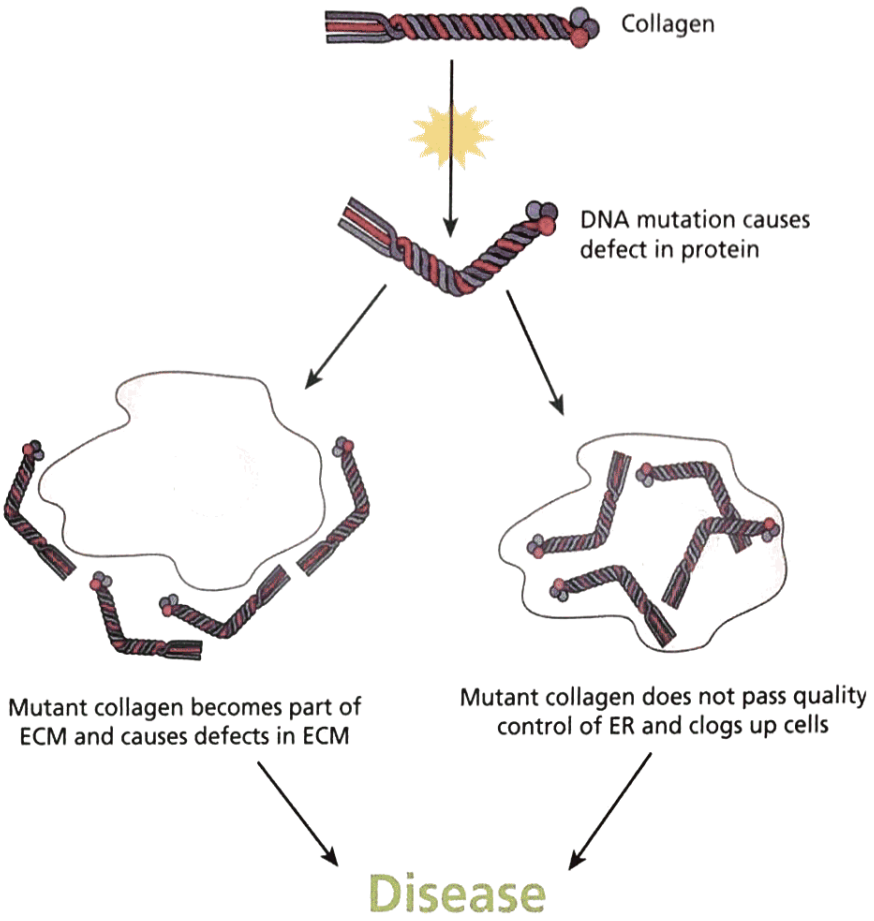


Figure 3 Overview of how some collagen mutations can cause disease

function makes this an exciting area of research. We have begun to appreciate the exquisite control our cells must maintain to protect us from the effects of mutations.

We understand how important research into molecular mechanisms of disease is to the future of medicine. We demonstrated this using collagen as an example, but the same journey from DNA mutation to protein shape, and ultimately changes in function, can be taken when considering the mechanism of any genetic disease. With the exponential growth of sequencing technology we are able to identify more genetic contributions to diseases than ever before. It is critical that we start using this information and are more creative with our drug design so that we can make a significant impact on treating and curing these diseases.

Lydia Murray is a PhD student and **Dr Tom Van Agtmael** is a lecturer at the University of Glasgow. Their group is interested in identifying how mutations in extracellular matrix proteins, such as collagen type IV, cause human diseases such as stroke.

Key points

- Most of the adult human body is extracellular matrix, and proteins need to be secreted from cells to be incorporated into the matrix.
- Collagen is the most abundant protein in the human body and defects in collagen can affect many different organs and tissues.
- Secreted proteins need to be folded in their correct shape. Folding happens in the endoplasmic reticulum.
- Mutations in secreted protein such as collagen can lead to human disease by affecting the extracellular matrix and/or by affecting the folding and secretion of proteins.