# A VERTEBRAL VARIATION MYSTERY:
# The Case for Missing COLLAGEN-8A1

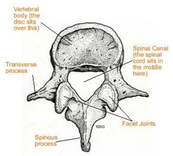
Wauwatosa West SMART Team: Paige Bonner, Zaynab Hassan, Annalise Ho, Alec Lau, Zoe Stack, and Aleksandra Zielonka
Advisor: Mary Haasch  Mentor: Michael Pickart, Ph.D.
Concordia University Wisconsin School of Pharmacy

## Abstract

Scoliosis affects 6 to 9 million people in the United States, and collagen-8a1 may contribute to the disease's development. Collagen-8a1, a structural protein, is found throughout the body, generally serving as a connection at the base of endothelial cells, which line blood vessels and are critical to immune response and growth regulation. The molecule plays a role in angiogenesis, the development of new blood vessels, and smooth muscle cell migration. Collagen-8a1 is a highly conserved protein, meaning there are few variations of the amino acid sequence in different organisms. The only crystallized part of the molecule is a wide conical shape at the end of the uncrystallized rope-like structure (1). A trimer made of chains a, b, and c, collagen-8a1 is held together by hydrogen bonds among sidechains such as tyr660 and tyr738 and water molecules in the central shaft. A single-point mutation at tyr660 on the c chain of the molecule results in a mutant called gulliver in zebrafish (2), causing a distortion of the notochord. Thus, preliminary research in zebrafish suggests a new role for collagen-8a1 in bone formation during development of vertebrae. Research is currently in progress to understand how the absence or mutation of the molecule results in spinal malformations in zebrafish and if this is true for other organisms, including humans. This research could result in further knowledge as to whether dysfunctional collagen-8a1 results in spinal deficiencies. The Wauwatosa SMART Team modeled collagen-8a1 using 3D printing technology

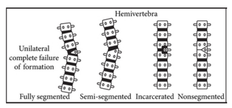
## Misaligned Spine

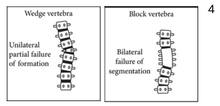
Scoliosis is an abnormal curvature of the spine. [3] Treatments include braces and surgery; however, presently there is no permanent solution. Curvature of the spine can result in pressure on internal organs, the shifting of internal organs' positions, and alteration of gait. No single gene alone appears to cause scoliosis, but a number of genes that are passed down through generations may contribute to the occurrence of families with scoliosis. Environmental factors likely play a significant role alongside the genetics.




Normal Vertebra

Malformed vertebrae is one cause of abnormal curvature of the spine. Different types of vertebral deformations include: hemi, block and butterfly. Hemi vertebrae are wedged shaped, caused by lack of blood supply. Block vertebrae exist when there is a failure in separation between two or more vertebrae. Butterfly vertebrae are missing bone from both sides causing the butterfly shape. [4]

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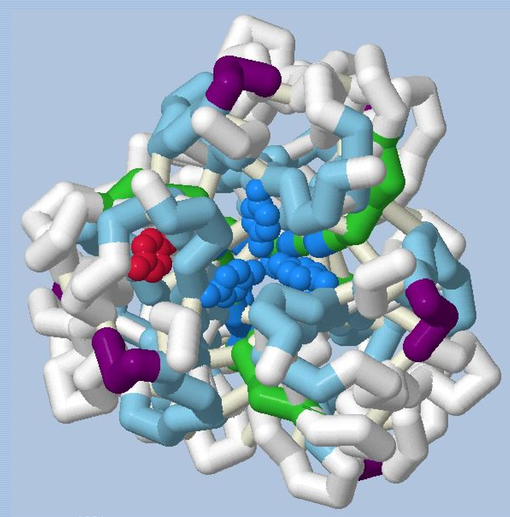
## 3D Model Collagen-8a1

Misshapen vertebrae cause spinal curvature in zebrafish. Preliminary research indicates when collagen-8a1 is knocked out, misshapen vertebrae and spinal curvature develop. Exactly how collagen-8A1 contributes to vertebrae development is not known. Collagen proteins are highly conserved among vertebrates, including zebrafish and humans.



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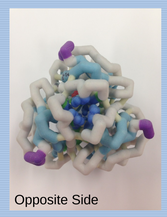


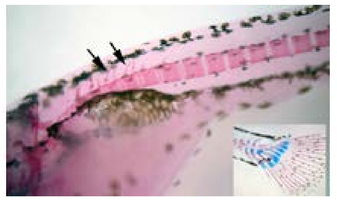
Opposite Side

**Tyr660*:** crimson - amino acid that mutates - single point mutation on the c chain
**Tyr738*:** dodger blue - on chains a, b, and c blocks central shaft
**Lys692*:** dodger blue - on chains a, b, and c, blocks other end of central shaft and hydrogen bonds to carbonyl oxygens.

**Hydrophobic amino acids:** limegreen
**Hydrophilic amino acids:** dodger blue
involved in hydrogen bonding, holding the chains together.
**Helices:** purple
**Beta sheets:** sky blue
**Note:** model based on crystal structure of the globular end of the mouse protein only.
*holds the chain together

## Development of Vertebrae with/without Collagen-8a1

**Wild-type (untreated)**



A: 17 days post fertilization (dpf), Normal vertebrae shape and alignment. Arrows point to normally developing spinal processes.
In preserved larval tissue:
- Calcified bone is stained alizarin red
- Cartilage is stained alcian blue

**Treated (Lacking Col-8A1 both panels below)**
Both: Have been injected with an antisense chemical-morpholino- that interferes with expression of collagen-8a1. Thus, treated larva are lacking the collagen-8a1 protein.


B : 3 dpf, Lack of collagen-8a1 results in a distorted notochord and scoliosis (see insert)

C: 19 dpf, Lack of collagen-8a1 results in abnormally structured and aligned vertebrae.

**Note:** data (unpublished) provided from Abbie R. Collova and Michael A. Pickart at Concordia University Wisconsin

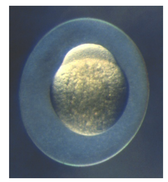
## Ongoing Collaborative Investigation

Dr. Pickart will provide M. Haasch and students larval zebrafish to address the hypothesis that lack of collagen-8a1 contributes to abnormally formed vertebrae and scoliosis.

### Experiment

Step 1: Obtain fertilized eggs with collagen-8a1 removed.

Step 2: Grow and observe eggs, recording normal and mutated outcomes in notochord, individual vertebrae, and overall spinal column shape.



Fertilized egg at 1 cell, (back), with yolk, (front).

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4. McMaster, M.J., "Congenital Deformities of the Spine, *J.R. Coll. Surg. Edinb., 47,April 2002, 475-480*

# Notes

- **Intro needs:**
- **PAIGE: Bone development (long bone development picture)**
- **ZAYNAB: Disease/abnormalities, scoliosis**
- **ALEKS: Collagen8a1 breakdown (insert picture)**
- **ALEC: model**


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