NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

What's Wrong with Our Son?

An Integrative Case Study in Genetics, Molecular and Cell Biology*

by
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Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature, by the careful investigation of cases of rarer forms of disease. —William Harvey, 1657

Part I – Growing Pains

"Your baby's spine is not developing properly and he needs surgery."

This one sentence by the doctor changed Nick and Tracie Patterson's lives forever. Alexander, their baby boy, was just four months old. The family had already been through one ordeal, as Alexander was born six weeks early and he spent the first three weeks of his life in the neonatal special care nursery of the hospital. Now, less than three months later, his parents were receiving the incomprehensible news that Alexander would need a bone graft to stimulate the growth of one of the vertebrae in his spine. The doctors predicted that without the procedure, Alexander's spine would continue to bend, resulting in an excessively rounded back. The doctors also told Nick and Tracie that in extreme cases, a reduction in chest volume could result in heart and lung problems and the spinal cord could become compressed, leading to nerve and muscle problems. Nick and Tracie agreed to the surgery, waiting anxiously for the graft to take hold and the vertebra to grow. However, the next report from the doctors was equally difficult for Nick and Tracie to hear.

"We're sorry to say that the graft did not have the desired effect, and we'll have to repeat the operation" said one of Alexander's doctors. "This time, we'll graft a larger amount of bone from Alexander's right femur [thighbone]."

Thankfully, the second spinal graft was a success. Sadly, however, this was just the beginning of a long series of heartbreaking trips to many different doctors for Nick and Tracie and their son. As his parents would soon find out, the early bending of Alexander's spine was only one of many symptoms to manifest themselves, and many other parts of his skeleton would also fail to develop properly. Although Alexander's arms appeared to grow in proportion to his torso, he was slow to grow taller; his doctors estimated that he would probably not exceed much more than four and a half feet in overall height. More painful, however, would be Alexander's abnormal joints, which would require repeated, corrective operations on both of his hips, knees and ankles. In sum, Alexander would have close to a dozen operations spanning his childhood and young adult years. Following each operation, Alexander would be in a body or leg cast, and he would have to teach himself to walk all over again each time the casts were removed.

Questions

1. List the developmental problems that Alexander has had in his life.

2. Is there one particular type of tissue or organ that seems most affected in his disorder?

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*The information presented in this case study is based on fact, although the names of the characters have been changed for reasons of privacy.

Alexander's Aunt Janet is a biologist, and a few years after his birth, Tracie told Janet that a specific diagnosis had finally been made.

"Alexander has a disorder known as spondyloepiphyseal dysplasia congenita (SEDC)," said Tracie.

With this diagnosis in hand, Janet went immediately to the Internet to learn more about SEDC, with the hopes of better understanding the biological basis of Alexander's condition.

Questions

3. One can learn a lot about SEDC by first considering the meaning of the word roots that make up its name. For each of the word woots below, write down a brief definition in your own words, using terms that you understand. For help, use one or both of the following on-line medical dictionary sites:

   Merriam-Webster Medical Dictionary at <https://www.merriam-webster.com/medical>

   The FreeDictionary's Medical Dictionary at <https://medical-dictionary.thefreedictionary.com/>

   a. *spondylo-*

   b. *-epiphyseal* [see also *epiphysis* and *epiphyseal plate*]

   c. *dysplasia*

   d. *congenita[l]*

4. Putting the above definitions together with your answers to Questions 1 and 2, what appear to be the primary (defining) features of SEDC?

Part II – Genetic Basis of the Disorder

As Alexander's family and you have now discovered, spondyloepiphyseal dysplasia congenita (SEDC) is a genetic disorder in which the primary deficits are abnormal growth of one or more spinal vertebrae and the growing ends of the femurs, resulting in disproportionate, short-trunk dwarfism (Parikh & Crawford, 2017). Interestingly, both of Alexander's parents, his grandparents and an older sister are of normal height. Therefore, what do you think the mode of inheritance is for SEDC?

Let's make a couple assumptions at this time. First, assume that a mutation in a single gene is responsible for Alexander's condition. Second, assume that the affected gene is on an autosome (non-sex chromosome). Do you think that the mutant allele that has caused Alexander's condition is dominant or recessive to the normal (wild-type) allele for this gene? Test these two alternative hypotheses by drawing two copies of a small family pedigree according to the questions below that includes Alexander, his sister and each of his parents.

Questions

1. For one pedigree, draw the symbols and add the likely genotypes of Alexander, his sister and each of his parents, as if SEDC is an autosomal *dominant* condition. Use the letters "D" and "d" to represent the dominant and recessive alleles, respectively, for the affected gene.

2. For the second pedigree, draw the symbols and add the likely genotypes of Alexander, his sister and each of his parents, as if SEDC is an autosomal *recessive* condition. Use the letters "D" and "d" to represent the dominant and recessive alleles, respectively, for the affected gene.

3. Which hypothesis do you think is supported by Alexander's family pedigree?

As you will soon learn, SEDC is actually an autosomal dominant disorder. Given that both of Alexander's parents are normal height (i.e., neither has SEDC), how is it possible that Alexander came to have an autosomal *dominant* disorder? This is a question that Alexander's father has also wondered about.

Question

4. How would you explain this to Alexander's father? In other words, what are some possible causes of a new mutation, and when and where (i.e., in what cell type) might Alexander's SEDC mutation have appeared? Brainstorm possible answers to these questions and list some possibilities.

Part III – The Affected Gene and its Encoded Protein in SEDC

Another question that Alexander's parents asked Janet early on is "Why is Alexander's skeleton not developing correctly?" The number of genes that affect bone growth and development is quite large however, and it was difficult initially for Janet to determine which gene might be the culprit. With Alexander's diagnosis of SEDC a few years later, Janet was then able to quickly discover the identity of the affected gene: *COL2A1*. Knowing the identity of the affected gene then enabled Janet to explore the biological basis of the disorder in some depth.

Use the following Web sites and questions to guide your own explorations into the biological causes of SEDC.

- National Center for Biotechnology Information (NCBI) Gene database – Gene ID 1280. <https://www.ncbi.nlm.nih.gov/gene/1280>
- NIH – U.S. National Library of Medicine – Spondyloepiphyseal dysplasia congenita. <https://ghr.nlm.nih.gov/condition/spondyloepiphyseal-dysplasia-congenita>
- Medscape – Spondyloepiphyseal Dysplasia. <https://emedicine.medscape.com/article/1260836-overview>
- MedicineNet.com – Medical Definition of *COL2A1*. <http://www.medterms.com/script/main/art.asp?articlekey=34100>
- Wikipedia – Collagen. <http://en.wikipedia.org/wiki/Collagen>
- Little People of America. <http://www.lpaonline.org/>

Questions

1. What is the name of the polypeptide that is encoded by the *COL2A1* gene?

2. What multimeric protein does this polypeptide assemble into? Is this a rare or abundant protein? Is it a unique protein or part of a large family of proteins?

3. Where in the body (i.e., in what tissues or organs) is the *specific* protein encoded by the *COL2A1* gene normally expressed (i.e., present) at high levels?

4. Where *specifically* in a tissue is the mature/functional protein found?

5. What does the protein do there?

6. Is there any discrepancy between the distribution of type II collagen in the body and Alexander's symptoms? If so, what symptoms do some other individuals with SEDC have that Alexander (thankfully) does not?

Extension Questions

7. How do individuals with SEDC differ in physical appearance from individuals with other types of dwarfism (such as Peter Dinklage from the HBO series *Game of Thrones*)?

8. What is a biological explanation for the different types of dwarfism?

9. Is dwarfism in general a relatively common or uncommon disorder in the U.S. population?

Part IV – From Genotype to Phenotype for SEDC

Overview of Janet's Initial Research on the COL2A1 *Gene and Its Protein Product*

Remember from Part II that SEDC is an autosomal dominant disorder. This means that all of Alexander's cells contain one unaltered (wild-type) allele as well as the mutant allele for the *COL2A1* gene. In contrast, most heterozygous individuals who are carriers for recessive disease/disorder alleles (such as for cystic fibrosis) are unaffected and healthy. As a biologist, Janet wanted to understand why Alexander's heterozygous *COL2A1* genotype resulted in a mutant (affected) phenotype (SEDC). By doing additional research using on-line databases, Janet hoped to use what she learned to explain to Alexander's family why the protein encoded by Alexander's wild-type *COL2A1* allele was not able to carry out its normal function as his skeleton developed and grew.

To understand possible answers to her questions, Janet examined the structure of the *COL2A1* gene, the sequence of amino acids encoded by that gene, the cellular pathway of protein synthesis and secretion, and the three-dimensional structure of a type II collagen molecule and fibril in more detail. This is what Janet discovered.

- *COL2A1* is not a simple gene: it is over 30 kilobases (kb) in length and has 53 introns and 54 exons! The coding region of the corresponding mRNA molecule is 4,461 base pairs (bp) long, resulting in a polypeptide chain that contains 1,487 amino acids (RSCB Protein Data Bank, <http://www.rcsb.org/pdb/gene/>; *COL2A1* and select "Protein Feature View" link).
- As a protein destined for secretion into the extracellular space, the polypeptide chains are deposited into the lumen of the rough endoplasmic reticulum (RER) as they are synthesized by ribosomes.
- The amino acid sequence of the nascent polypeptide chains can be subdivided into three regions: an N-terminal prepropeptide (amino acids 1–181); a long central region (amino acids 182–1241); and a C-terminal propeptide (amino acids 1242–1487) (UniProt Knowledgebase, < https://www.uniprot.org/>, P02458, COL2A1_Human).
- Extensive post-translational processing of the polypeptide chains occurs in both the RER and Golgi apparatus prior to secretion, and additional processing of the protein occurs immediately following secretion to form a mature type II collagen molecule (Lodish *et al.*, 2000).

Given the complex structure of the *COL2A1* gene and the high degree of processing needed to synthesize both its mature mRNA and protein, Janet was not surprised to discover that mutations in the *COL2A1* gene lead to a number of different clinical disorders in human cartilage and bone development and growth. For SEDC alone, at least 56 different mutations in the *COL2A1* gene had been identified in SEDC individuals worldwide by 2016, and new mutations continue to be discovered (Xiong *et al.*, 2018). The next challenge for Janet was to understand what the molecular, cellular and tissue impacts might be for some of these different SEDC mutations.

Student Research on COL2A1 *Gene Mutations in SEDC Individuals*

Once again, put yourself in Janet's shoes to next examine the possible effects of *COL2A1* gene mutations in SEDC individuals. Building on the above overview of the *COL2A1* gene and the polypeptide chain that it encodes, use the questions that follow to guide your analyses. Research answers to these questions by consulting the previous links and additional sources of information listed below. You are encouraged to consult other sources, including your instructors, for help as needed. Our hope is that this process, though not easy, will be interesting and provide you with a deeper understanding of the genotype to phenotype relationships that are so vital to the inner workings of living organisms.

Information Sources

- Ricard-Blum, S. 2011. The collagen family. *Cold Spring Harbor Perspectives in Biology* 3:a004978. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3003457/>
- NIH—U.S. National Library of Medicine. *COL2A1* gene. <https://ghr.nlm.nih.gov/gene/COL2A1>
- National Center for Biotechnology Information (NCBI). Structure database: PDB #1K6F. <https://www.ncbi.nlm.nih.gov/Structure/pdb/1K6F>. (Examine a short, artificial 3-D model of collagen.)
- Review the amino acid and protein structure pages in your textbook, as needed.
- Mescher, A. L. 2016. *Junqueira's Basic Histology* 14th ed. McGraw-Hill Education. <https://accessmedicine.mhmedical.com/book.aspx?bookID=1687>
- Online Mendelian Inheritance in Man (OMIM). #183900 Spondyloepiphyseal dysplasia congenita; SEDC. <http://omim.org/entry/183900>
- National Center for Biotechnology Information (NCBI). Gene database: *COL2A1* collagen type II alpha 1 chain [*Homo sapiens* (human)]. <https://www.ncbi.nlm.nih.gov/gene/1280> (Scroll to "Variation" section, select "See variants in ClinVar" link.)
- RSCB Protein Data Bank. Collagen alpha-1(II) chain – P02458 (CO2A1_HUMAN). <http://www.rcsb.org/pdb/protein/P02458>
- UniProt Knowledgebase. UniProtKB – P02458 (CO2A1_Human). <http://www.uniprot.org/uniprot/P02458#PRO_0000005730>

Important Notes: Do not copy and paste directly from another source to answer the following questions. Instead, write answers in your own words, using terms that you understand. If you use a source other than those listed in this case study, please cite that source. You are welcome to supplement your answers with diagrams or images. Remember to also cite the sources of all figures (if not created by you).

Questions

Section A – Building a Type II Collagen Molecule

1. All collagen molecules share the same three-dimensional (3-D) arrangement of collagen polypeptide chains (for at least part of their length). Briefly describe this 3-D structure.

2. How does the primary (amino acid) sequence of the individual alpha-1(II) polypeptide chains direct assembly into a type II collagen molecule? How much of a mature type II collagen molecule has the tri-helical structure?

3. Examine a 3-D model of a short artificial collagen triple helix, created from crystals of three identical chains, each with ten Pro-Pro-Gly repeats. Using NCBI's 3-D protein imaging software (Cn3D) at <https://www.ncbi.nlm.nih.gov/Structure/pdb/1K6F>, launch the full-featured 3D viewer.
   a. Select Style-> Proteins -> Ball and Stick to add the proline side chains and peptide bond atoms to the model. Copy and paste a screen shot of your model into your report.
   b. Next, select Color -> Atom. The oxygen atoms of each peptide bond now appear red and the nitrogen atoms are blue. Select one P(ro)-P(ro)-G(ly) repeat of one chain in the Sequence viewer, which will highlight those same three residues in the model. Copy and paste a screen shot of your model into your report. How are the proline side chains oriented as compared to the alpha carbon atoms of the glycine residues?
   c. In looking at these models, imagine how polar –OH groups that protrude out from hydroxyproline side chains in native collagen molecules would provide additional sites for interchain interactions to stabilize the triple helix.

Section B – Assembling a Type II Collagen Fibril

4. Briefly describe the process by which collagen molecules assemble into fibrils, which are the functional units of type II collagen.

5. How is the three-dimensional structure of type II collagen fibrils well suited to their function during bone growth and development?

Section C – Effects of Mutations in the COL2A1 *Gene of SEDC Individuals on Type II Collagen Structure*

6. Pick two different mutations in the *COL2A1* gene that are present in patients with SEDC. How and where is the amino acid sequence of the collagen type II alpha 1 polypeptide chains changed for each of these two mutant alleles?

7. As previously discussed, Alexander is likely heterozygous at the *COL2A1* gene locus, with one mutant and one wild-type allele. Therefore, his epiphyseal cartilage cells should have produced significant amounts of normal collagen type II alpha 1 polypeptide chains. Using what you learned in Sections A and B about the assembly of type II collagen molecules and fibrils, formulate a hypothesis for how the presence of both normal and abnormal collagen type II alpha 1 polypeptide chains for one of the mutant alleles that you described in Question 6 could lead to abnormal epiphyseal cartilage and perturbed bone growth.

   Note: this answer will require significant thought and should be presented as a detailed, multi-part hypothesis, in which you address the following topics.

   - The composition of type II collagen molecules and fibrils in a heterozygous individual. In other words, what is the probability of assembling totally normal type II collagen molecules or fibrils in SEDC individuals?
   - The nature of the specific mutation with regards to the amino acid sequence of the collagen alpha 1(II) polypeptide chains and its possible effects on the structure and function of the mutant type II collagen molecules and fibrils.
   - The role of the RER in protein folding and processing.

8. What is one remaining question that you have about the correlation between mutations in the *COL2A1* gene and the incidence of SEDC?

Part V – Living with SEDC and Future Prospects

When Janet was searching the Internet for more information about SEDC, she discovered that the National Institutes of Health (NIH) in Bethesda, Maryland was running a large clinical trial of patients with various skeletal disorders, including SEDC. Janet immediately called Tracie to let her know about the study, so that Nick, Tracie and Alexander could apply to participate. If accepted into the trial, Alexander would travel from Massachusetts to Bethesda on one or more occasions to undergo a detailed physical examination. Since there are many different mutant alleles of the *COL2A1* gene that can cause SEDC, Alexander would also undergo genetic testing to pinpoint the nature of the specific mutation that he carries in his *COL2A1* gene. Alexander (and his parents) would then receive genetic counseling and other follow-up care. Alexander was twelve years old at the time and settling back into school after one of his many surgeries.

"The decision is up to Alexander as to whether or not he wants to participate," Tracie told Janet.

Questions

1. If this had been your choice at the time, what would you have done?

2. After considering your own decision, discuss this question with your groupmates. How is your choice similar or different than your groupmates' choices?

3. As a 12-year old, Alexander decided against joining the trial, as he wanted to settle back into as normal a life as he could after his most recent surgery. Discuss with your groupmates why Alexander might be interested in receiving genetic counseling and genetic testing later in his life, as a young adult.

References

Little People of America. *n.d.* [Website]. <http://www.lpaonline.org>.

Lodish, H., A. Berk, S.L. Zipursky, P. Matsudaira, D. Baltimore, and J. Darnell. 2000. *Molecular Cell Biology,* 4th ed. W.H. Freeman. Searchable at: <https://www.ncbi.nlm.nih.gov/books/NBK21582/>.

Mescher, A. L. 2016. *Junqueira's Basic Histology,* 14th ed. McGraw-Hill Education. <https://accessmedicine.mhmedical.com/book.aspx?bookID=1687>.

MedicineNet.com – Medical Definition of *COL2A1*. <http://www.medterms.com/script/main/art.asp?articlekey=34100>.

Medscape – Spondyloepiphyseal dysplasia. <https://emedicine.medscape.com/article/1260836-overview>.

Merriam-Webster Medical Dictionary: <https://www.merriam-webster.com/medical>.

National Center for Biotechnology Information (NCBI) Gene database: Gene ID 1280: <https://www.ncbi.nlm.nih.gov/gene/1280>.

National Center for Biotechnology Information (NCBI). *n.d.* Structure database: PDB #1K6F. <https://www.ncbi.nlm.nih.gov/Structure/pdb/1K6F>.

NIH – U.S. National Library of Medicine. *n.d*. *COL2A1* gene. <https://ghr.nlm.nih.gov/gene/COL2A1>.

NIH – U.S. National Library of Medicine. *n.d.* Spondyloepiphyseal dysplasia congenita. <https://ghr.nlm.nih.gov/condition/spondyloepiphyseal-dysplasia-congenita>.

Online Mendelian Inheritance in Man (OMIM) – #183900 spondyloepiphyseal dysplasia congenita; SEDC. <http://omim.org/entry/183900>.

Parikh, S., P. Batra, and A.H. Crawford. 2107. Spondyloepiphyseal dysplasia. WebMD. <http://emedicine.medscape.com/article/1260836-overview>.

Ricard-Blum, S. 2011. The collagen family. *Cold Spring Harbor Perspectives in Biology* 3:a004978. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3003457/>. DOI: 10.1101/cshperspect.a004978.

RCSB Protein Data Bank (PDB). *n.d. COL2A1*: collagen type II alpha 1 chain. <http://www.rcsb.org/pdb/gene/>. (Search *COL2A1* and select Protein Feature View link therein.)

The FreeDictionary by Farlex: Medical Dictionary. <https://medical-dictionary.thefreedictionary.com/>.

The Works of William Harvey. <https://en.wikisource.org/wiki/Page:The_Works_of_William_Harvey_(part_2_of_2).djvu/98>.

Wikipedia – Collagen. <http://en.wikipedia.org/wiki/Collagen>.

UniProt Knowledgebase (UniProtKB). *n.d.* P02458 (CO2A1_Human). <http://www.uniprot.org/uniprot/P02458>.

Xiong, Q., Y. Liu, Y. Xue, J. Wang, P. Li, C. Wu, Y. Yang, and H. Xiao. 2018. A novel *de novo* mutation in COL2A1 leading to spondyloepiphyseal dysplasia congenita in a Chinese family. *Human Genome Variation* 5: 17059. DOI:10.1038/hgv.2017.59.

Internet references accessible as of January 2, 2020.