

# COPA Syndrome: Adventures in Intracellular Transport

by

Madison Hossack and Kenneth W. Yip  
Department of Cell and Systems Biology  
University of Toronto, Ontario, Canada



## Part I – “I’ve Never Seen This Before . . .”

*The following case study is based on a true story and presented here with the permission of the affected family. The names of the family members have been changed.*

### Ethan

A seemingly casual comment from the community radiologist reverberated in Cathy’s head.

Cathy was not a scientist, but she knew that something was wrong with her four-year-old son Ethan. Even though his parents and grandparents were of above average height and weight, Ethan was always shorter and thinner than the other children. Their family doctor told Cathy that Ethan was in the bottom third percentile for his age group, or less, and suggested that he was demonstrating a “failure to thrive.”

Recently, Ethan developed a recurring cough, particularly when running around during recess and while laughing with his friends. His family doctor suspected asthma, but asthma inhalers did not help. His family doctor then sent him for a chest X-ray.

The radiologist saw blurry areas in Ethan’s lung on the X-ray (Figure 1, next page). He called these “diffuse lung markings,” and said he had never seen them before. He referred Ethan to a special pediatric hospital, the Hospital for Sick Children. There, further imaging (X-ray and CT) suggested interstitial lung disease (ILD). A surgical biopsy and a team of pathologists confirmed the diagnosis as follicular bronchiolitis, a rare form of ILD in which the bronchioles become inflamed and blocked by small growths filled with immune cells.

Gene sequencing was done to further investigate the cause. Upon sequencing of Ethan’s DNA, no mutations were detected in any of the 30 genes that are commonly sequenced to diagnose ILD. After more extensive sequencing, Ethan was found to have a heterozygous mutation in the *COPA* gene, changing the 239<sup>th</sup> amino acid of the protein from an alanine to a proline.

### COPA Syndrome

At a glance:

- COPA syndrome is a rare inflammatory autoimmune disease.
- COPA syndrome results from a single mutation in the *COPA* gene, which is inherited in an autosomal dominant manner.
- The lungs, joints, and kidneys are commonly affected by variable symptoms.
- Patients present early in childhood, at an average age of approximately four years.

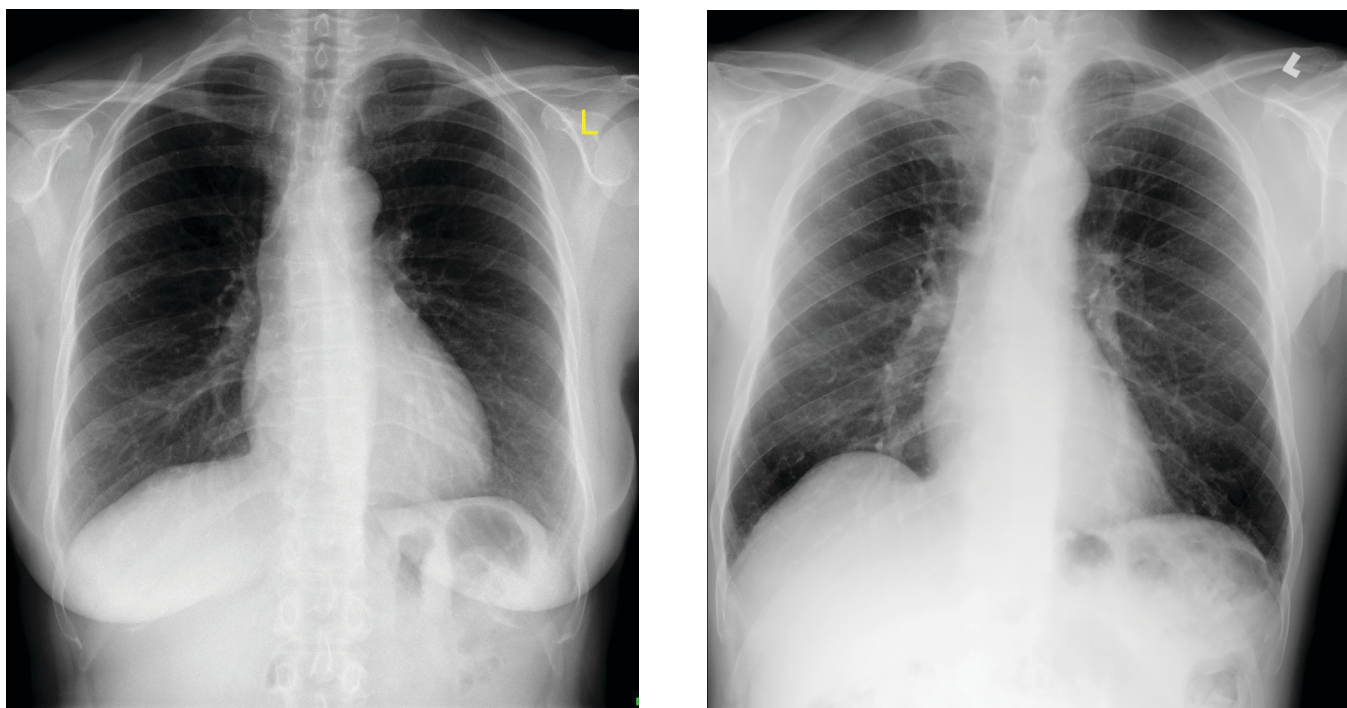


Figure 1. Chest X-rays of normal lungs (left) and lungs of a follicular bronchiolitis patient (right). Note the white-coloured material visible in the lungs of the follicular bronchiolitis patient in comparison to the “clarity” of the normal lungs. Image credits: Left panel from Knipe, Radiopedia.org, 2014, CC BY-NC-SA 3.0. Right panel from Salam, Radiopedia.org, 2016, CC BY-NC-SA 3.0.

COPA syndrome is a *monogenic disease*, meaning that it is caused by a single mutation in one gene, *COPA*, which encodes the  $\alpha$  subunit of the COPI protein complex. Seven different mutations in *COPA* have been documented in relation to COPA syndrome, and any one of these mutations can cause the disease.

Mutations in *COPA* follow an *autosomal dominant* inheritance pattern. COPA syndrome displays *incomplete penetrance*, which means that some individuals will inherit a mutation and display no symptoms. There is also *variable expressivity* among patients, which means that individuals with symptoms will display them with varying degrees of severity. For example, joint symptoms can range from mild pain to osteonecrosis (bone tissue death); patients with pulmonary symptoms may require interventions ranging from medications to lung transplants. In addition to being heritable, mutations in *COPA* can arise *de novo* in the germline of an individual with no family history of COPA syndrome.

COPA syndrome patients often present early in childhood with *interstitial lung disease (ILD)* and *juvenile arthritis*.

- ILD is associated with lung inflammation and an aberrant immune response leading to scarring, which can affect a patient’s ability to breathe properly, particularly during exertion. Several distinct lung diseases fall under the category of ILD, and they range in severity: some patients experience only exercise-induced breathing difficulties, while others require supplementary oxygen to breathe comfortably, and some must even undergo a lung transplant.
- Arthritis is characterized by inflammation of the joints, and manifests as swelling and pain at the affected joint. Patients often find it difficult to use the arthritic joint for movement, which can affect their mobility to varying degrees depending on the severity of the arthritis. Juvenile arthritis refers to arthritis presenting in childhood, which is when COPA syndrome is typically diagnosed.

The exact mechanisms of immune dysregulation in COPA syndrome are unknown, and occupy the intense focus of researchers studying COPA syndrome. In brief, the leading hypothesis to date is that COPA syndrome mutations initiate errant immune signalling leading to consistent immune overactivity and high levels of immune cells. In the context of the lungs, this damages the delicate respiratory passages and membranes of the lung, leading to fibrosis, or scarring. Fibrosis of the lung is known to impede gas exchange and cause the effects observed in patients.

If COPA syndrome is detected early, symptoms can be managed more effectively. For example, the lasting damage to the lungs caused by ILD-related scarring can be mitigated through anti-inflammatory treatment. The earlier that COPA syndrome is diagnosed, the greater the chance that symptom severity and discomfort can be reduced for the patient.

### Questions

(The following questions are not based on the true story related to the above case.)

1. Ethan's father, Rob, has taken high school biology. Rob remembers learning that autosomal dominant disorders would be observed in one of the parents. Rob is curious as to how Ethan has COPA syndrome given that neither he nor Cathy have any signs of the disease. What might be some explanations?
2. When she learns of Ethan's condition, Rob's mother Sharon says that it reminds her of her older brothers, Tom and Alex. They passed away before Ethan was born, but they had both always struggled to keep up with the other neighbourhood boys when they would play street hockey. She remembers her parents often being worried about the boys' strength, especially when the doctor told them that Tom appeared to have childhood arthritis. Given this information, draw a possible pedigree for Ethan's family that explains how he inherited the COPA syndrome mutation. Indicate the family members displaying the COPA syndrome phenotype with an asterisk (\*) beside their name.
3. The hospital is offering to sequence the *COPA* gene of Ethan's older sister Sarah, as well as their biological parents, aunts, uncles, and other family members. Suppose that you are a member of Ethan's family. Would you want to have your *COPA* gene sequenced? Why or why not? What issues do you need to consider and what factors would contribute to your decision?
4. Sarah is sixteen years old and adamantly states that she does not want to have her *COPA* gene sequenced because she is afraid to find out if she also has COPA syndrome. If you were one of Ethan and Sarah's parents, what would you say to Sarah?

## Part II – Moving Deeper

### *Molecular and Cellular Biology of Endomembrane System Vesicular Transport*

The transport of proteins, nutrients, and other critical materials within cellular compartments and between the cell and its external environment is essential for the proper functioning of cells, tissues, and organisms. Intracellular transport is carried out by membrane-bound *transport vesicles*, which enclose cargo materials and carry them from the *donor compartment* (from which vesicles originate) to a *target compartment* (where cargo materials are required to carry out a function or are degraded). Vesicles are formed from the membrane of the donor compartment through a process called *budding*, and *fuse* to the target compartment to release their cargo to its destination (Figure 2).

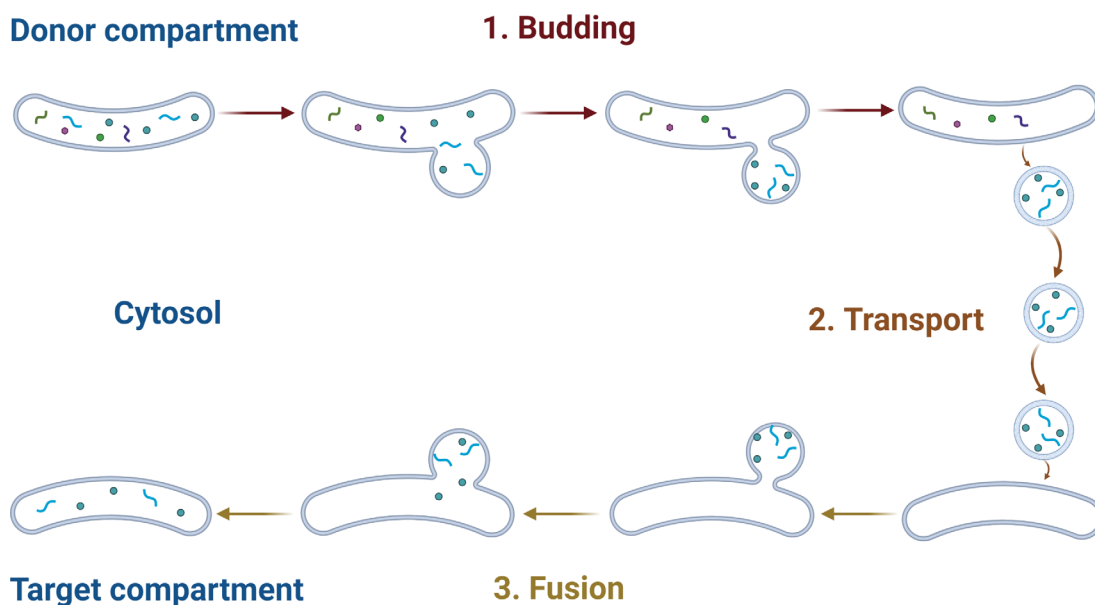


Figure 2. Transport vesicle budding and fusion.

Transport vesicles must be highly *specific* to function properly: they must extract only specified materials from a donor compartment, and deliver their cargo only to the specified target compartment. This specification is mediated by the *coatome* complex on the surface of the vesicle. The constitutive *coat proteins* of the coatome selectively bind the appropriate material for transport, remodel the donor membrane to create a vesicle (budding), carry out transport to the appropriate target compartment (transport), and selectively fuse to the membrane of the target compartment (fusion). The donor and target compartments can be the membrane of an organelle, or the membrane of the cell itself, since intracellular transport involves both transport between organelles and the exchange of materials with the external environment via the plasma membrane. The particular combination of coat proteins present in the donor membrane confers these specifications so that each piece of material in the cell is transported efficiently as needed.

Coatomers can be divided into three major categories based on the routes through which they direct vesicular transport (Figure 3, next page).

- *Clathrin*-coated vesicles transport materials from the plasma membrane to organelles within the cell, and between the Golgi apparatus and endosomes.
- *COPI*-coated vesicles transport materials between Golgi cisternae and from the Golgi apparatus to the endoplasmic reticulum (ER).
- *COPII*-coated vesicles transport materials from the ER to the Golgi apparatus.

Movement away from the extracellular space/plasma membrane is termed *retrograde* transport, while movement towards the extracellular space/plasma membrane is termed *anterograde* transport.

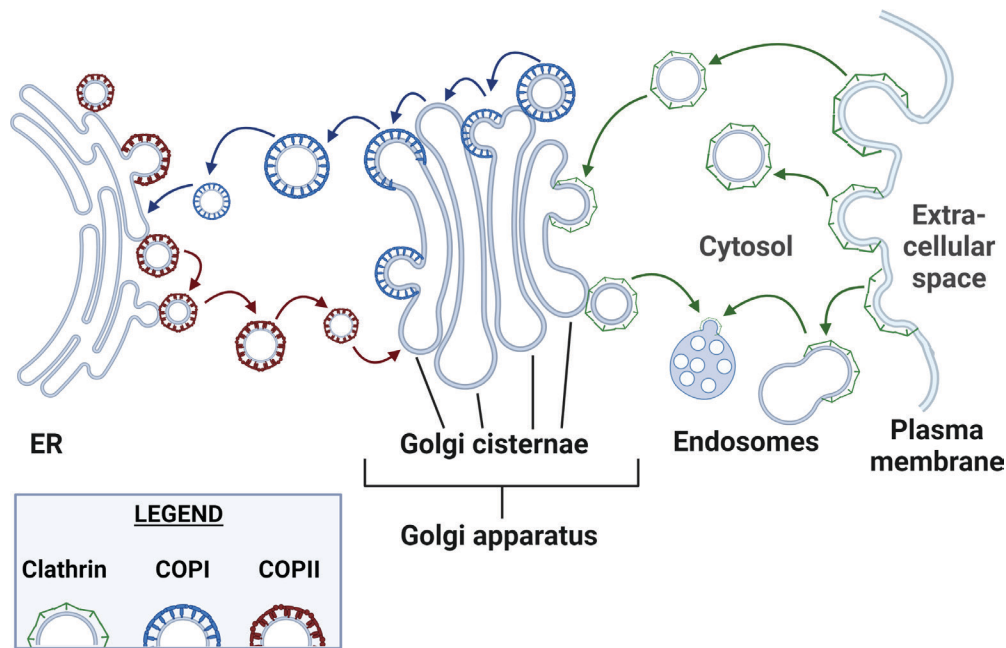


Figure 3. Coat proteins in vesicular transport.

Anchored within coatomers are *cargo receptors*, which face the lumen of the donor compartment and selectively bind proteins therein (Figure 4). Different coatomers bind to different cargo receptors. Each cargo receptor recognizes a different *signal sequence*, which is an amino acid sequence in a protein's code that indicates the compartment it needs to go to (i.e., its target compartment). Proteins are synthesized into the ER, but some must be transported to other organelles or to the cell surface to carry out their functions. These proteins display an *exit signal sequence* to which COPII coat proteins bind and facilitate anterograde transport.

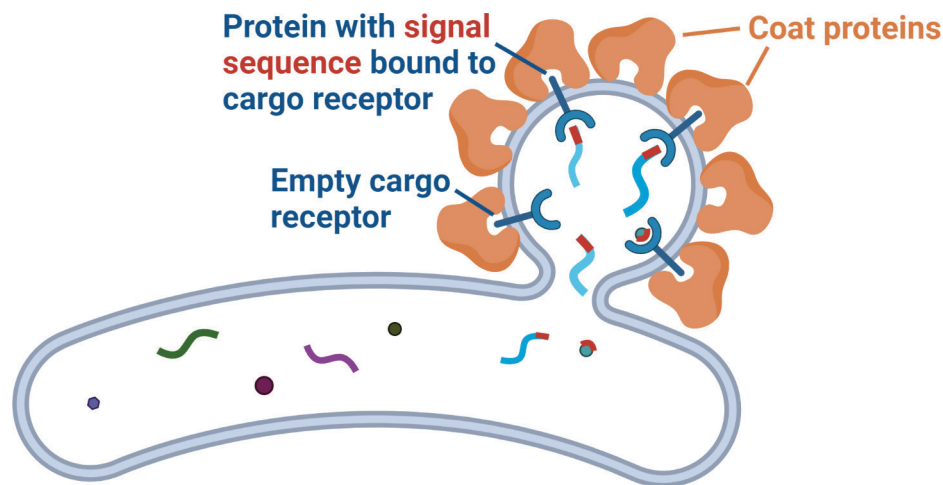


Figure 4. Cargo receptor binding on a budding vesicle.

Some proteins are made to function in the ER. These “ER resident” proteins have no exit sequences, but may become trapped in vesicles and need to be returned to the ER. In this situation, these proteins contain ER retrieval signal sequences, to which the COPI coatomer can bind to facilitate retrograde transport back to the ER. Proteins that participate in COPII-mediated transport also contain these sequences so that they can be “recycled” back to the ER to perform their functions again.

### The COPA Mutation

The COPI coatomer complex is composed of seven coat protein subunits, named  $\alpha$ -,  $\beta$ -,  $\beta'$ -,  $\gamma$ -,  $\delta$ -,  $\epsilon$ -, and  $\zeta$ -COPI. Each of these subunits performs functions that are critical to proper endomembrane transport, but we are specifically interested in  $\alpha$ -COPI, which is encoded by the *COPA* gene (Figure 5). Mutations in *COPA* can lead to COPA syndrome, and researchers believe that *COPA* mutations cause disease by disrupting COPI-mediated intracellular transport.

To date, five different mutations in *COPA* have been found to cause the symptoms of COPA syndrome (Table 1). Patients with different mutations display different symptoms, and different levels of symptom severity. One previously discussed mutation, which has been observed to cause moderate disease, substitutes an alanine at position 239 for a proline. This is the mutation that Ethan carries.

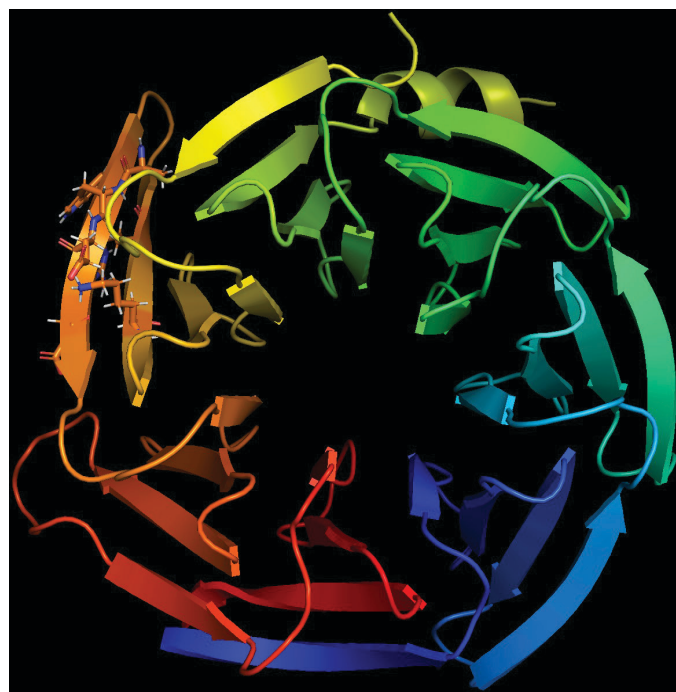


Figure 5. The wild-type  $\alpha$ -COPI protein, with the side chains of residues mutated in COPA syndrome displayed in the top left.

Table 1. Observations of symptoms in COPA syndrome patients.

<i>Mutation</i>	<i>Proportion Symptomatic</i>
K230N	2/3
R233H	11/12
W240R	1/1
E241K	8/11
D243G	6/6

Figure 6 depicts the wild-type  $\alpha$ -COPI protein, which contains an *alanine* residue at position 239. This represents the  $\alpha$ -COPI subunits in the cells of an individual without COPA syndrome, such as Ethan's mother.

Figure 7 depicts a mutant  $\alpha$ -COPI protein containing a *proline* at position 239. This represents the  $\alpha$ -COPI subunits in the cells of an individual who may have COPA syndrome, such as Ethan.

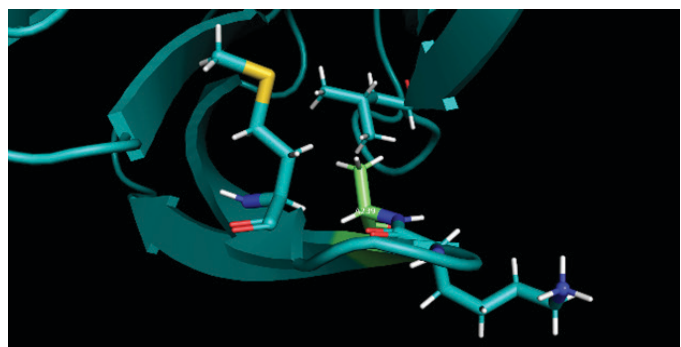


Figure 6. Wild-type  $\alpha$ -COPI at position 239 (green).

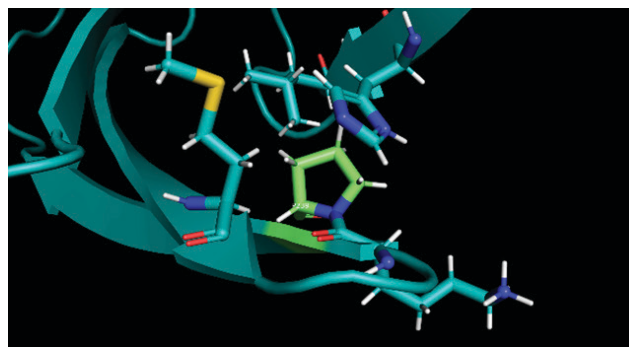


Figure 7. Mutant  $\alpha$ -COPI at position 239 (green).



## Part III – The Big Picture

COPA syndrome was first discovered in 2015 by Anthony Shum’s group at the University of California, San Francisco (Watkin, 2015). However, many people have long suffered from the symptoms attributed to COPA syndrome and have not been able to receive a definitive diagnosis for their condition. For example, interstitial lung disease often develops without a known cause.

As of 2021, only 17 families worldwide had been identified as carrying COPA syndrome mutations, though some researchers estimate that this rare disease is more common than we think (Frémond, 2021). Interstitial lung diseases, while still classified as a group of rare diseases, affect many more people. Some estimates place the prevalence of ILDs in the order of millions worldwide (Choi, 2018). Furthermore, idiopathic pulmonary fibrosis, or stiffening of the lungs with no known cause, is among the most common forms of ILD. This form of ILD is also observed in COPA syndrome patients (Kaul, 2021).

### *Question*

1. There is an argument to be made that rare diseases with common symptoms should be grouped together. For instance, a category of conditions causing idiopathic pulmonary fibrosis could include rare diseases (such as ILD) and extremely rare diseases (such as COPA syndrome). This group of conditions is much more prevalent than its constituent diseases, and this may increase the resources available to address them. Do you think that this is a valuable strategy? Explain why or why not.