Introduction
Diagnostic odyssey, a deep dive into patient symptomology and disease state pathophysiology, is often performed in children with undiagnosed genetic diseases who face uncertainty in daily life. In these cases patients have exhausted all other medical avenues, and despite careful clinical observation, remain undiagnosed. Through diagnostic odyssey, the proband (patient) undergoes targeted disease gene sequencing in order to rule out disease mutations through processes of genotyping all genes in pathways possible to the proband’s symptomology. Genetic mapping, an emerging tool and technique, allows for this insight into disease state pathophysiology through the lens of genetic variation. Utilizing a systems biology algorithmic approach based on mutation analysis via whole exome sequencing data in VariantGuide allows end users to go beyond single gene effects, and look further into metabolic and signaling pathways, which could explain unknown causes of symptomology. In this research project, the group was presented with a young child who had multiple ICU hospitalizations due to respiratory illness. Over the course of these hospitalizations, he had been treated for respiratory infection. However, all labs and cultures returned negative for infectious organisms. Although the patient is medicated with ultrasonic, antibiotic therapy, and other interventions, the patient’s symptomology remains severe and persistent, further exacerbating the symptoms identified at the first visit. The patient’s family history is not uncommon for children with respiratory medical therapies, but rather to seek-out possible pathologies of the proband’s disease state based off the symptomology, family history and genetic make-up. Therefore, our research team decided to use a list of genetic pathways utilizing a trios analysis approach which could provide better insight to properly diagnose the proband.

Methods
After informed consent under a clinical research program at the St. Louis Children’s Hospital Pulmonary clinics, and followed by HFPA release consent by parents for release of proband medical records, as well as for release of whole exome sequence data from both parents and proband, the trios was performed by Genesys and was covered by the patient’s family’s health insurance. Ferrelle genetic medical history was collected during interviews, while proband medical history was obtained by study of the proband’s medical history and consultation with the proband’s physician. Whole exome sequence data were uploaded to VariantGuide in the standard form of Variant Call Files. Whole exome analysis was performed on these variants. Following analysis, the research team identified those disease genes present on each genetic pathway of interest, and filtered out those that would be of low impact. Filters utilized during the research project included those focusing on impact of genetic variation on each genetic pathway of interest list the Primary Immunodeficiency Pathway (illustrated in Figure 2) has shown to be associated with many autoimmune disorders. Some of these disorders are treatable with the recombinant monoclonal antibodies, rituximab. This monoclonal antibody works to suppress the immune system in instances of organ transplant to prevent rejection. Given its effects specifically on RAGS and IL7 in this affected pathway it would stand to reason that cerdulimonab could provide benefit to the proband during instances of flare-ups. Additionally, as discussed with the Idiopathic Pulmonary Fibrosis Pathway, the HFE gene is known to be an important regulator of iron hemostasis. Given the proband’s familial history of these genetic variants were flagged and included in the final pathways of interest list. Currently there is no definitive diagnosis of interest list these genetic variants were flagged and included in the final pathways of interest list. Currently there is no definitive diagnosis

Results
Four pathways of interest were identified via VariantGuide to be potential causes of the proband’s symptomology. One add-on genetic mutation of the Phagosome pathway, the Primary Immunodeficiency pathway, the Staphylococcus aureus pathway, and the Complement and coagulation cascade pathway. The phagosome pathway details a list of genes that interest in a cascade to activate phagosomes of the immune system, and involved genetic mutation of the CLEC7A gene which is known to be a high impact gene in this pathway [Figure 1].

The Primary Immunodeficiency pathway was found to be a pathway of interest due to the genetic mutations present in multiple genes found early in this cascade (Figure 2). IL-7, alpha and RAGS were both found to have genetic mutation present in the proband with potential to upregulate both the innate and adaptive immune response to existing data suggesting an association of this gene with the proband. The Staphylococcus aureus pathway was found to have multiple mutated genes including FGCR3A, FGCR3B, CHI and MBL2 as detailed in Figure 3. Each of these four genes is tied to activity in the innate immune response with genetic mutations that can increase the body’s ability to log and kill foreign invaders. Idiopathic pulmonary fibrosis is linked to roughly 20 genetic mutations, one of which is the HFE genetic variant. While no cascade schematics were found through VariantGuide for this gene, the proband does carry the HFE genetic mutation. This gene is the principal regulator of iron hemostasis, and given a prior medical history significant for iron deficiency anemia and a correlation with pulmonary fibrosis, the HFE gene has been included as a gene of interest in this list. Alpha-1 Antitrypsin deficiency is a hereditary disorder that can provide potential pathology to our proband’s symptomology. This disorder results from a low level of Alpha-1 Antitrypsin in the body. This is produced by the liver and works to protect the body’s tissue against infection fighting agents produced by the body’s own innate immune response. With these low levels of the protein present in the body immune response is left unchecked. Ultimately, this deficiency has been linked to an autoimmune disorder characterized by the presence of several neutrophilic and neutrophil elastase in the lung epithelial lining causing lasting damage to the epithelial tissue. The Coagulation and Complement Cascade pathway was found to have multiple mutated genes including CH50, CH1 and MBL2 as detailed in Figure 4. Due to the nature of the diagnosis, the complement cascade was prioritized for analysis. This involves a cascade of pathways involving CH50, CH1 and MBL2 and CH50 genetic mutations were flagged as potential pathologies as detailed in Figure 4.

Discussion
The proband’s main symptomology consisted of severe episodes of peri-ocular paresthesia of dysregulated unresponsiveness of unknown etiology. With this in mind, the research team felt whole exome trio analysis was justified for locating the genetic variant that could give rise to altered immune response in this patient. Genetic data suggests an association of several of these genetic variants were flagged and included in the final pathways of interest list. Currently there is no definitive diagnosis for the proband in this research project. However, through the use of whole exome sequencing possible diagnoses have been discovered. Certain pathways are not found in the diagnostic odyssey yet to come, mark these potential diagnoses. Diagnostic odyssey such as this are proof of the potential benefit, which can be seen as healthcare continues to evolve into individualized medicine.