SYSTEMS BIOLOGY STUDIES OF WHOLE EXOME DATA FOR RESOLUTION OF A DIAGNOSTIC ODYSSEY INVOLVING RECURRENT, SEVERE RESPIRATORY DISTRESS

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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 studies, and despite consultations with top medical specialists, 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 relevant symptomology. Genetic mapping, an emerging technology in healthcare, allows for this insight into disease state pathophysiology through the lens of genetic mutation. Utilizing a systems biology algorithmic approach based on mutation analysis via whole exome sequencing data in iVariantGuide allows end users to go beyond single gene effects, and look further into metabolic and signaling pathways, which could be potential causes for patient symptomology. In this research project, the group was presented with a young child who had experienced 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 fluticasone, albuterol, montelukast and oral steroids the symptomology remains recurrent, severe periodic pulmonary dysfunction of unknown etiology. Additonally, the proband has a past medical history of iron deficient anemia, failed antibiotics during hospitalization and a family history of Alpha-1 antitrypsin deficiency. Despite seeing multiple medical specialists spanning multiple years the proband's illness remains undiagnosed. The intentions of this diagnostic odyssey are not to recommend medical therapies, but rather to seek out possible pathologies of the proband's disease state based off the symptomology, family history and genetic makeup. Through our research we plan to develop a list of genetic pathways utilizing a trio 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 Pulmonology clinic, and followed by HIPAA release consent by parents for release of proband medical records, as well as for release of whole exome sequence data from both parents and their child. Whole exome sequencing for the trio was performed by GeneDx and was covered by the family's health insurance. Familial genetic medical history was collected by interviews, while proband medical history was obtained by study of the proband's medical records after parental HIPAA release, along with interview of the mother. Whole exome sequence data were uploaded to iVariant Guide in the standard form of Variant Call Files. Whole exome analysis was conducted on the trio study subjects, proband, mother and father. Genetic information consisted of zygosities of specific genetic variants as well as genotyping information. A list of genetic variants was then formulated. From this list of 240,000 variants, searches were conducted to find those which could represent moderate to high effect on genetic pathways known to be involved in or related to pulmonary disease and immunopathophysiology. The full list of 240,000 variants was included in initial analysis, and utilizing filtering techniques provided in the iVariantGuide application, the list was narrowed to roughly 70 genetic variants of interest. Filters utilized during the research project included those focusing on impact of genetic variation on each genetic pathway of interest, and ranged from none to moderate to high impact. Additionally, only those gene mutations which were marked as pathogenic or likely pathogenic were included in final results. From this filtering iVariantGuide provided lists of genetic pathways of which the 70 genetic variants that fit these filters were involved in. From this list, only those pathways known to be involved in immune response or lung dysfunction were considered for further analysis. Lastly, analysis was conducted in this set of pathways of interest to examine the genetic variants involved in each pathway, and to determine the likelihood genetic variation could cause the symptomology exhibited by the proband. Those genes labeled as "high impact genes" were prioritized as most important.

Results

Four pathways of interest were identified via iVariant Guide to be potential causes of the proband's symptomology. One additional pathway was identified to be of interest due to existing data suggesting an association of 20 genetic variants with the disease that were all present in the proband. These are the Phagosome pathway, the Primary Immunodeficiency pathway, the Staphylococcus aureus pathway, and the Coagulation and complement cascade pathway. The phagosome pathway details a list of genes that interact in a cascade to activate phagosomes of the immune system, and involved genetic mutation of the CLEC7A gene which showed to be a high impact gene in this pathway (Figure 1).

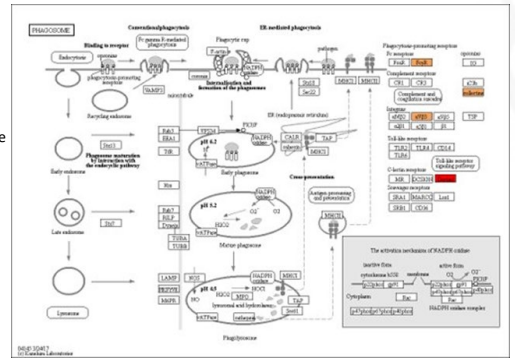

Figure 1: The Phagosome Pathway detailing placement and action of important genetic variant CLEC7A

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-7R alpha and RAG1 were both found to have genetic mutation present in the proband with potential to upregulate both the innate and adaptive immune response

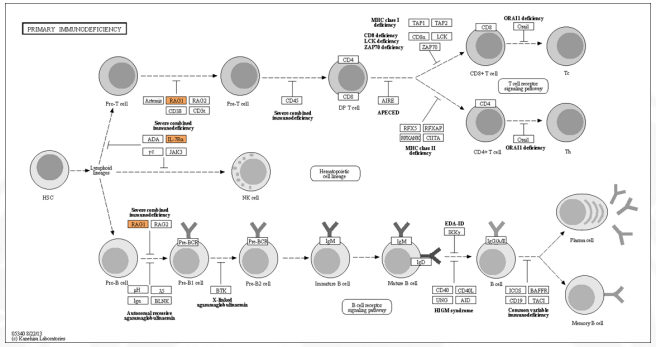

Figure 2: The Primary Immunodeficiency Pathway detailing the placement and action of important genetic variants RAG1 and IL7R.

The Staphylococcus aureus pathway was found to have multiple mutated genes including FGCR3A, FGCR3B, CFH and MBL2 as detailed in Figure 3. Each of these four genes are tied to activity in the innate immune response with genetic mutations that can increase the body's ability to tag 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 iVariant Guide for this gene, the proband does have mutations present in HFE. This gene is the predominant regulator of iron hemostasis, and given a prior medical history significant for iron deficient anemia and a correlation with pulmonary fibrosis, the HFE gene has been included as a gene of interest in this list.

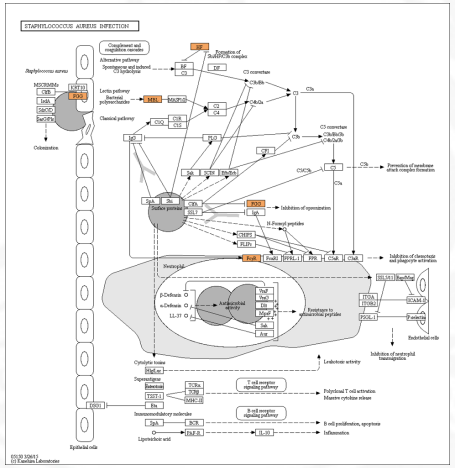

Figure 3: The Staphylococcus Aureus Pathway detailing the placement and action of important genetic variants.

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 via the immune system. 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 shown to increase levels of neutrophil and neutrophil elastase in the lung epithelial lining causing lasting damage to the epithelial tissue.

Last of the pathways found through iVariant Guide is the Coagulation and complement cascades. Due to the nature of the diagnosis, the complement cascade was prioritized for analysis. Similar to the Staphylococcus aureus pathway, the MBL2 and CFH genetic mutations were flagged as potentially pathogenic as detailed in Figure 4.

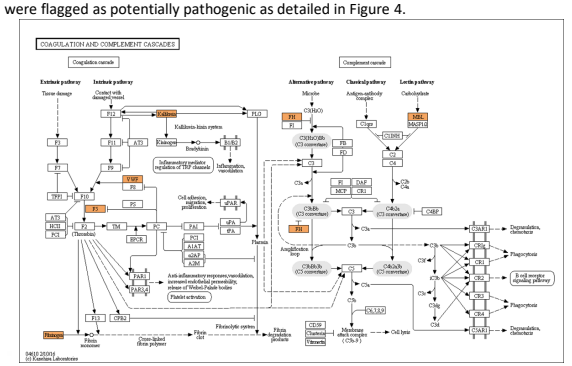

Figure 4: The Coagulation and Complement Cascade

Potential treatment options exist for some of the proposed pathophysiology already discussed. These treatments may not provide benefit given the speculative nature of our diagnoses. However, the research team feels they would be worth noting with a team of physicians regarding the findings of this diagnostic odyssey. With regard to our pathways 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 antibody, cedelizumab. This monoclonal antibody works to suppress the immune system in instances of organ transplant to prevent rejection. Given its effects specifically on RAG1 and IL7 in this affected pathway it would stand to reason that cedelizumab 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 past medical history of iron abnormalities, the metal chelating agent deferoxamine could provide benefit to a patient in which the HFE gene is not providing proper iron hemostasis. Given the Proband's familial history of alpha-1 antitrypsin deficiency the research team feels it beneficial to note existing treatment options for the condition. Current treatment options include Aralast and Glassia. These are recombinant and purified forms of the Alpha-1 Antitrypsin protein and work to correct the deficiency associated with this disorder. Other pathways associated with the Proband's symptomology and those included in the Pathways of Interest list showed to have no currently approved treatment modalities which could provide benefit to the Proband

Discussion

The Proband's main symptomology consisted of severe episodes of periodic pulmonary dysfunction of unknown etiology. With this in mind, the research team felt whole exome trio analysis was justified for locating de novo or homozygous recessive codon mutations which impacted metabolic or signaling pathways. The final pathways of interest list showed potential pathologies based upon significant genetic variants present in the proband which could give rise to altered immune response in those pathways. For example, CLEC7A genetic variants were present in the proband. This gene is specific for the activation of T-cells in the innate immune response and is a part of the toll-like receptor family of genes. CLEC7A binds T-cells and effectively recognizes both bacterial and fungal pathogens in the respiratory tract leading to increased T-cell activity in addition to an increase in toll-like receptor mediated inflammatory responses. As CLEC7A is well documented as being associated with infections of the lungs this genetic variant was of particular interest to the research team as a potential target for medical therapy in the future. Other notable genetic variants present in the proband include those in the RAG1 and IL-7R alpha genes as they are both found early in the production of both mature B cells and T cells. As noted in Figure 2 both genes are involved in important steps in the production of these cells, and therefore, increased levels of these genetic variants would lead to subsequent increased activation of both the innate and adaptive immune responses. Additionally, the FGCR3A/B CFH, and MBL2 genes are also involved in the activation of innate immune response which is why the relevant pathways associated with 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 of interest, which could give potential for future medical breakthroughs not only in the proband, but in future diagnostic odysseys yet to come, mark these potential diagnoses. Diagnostic odysseys such as this are proof of the potential benefit, which can be seen as healthcare continues to evolve into individualized medicine.