In this study ASXL3 ser2214pro mutation was examined using virtual structural assessment and analysis of previous research in order to better understand how this mutation in the gene sequence of ASXL3 would have on the structure and function of the protein. The main structure generating software used was Yasara (Yet Another Scientific Artificial Reality Application). A model for the wild type ASXL3 and the mutated ASXL3 were generated using Yasara (Krieger, 2014). Models from I-Tasser and Uniprot were created and compared using the Yasara homology modeling tool. Various models were used to further examine the effects of the mutation on the structure. A molecular simulation was carried out to assess the changes a mutation would have in this domain, and gain corresponding data. The function of ASXL3 was examined using the Allen Brain Atlas and examination of mouse gene knockout studies. The comparison of these models showed that the mutation in ASXL3 affects the structural integrity of the protein as well as presence in certain areas of the brain (Fig. 1).

Methods
An initial wild type protein model of ASXL3 (Huret et al., 2013) was used as a baseline for modeling and comparison, and the mutated form was first modeled using the computer program Yasara (Krieger, 2014) by substituting serine for proline using the swap residue function. (Fig. 1). A second structure of ASXL3 was created using data from I-Tasser (Yang et al., 2015), in which it was necessary to split the sequence due to size. This returned a flawed model, so the process was repeated to produce a better model, that was then aligned with the original model from PDB (Protein Data Bank) (Prokop, 2018). A third structure of ASXL3 was created via slow and fast homology modeling. A fourth round of structures of ASXL3 were modeled via molecular simulation in Yasara (Yet Another Scientific Artificial Reality Application). A model for the wild type ASXL3 and the mutated ASXL3 were generated using Yasara (Krieger, 2014). Models from I-Tasser and Uniprot were created and compared using the Yasara homology modeling tool. Various models were used to further examine the effects of the mutation on the structure. A molecular simulation was carried out to assess the changes a mutation would have in this domain, and gain corresponding data. The function of ASXL3 was examined using the Allen Brain Atlas and examination of mouse gene knockout studies. The comparison of these models showed that the mutation in ASXL3 affects the structural integrity of the protein as well as presence in certain areas of the brain (Fig. 1).

Results
The conclusions obtained through the study are based around the idea of the zinc finger structure in ASXL3, its stability, and the amino acid changes that would have a destabilizing impact on its conformation. Through structural modeling, it was found the mutation of Serine to Proline at the 2214th position in ASXL3 may have an effect on the overall protein structure (Fig. 1). In I-Tasser models, it can be seen that the important ligand, Zinc, is not present. When aligned with the original ASXL3 PDB model, the RMSD value was 0 and there was no sequence identity. This was most likely because the zinc atom is present in the original model, not the I-Tasser model and is necessary for the overall structure of the domain being analyzed. Previous research has found that the mutation prevents the attachment of the zinc finger within the protein because of the cyclic structure of proline which creates a more rigid bond than serine. When a mutation is present, the zinc finger is not attached, and the section of the protein containing it is allowed to move freely causing the protein the be less structurally sound and to be less condensed. The wild type ASXL3 is much more stable than that of the mutated gene. Its radius of gyration has a smaller range of motion than the mutated form, leading to the assumption that the mutated form is more mobile, despite the proline mutation that causes a more rigid bond (Fig. 3). Analysis of information provided by the Allen Brain Atlas indicated moderate levels of ASXL3 expression in both the Anterior Cingulate Cortex (ACC) as well as the Primary Visual Cortex (PVC) values of which can be seen in figure 2. This suggests that a mutation in the PDB domain of ASXL3 may be the cause of certain aforementioned developmental diseases. Higher levels of expression of ASXL3 was shown in the ACC, than PVC. The differences discussed above support the conclusion that the mutation of serine to proline at the 2214th position of ASXL3 may have an effect on the structure of ASXL3. The data that lead to these conclusions indicates that the mutation in question may have significant loss of function properties, and may be the cause of certain diseases in correlating regions of the brain.

Conclusions
• The mutation may alter the function and folding of the protein.
• ASXL3 exhibits significant expression in regions of the brain such as Primary Visual Cortex and Anterior Cingulate Cortex.
• The mutation in question is almost certainly damaging.

Forthcoming Research
We plan to continue to organize and interpret the information from the Allen Brain Atlas specifically identifying the cell types affected. We want to determine if the cell types associated with ASXL3 and its associated proteins are also associated with the phenotype of patient with mutation in question.

References

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