# Insights from Metabolomics analysis of Pseudoexfoliation and Primary Open Angle Glaucoma Aqueous Humor Samples.

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ABSTRACT

Pseudoexfoliation (PEX) is a known cause of secondary open angle glaucoma. PEX glaucoma is associated with structural and metabolic changes in the eye. Despite similarities, PEX and primary open angle glaucoma (POAG) may have differences in the composition of metabolites. We analyzed the metabolites of the aqueous humor (AH) of PEX subjects sequentially first using nuclear magnetic resonance (1H NMR: HSQC and TOCSY), and subsequently with liquid chromatography tandem mass spectrometry (LC-MS/MS) implementing isotopic ratio outlier analysis (IROA) quantification. The findings were compared with previous results for POAG and control subjects analyzed using identical sequential steps. We found significant differences in metabolites between the three conditions. We used machine learning algorithms and a percentage set of the data to train and utilized a different or larger dataset to test whether a trained model can correctly classify the test dataset as PEX, POAG or control. Three different algorithms: linear support vector machines (SVM), deep learning, and a neural network were used for prediction. They all accurately classified the test datasets based on the AH metabolome of the sample. We next compared the AH metabolome with known AH and TM proteomes and genomes in order to understand metabolic pathways that may contribute to alterations in the AH metabolome in PEX. We found potential protein/gene pathways associated with observed significant metabolite changes in PEX.
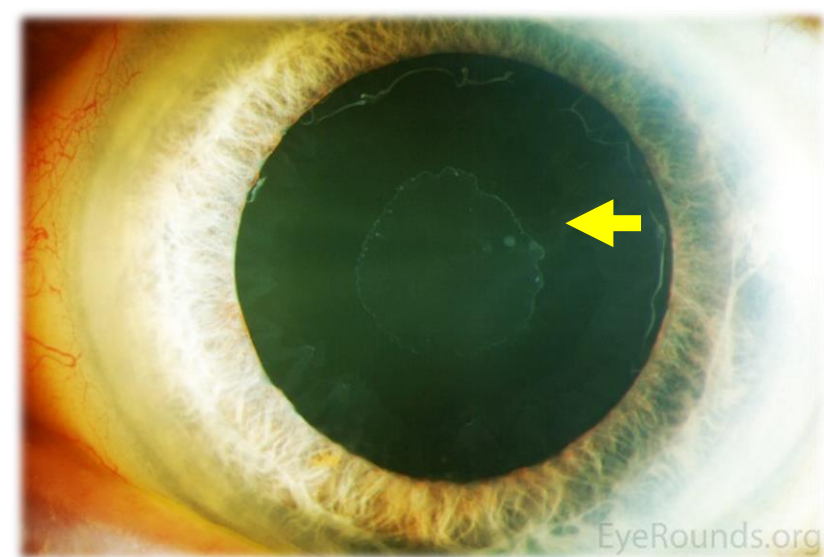
A photograph showing psedoexfoliation (PEX) material (arrow) in the eye of a patient.

MATERIALS AND METHODS
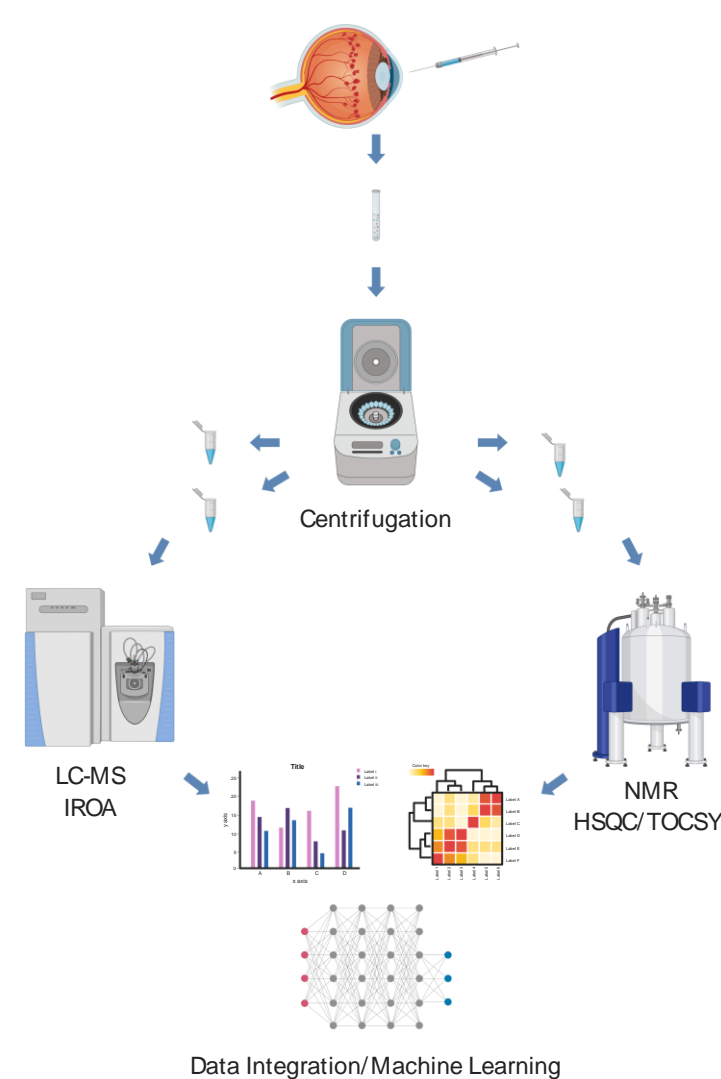
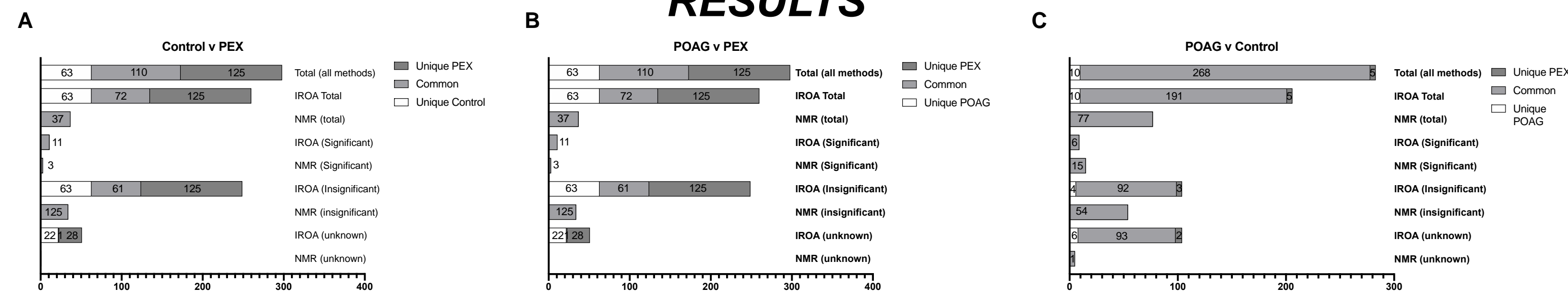A schematic diagram depicting methods used for the analysis.

RESULTS

Figure 1.The number of common and unique metabolites found (**A**) Control vs PEX (**B**) POAG vs PEX. (**C**) POAG vs Control
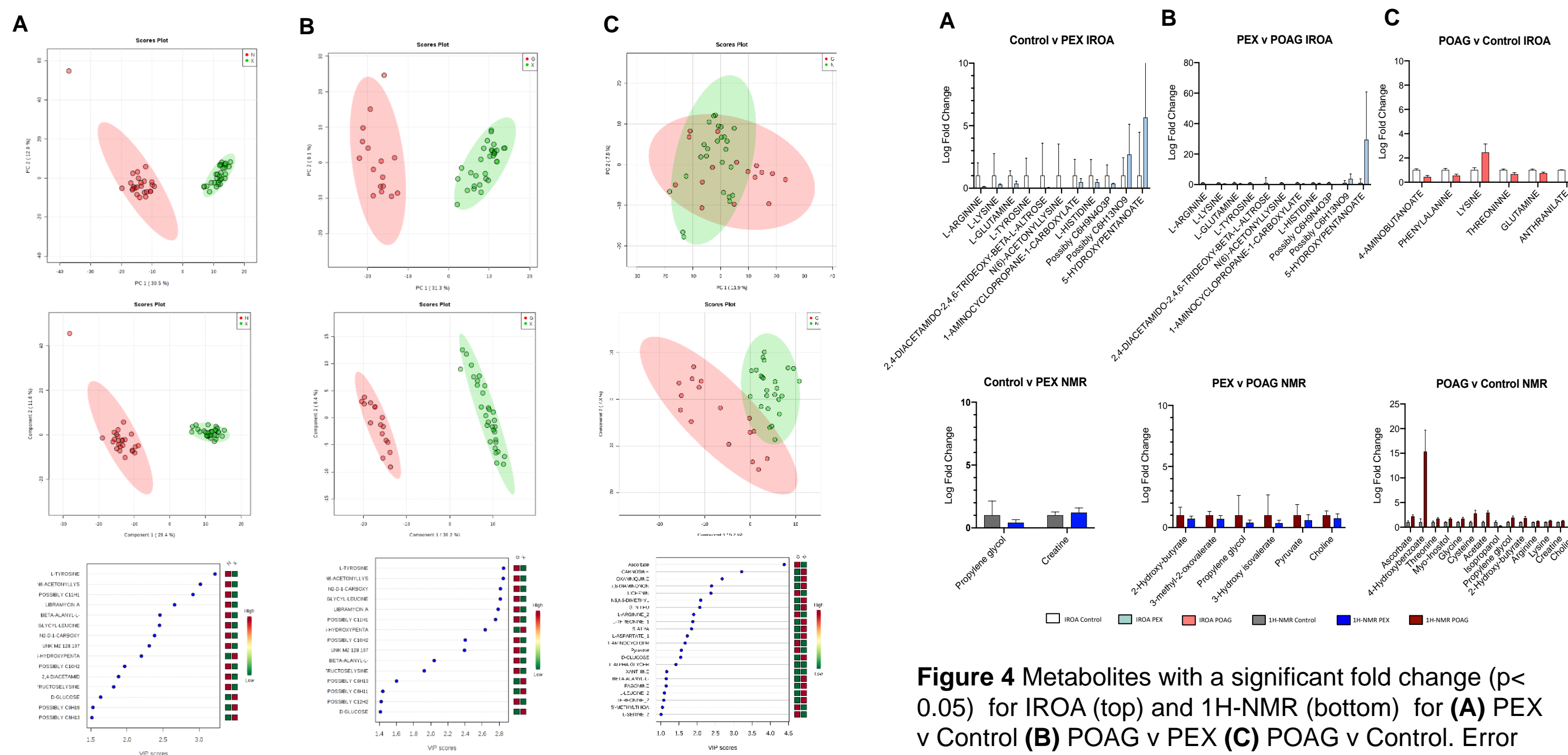

Figure 2. PCA (top) for the three experimental conditions. The PCA helps to show the grouping as a result of 2 primary principle components. PLS-DA (middle) for the three conditions. VIP scores (bottom) for all three conditions showing the metabolites that contribute most to the separation of conditions. (**A**) PEX v Control. (**B**) PEX v POAG (**C**) POAG v Control
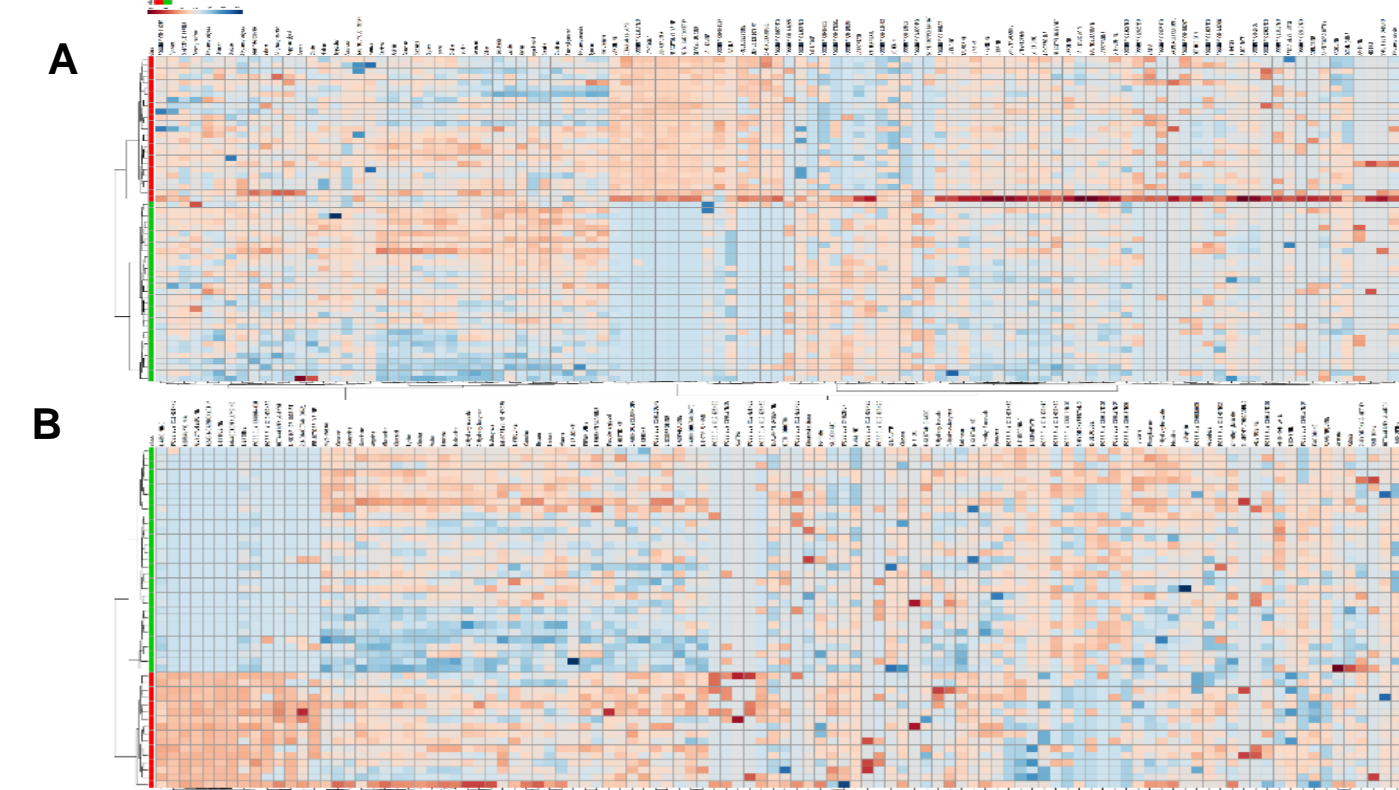

Figure 3. Heat maps (showing the over and under expression of the metabolites analyzed. (**A**) PEX v Control. (**B**) POAG.v PEX.
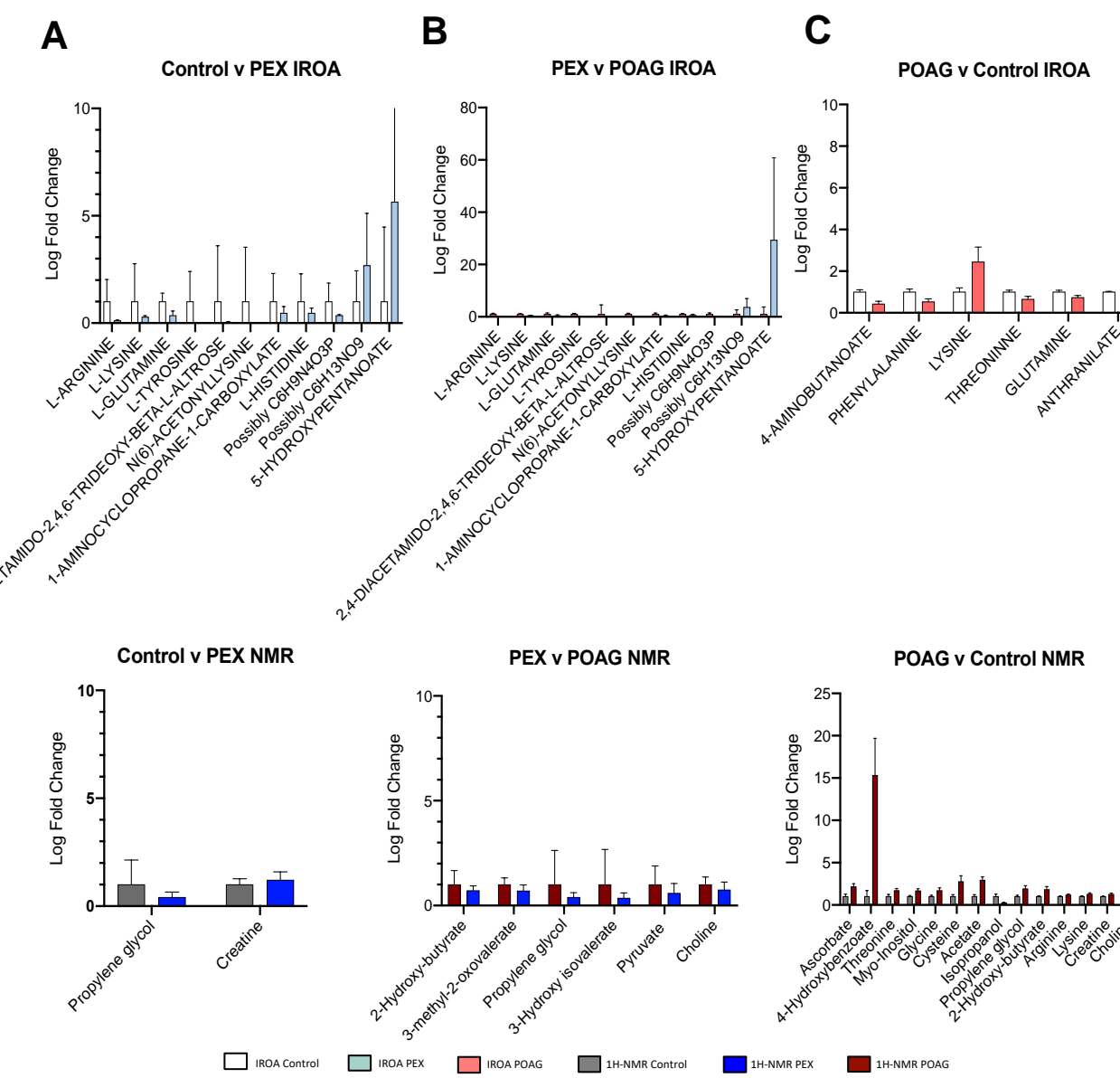

Figure 4 Metabolites with a significant fold change (p< 0.05) for IROA (top) and 1H-NMR (bottom) for (**A**) PEX v Control (**B**) POAG v PEX (**C**) POAG v Control. Error bars indicate standard deviation from the mean.
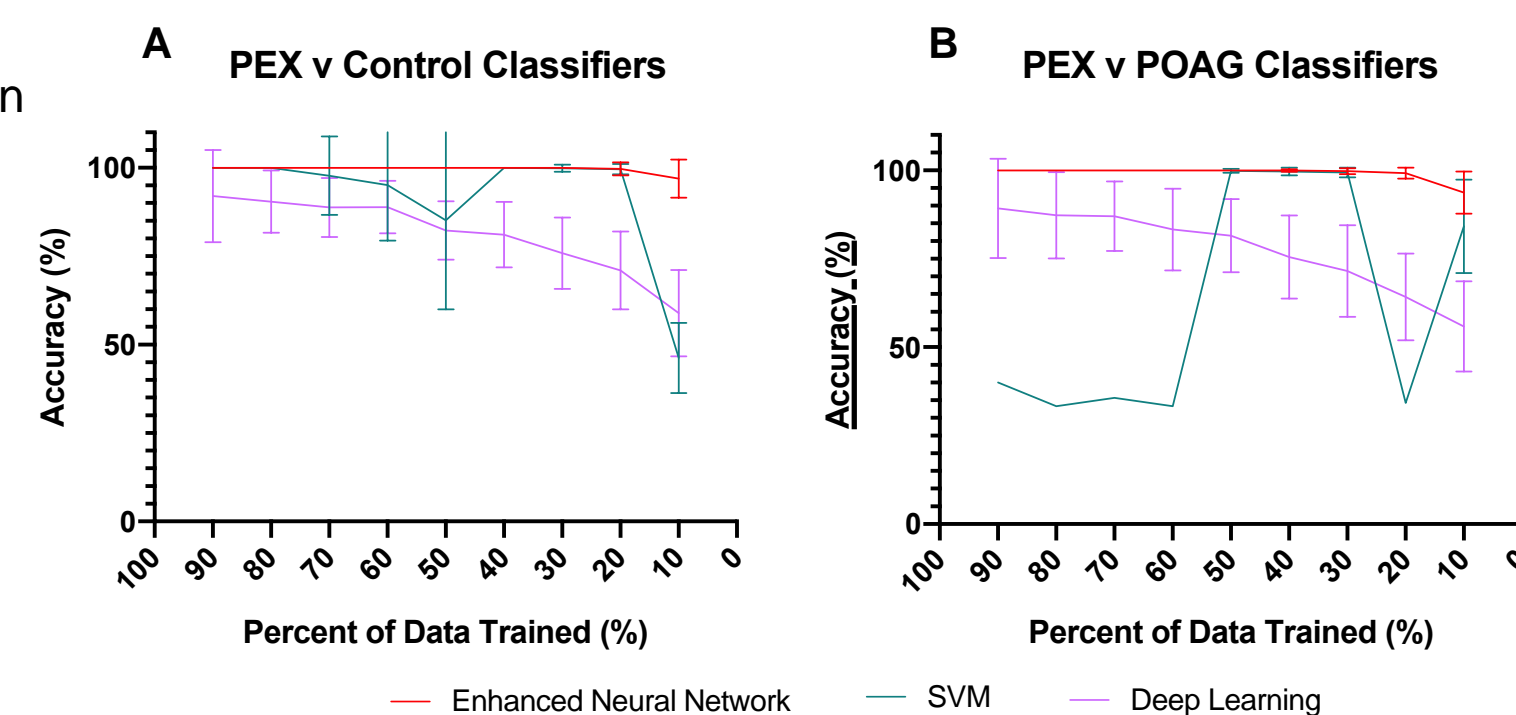

Figure 5. Machine learning performance analysis showing accuracy as a function of split-ratio validation. (**A**) PEX vs Control Classifiers (**B**) PEX vs POAG Classifiers. The results show that an enhanced neural network is the most robust in response to changes in sample size used for the training set, despite the fact that it required the most computational time.
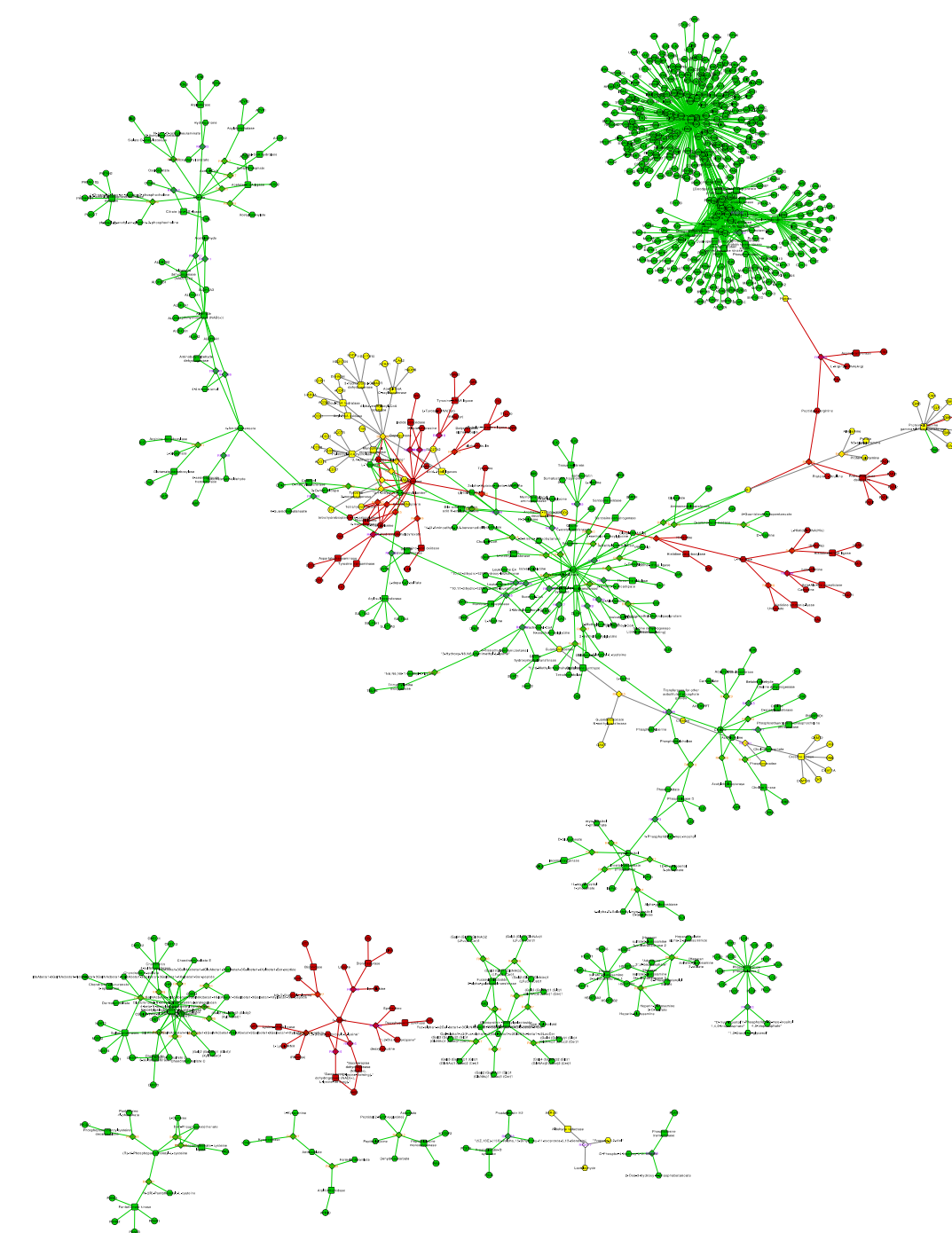

Figure 6. Differential network analysis. Green shows pathways involved in PEX, red shows pathways involved in POAG. Yellow shows consensus pathways.

CONCLUSION

The results indicate clear differences in metabolite expression for PEX and POAG. In the PEX-Control test, tyrosine had the most significant fold change. In the PEX-POAG test, tyrosine was highly expressed in PEX but not POAG. In the POAG-Control test, Ascorbate had the highest VIP score in the PLS-DA analysis. Ascorbate is common in the AH[1], but elevated levels of this compound appear in POAG samples. Finally, ML comparison indicates sample size impact. Linear SVMs are traditionally used, however the results indicate the robustness of neural networks. The comparisons in the proteome-metabolome networks showed common pathways. These results have many therapeutic development applications.

ACKNOWLEDGMENTS

REFERENCES

1. Ringvold A. The significance of ascorbate in the aqueous humour protection against UV-A and UV-B. Exp Eye Res. 1996 Mar;62(3):261-4. doi: 10.1006/exer.1996.0031. PubMed PMID: 8690035.