Analysis of CNS Disorders Using Active Kinome Profiles

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Background

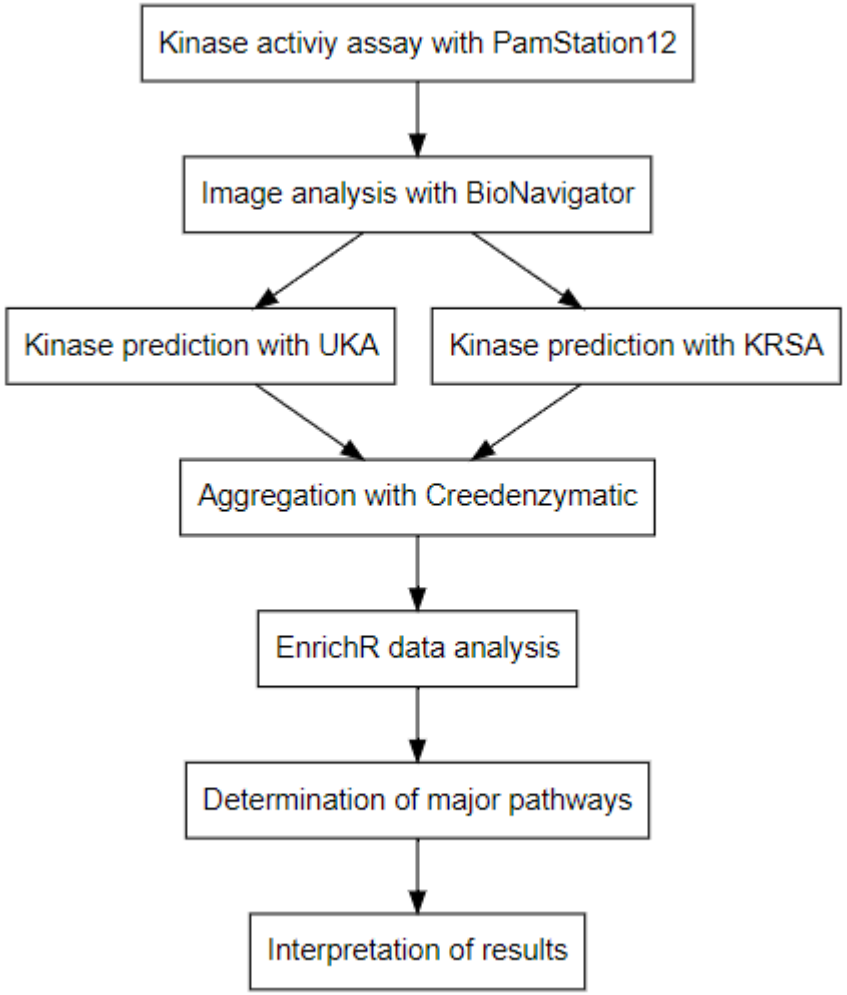
- Disorders of the central nervous system (CNS Disorders) such as Alzheimer's Disease and schizophrenia (SCZ) often have multiple different causes, however, research suggests common mechanisms between the two [1,2] along with the involvement of protein kinases [3,4].
- Most work focuses on measures of gene expression, including mRNA, protein, or phosphoprotein levels in a biological sample. We sought to investigate a more functional measures of gene expression, protein kinase activity. Recent technologies allow for real-time analysis of protein kinase activity, giving further insight into how kinases regulate the underlying pathways behind these complex disorders.
- Our approach permits simultaneous assessment of 100's of protein kinases, yielding datasets that inform the "Active Kinome" in our postmortem samples.
- Bioinformatic analyses of active kinome data may provide important clues regarding the similarities and differences between Alzheimer's Disease and schizophrenia .

Materials and Methods

- Samples from the dorsolateral prefrontal cortex (DLPFC) of postmortem brains were analyzed using the PamStation12.
  - AD samples were run at the university of Toledo and included mild cognitive impairment (MCI) in addition to AD.
  - SCZ samples were run at the University of Alabama Birmingham

- Image analysis was done using BioNavigator by PamGene. Following this, two separate deconvolution methods were used:
  - UKA (upstream kinase analysis, PamGene): predicted exact kinases.
  - KRSA (kinome random sequence analyzer, K. Alganem): predicted kinase families through random sampling.

- Next, the R. Package Creedenzymatic (J. Creeden) aggregated the results of the two deconvolution analyses together. Kinases were assigned a ranked, ordered, composite score based on their original UKA/KRSA scores and organized in quartiles. Kinases that appeared in both KRSA and UKA from the first quartile of the data pool were considered to be significant hits and were selected for further study.
- Data enrichment was done through the EnrichR [1] web server. For pathway analysis, the BioPlanet 2019 database was used while the Human Gene Atlas was used for expression level analysis.



Kinase Analysis Results

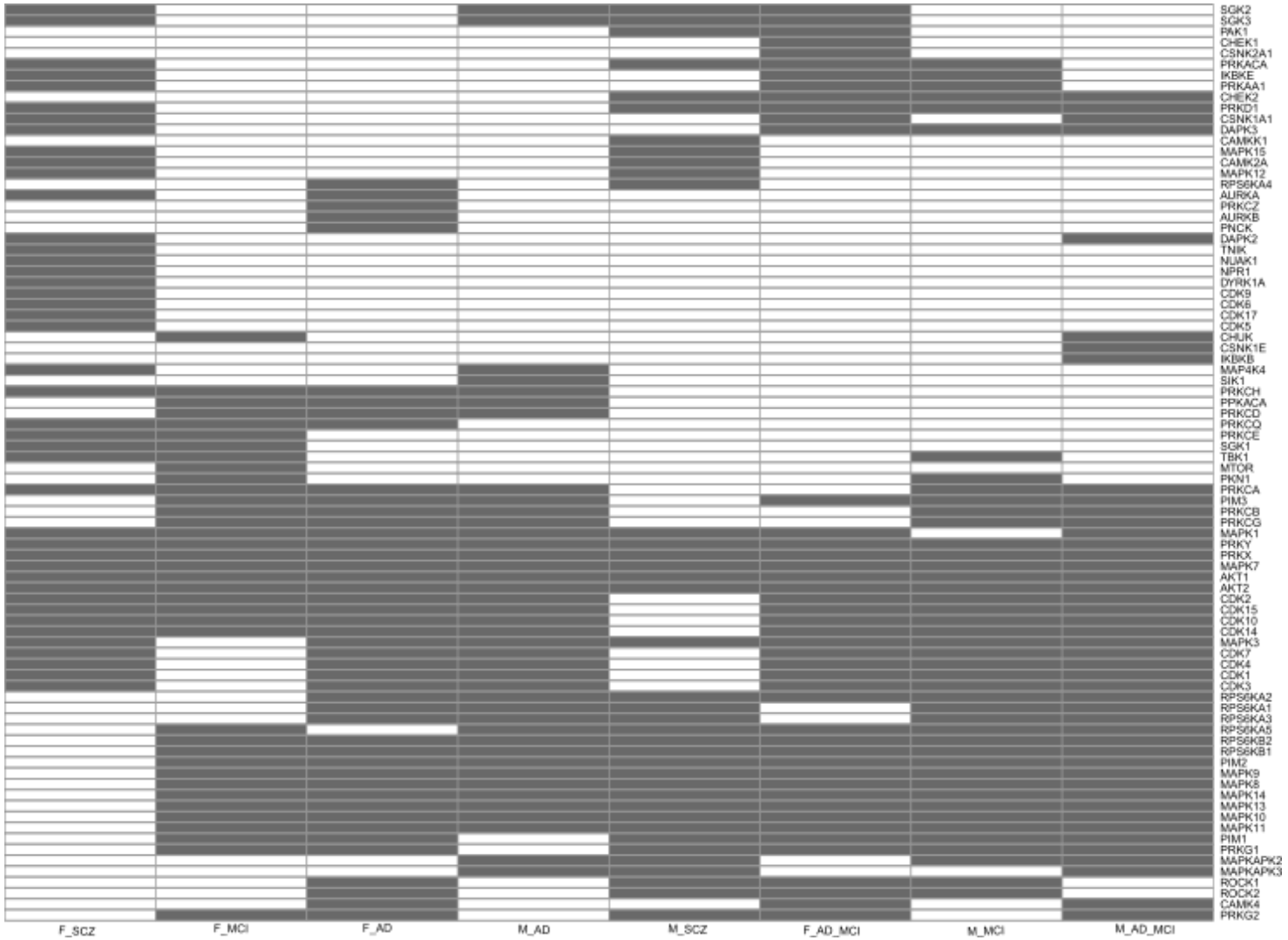
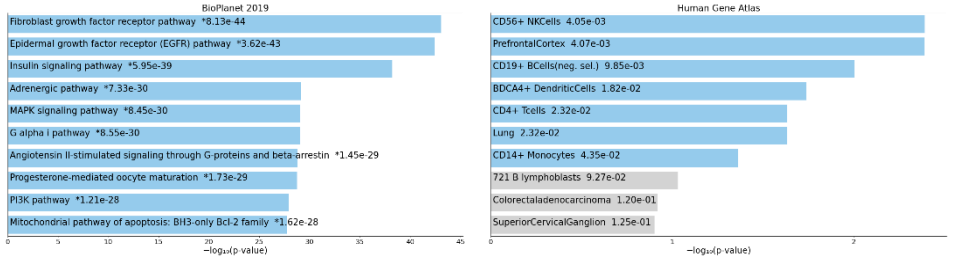


Figure 1:
Plot showing the kinase hits in each of the eight groups following deconvolution and data aggregation. Grey boxes represent the kinase is included in that group while white boxes show that it is not.
Hits were determined based on quartile-rank plots using Creedenzymatic scores which combined the results of both KRSA and UKA. A kinase had to be present in both deconvolution methods to be considered a hit. The table below is an example using the female AD group that shows the top 5 UKA results and the top 5 KRSA results ranked according to their Creedenzymatic score.

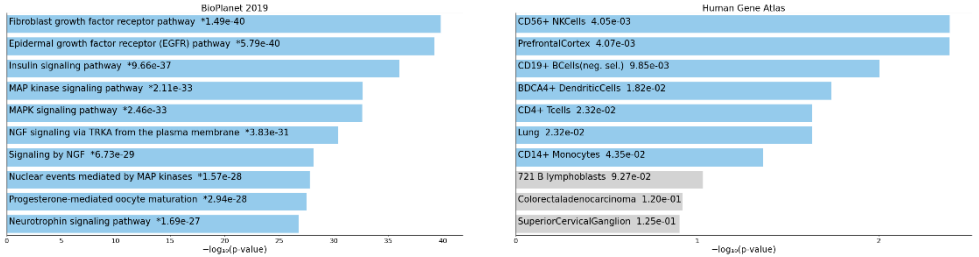
Female AD

Hgnc Symbol	Score	Rank	Method
ROCK1	2.238561	1	UKA
COQ8A	1.853872	2	UKA
PIM1	1.79588	3	UKA
AURKA	1.427903	4	UKA
PRKCQ	1.427903	4	UKA
PRKY	1.390761	5	UKA
MAPK	-5.49673	1	KRSA
DYRK	-4.91122	2	KRSA
JNK	-4.56775	3	KRSA
RSK	4.011	4	KRSA
DMPK	3.526	5	KRSA

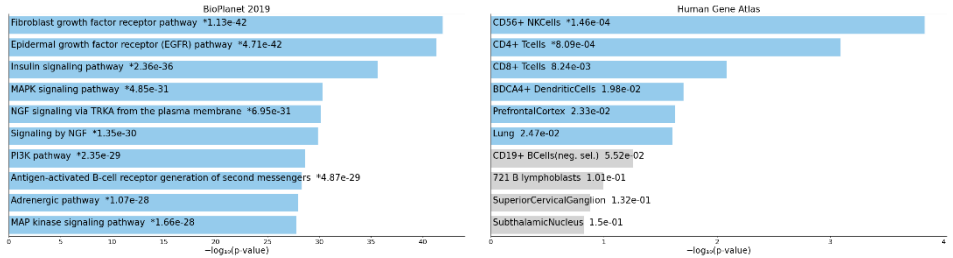
2a. Female AD



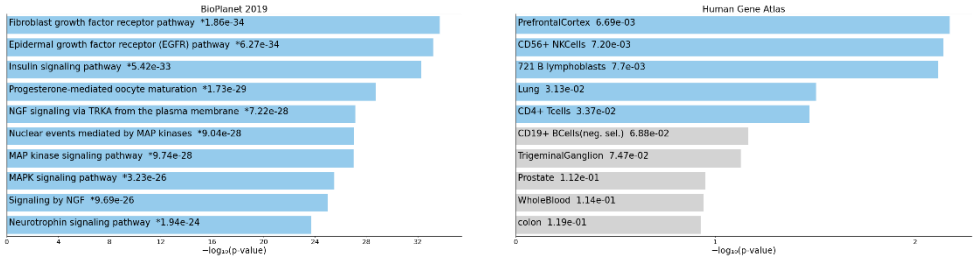
2b. Male AD



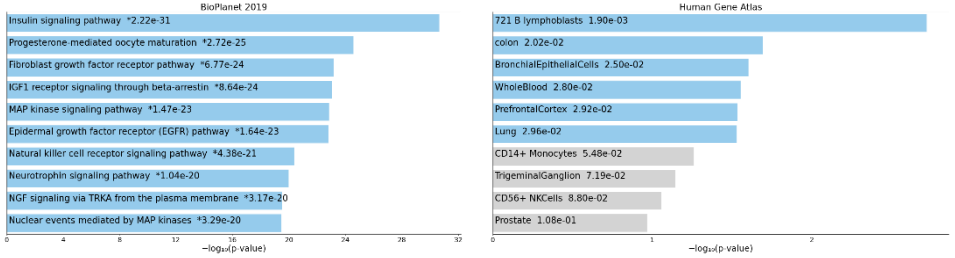
2c. Female MCI



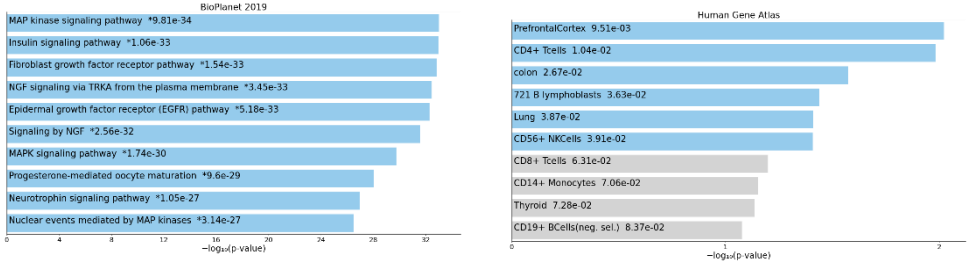
2d. Male MCI



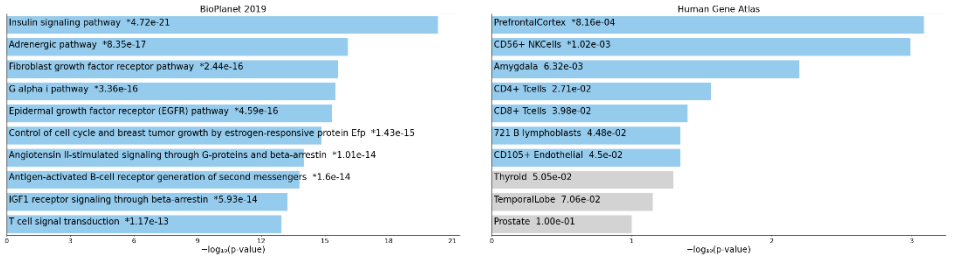
2e. Female AD/MCI



2f. Male AD/MCI



2g. Female SCZ



2h. Male SCZ

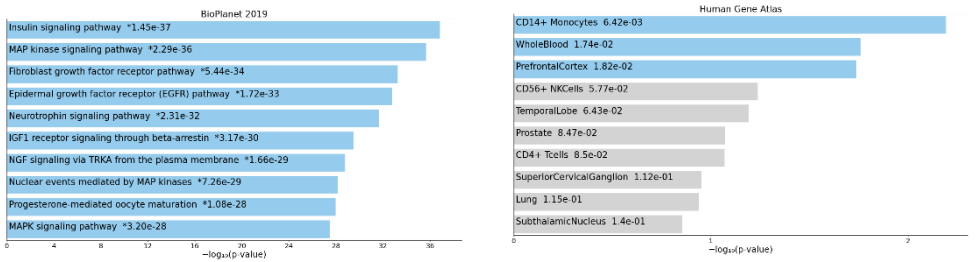


Figure 2:
Top 10 Enrichr results for each of the eight groups. Within each group, the left graph corresponds to the BioPlanet 2019 pathways while the right graph corresponds to the Human Gene Atlas expression locations. All bars are sorted by lowest p-values.

Pathway Analysis Results

Figure 3: Plot showing the pathway results. Blue boxes represent significant pathways in each group based on their p-values from figure 2. White boxes indicate that a particular pathway was not significant in that group.

Expression Analysis Results

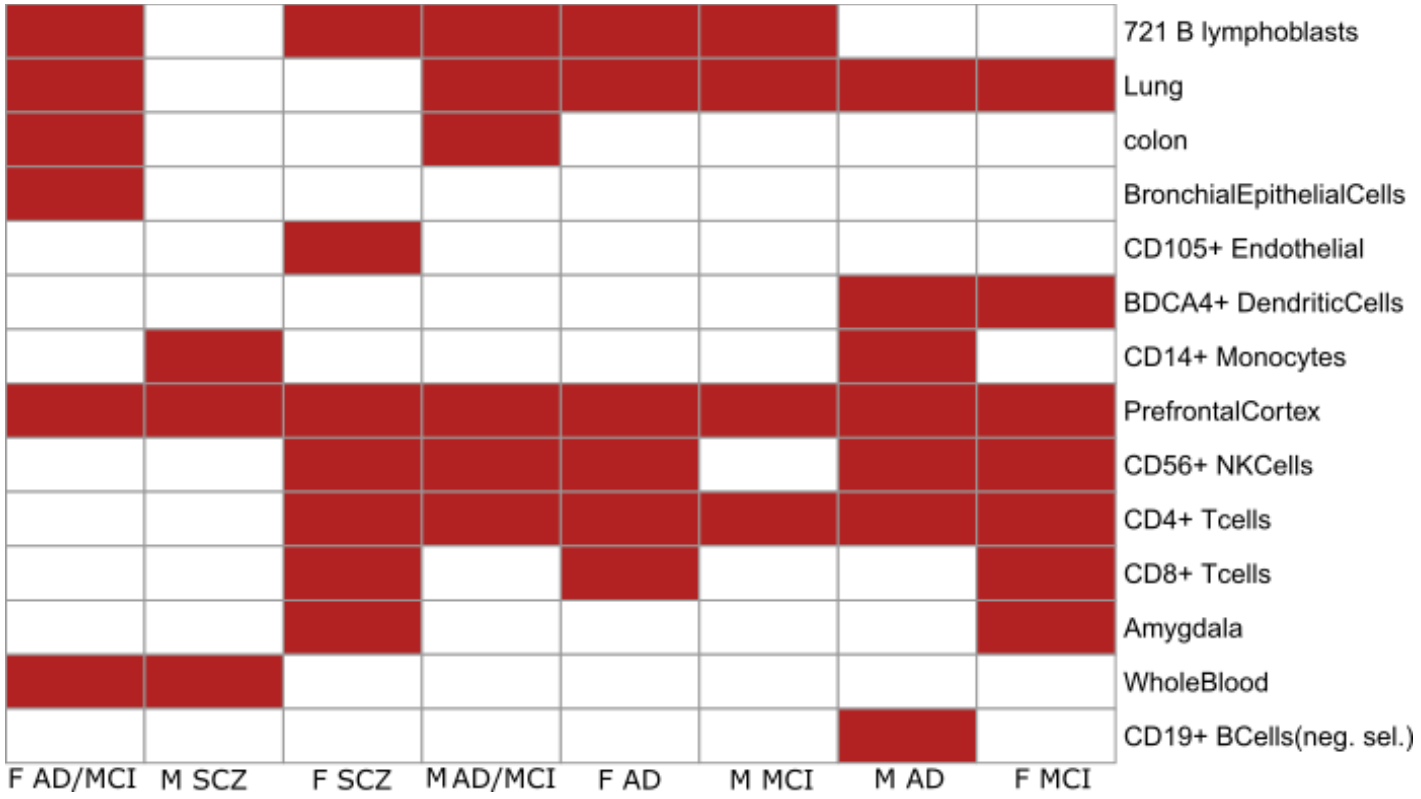


Figure 4: Plot showing the expression level results. Red boxes represent significant cell/tissue types in each group based on their P-values as indicated in figure 2. White boxes Indicate that a cell/tissue type was not significant in that group.

Discussion/Conclusion

Kinase Analysis (figure 1)

- Figure 1 indicates that gender differences between groups has a large impact; female SCZ appears to contain the largest differences in kinase content compared to others as it contains a large amount of unique kinases, denoted by 'F' in the lists below. Kinases only found in male groups are denoted by 'M'. Kinases found in both male and female groups are denoted by 'F/M'.
- Kinases exclusive to AD and MCI include: CHEK1(F), CSNK1E(M), MTOR(F), and PIM3(F/M).
- Kinases exclusive to SCZ include: CDK5(F), CDK6(F), CDK9(F), DYRK1(F), MAPK12(F/M), MAPK15(F/M), NPR1(F), NUAK1(F), TINK1(F), and CAMKK1(F).
- Kinases found in all groups include: AKT1, AKT2, PRKX, PRKY, and MAPK7.
- Interestingly, female SCZ does not contain any members of the RPS6K family while male SCZ does not contain any members of the CDK family; this may be something that could be investigated further.

Pathway Analysis (figure 2 and figure 3)

- Insulin/EGFR/Fibroblast signaling was dysregulated in all samples while neurotrophin signaling was dysregulated in all except for female MCI, SCZ, and AD.
- Fibroblasts derived from neurons show AD-like pathologies, allowing them to be used as potential biomarkers for AD [5,6,7]. Additionally, insulin signaling dysregulation along with diabetes has been shown to be a risk factor for both AD and SCZ [8,9].
- IGF-G signaling appears to be dysregulated in both male and female SCZ along with late-stage female AD (AD/MCI); overall, the pathways between SCZ and AD appear to be similar when accounting for gender differences.

Expression Level Analysis (figure 2 and figure 4)

- The Prefrontal cortex was enriched in all groups; additionally, many of other hits from the Human Gene Atlas were immune cells such as CD4+/CD8+ T cells, NK cells, and lymphocytes. These results are consistent with both the location of the samples (DLPFC) and the fact that inflammation is associated with both AD and SCZ [10,11] .

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