Research Grants 2025

Neuroimmunology | Development of a PCR-Based Host Transcriptomics Assay for Rapid Differentiation of Encephalitides

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A quick and accurate diagnosis of brain inflammation and the cause of inflammation is essential for patient care, but current testing methods are often slow. Due to this delay, doctors need to relay on clinical evaluation to help identify the correct diagnoses. This can be very difficult as patients present to hospital with similar signs and symptoms regardless of the underlying cause. Delays in the investigations and difficulty in discerning the cause through clinical evaluation, can often lead to wrong diagnoses, and therefore the wrong treatment. Incorrect or delayed treatment often leads to poorer outcomes for patients.

Our research aims to address this by developing a simple, rapid, cost-effective diagnostic test that enables clinicians to distinguish between different types of brain inflammation, particularly infections versus autoimmune causes.

We will study fluid samples from patients who already have confirmed diagnoses. Using advanced sequencing techniques and computational analysis, we will find unique inflammation patterns that help identify different types of brain inflammation. We will then turn these patterns into a quick test that doctors can use in hospitals and clinics when a diagnosis is uncertain.

This incredibly novel method could greatly improve patient care by giving doctors a fast and reliable way to make diagnoses. When doctors can quickly figure out what type of brain inflammation a patient has, they can start the right treatment sooner. For infections this means antibiotics or antivirals, and steroids for autoimmune causes. This would mean better results for patients and lower healthcare costs. Our research fills an important gap in brain health care and could set a new standard for diagnosing brain inflammation.

Grant $40,000

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Summary

To date, 10 cases of orthogonally-confirmed infectious encephalitis have been recruited, and a novel viral cause of encephalomyelitis has been identified in the last week (pending further confirmation). 34 autoimmune encephalitis and encephalitis mimics have been recruited (11 seropositive for antibodies and 7 with other confirmed diagnoses). Several additional cases of confirmed CNS infection have also been recruited, including 11 bacterial meningitis cases. These samples underwent RNA sequencing.

Although recruitment numbers continue to increase, sample accumulation remains a bottleneck because CSF collection volumes are often limited and, in many cases, no sample remains for research following necessary clinical testing. To rectify this issue, an ethics amendment was submitted and has been approved in recent weeks. This amendment permits analysis of the host transcriptome in multiple stored specimens at VIDRL. This will permit the use of dozens of additional cases for transcript classifier discovery, which can be tested on our recruited case samples, allowing us to move forward quickly with the project in a short timeframe, with a projected assay completion date of late 2026.

To progress with the project while recruitment is ongoing, an external dataset was acquired through correspondence with the publishing author of a case series of 70 paediatric meningitis cases with raw CSF transcriptomic data available.12 We had the aim of developing a proof-of-concept PCR assay for transcriptomic diagnosis in CSF, to establishing a workflow for our own assay's development and testing.

Sample selection

The de-identified external dataset contains sample-level gene counts and individual patient metadata from a paediatric cohort of meningitis patients. Samples were rapidly frozen to preserve genomic data and were processed in small batches to reduce batch effects. Of 70 patients aged <1 year to 18 years (41 male), 10 had culture-confirmed bacterial meningitis and 12 had PCR-confirmed viral meningitis. These 22 cases formed the cohort used in our assay design.

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Differential gene expression analysis

A custom R script was developed for differential expression analysis. After data import and alignment, the script adjusts for log-transformed CSF cell counts as covariates and filters lowly expressed genes. Age and sex were not included as covariates because of the limited sample size and the risk of reducing biological signal through over-adjustment. The ZINB-WaVE algorithm13 was then used to model transcriptomic data and generate per-gene observational weights to account for the high proportion of unexpressed genes, a typical finding in low-biomass CSF samples. These weights were incorporated into a generalised linear model (edgeR DGEList) to produce differential expression (DE) statistics. RUVSeq subsequently was used to screen for candidate PCR assay housekeeping reference genes with low variance and high expression.

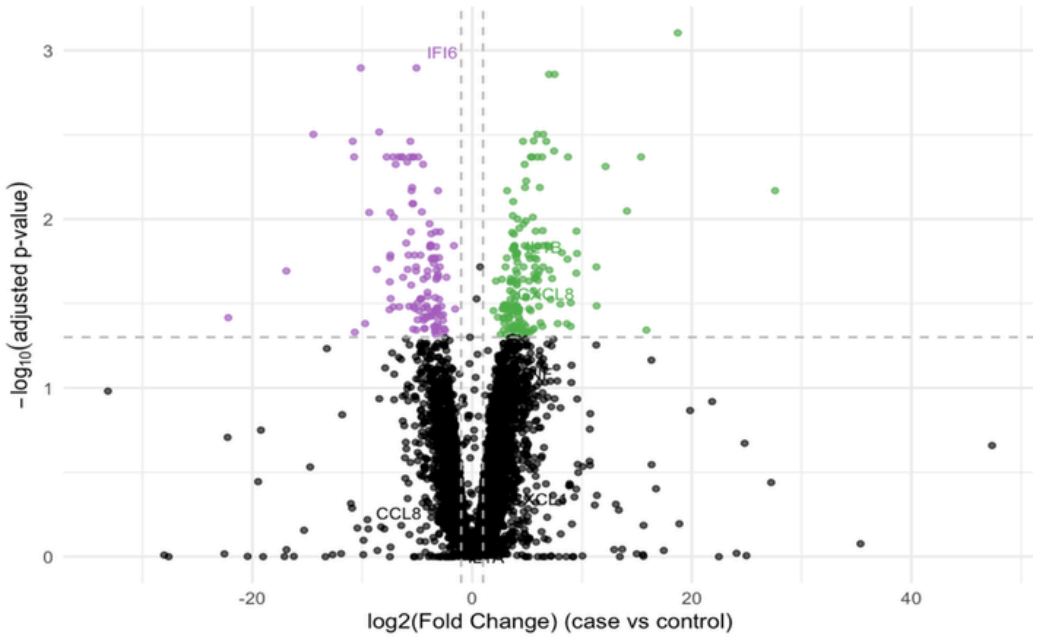

Figure 1a: Volcano plot plotting log-fold change vs significance. Genes plotted above the horizontal dashed line pass a significance threshold (FDR-adjusted P<0.05). Green dots denote significantly overexpressed genes in bacterial cases; purple dots denote genes overexpressed in viral cases. (FDR, false-discovery rate)

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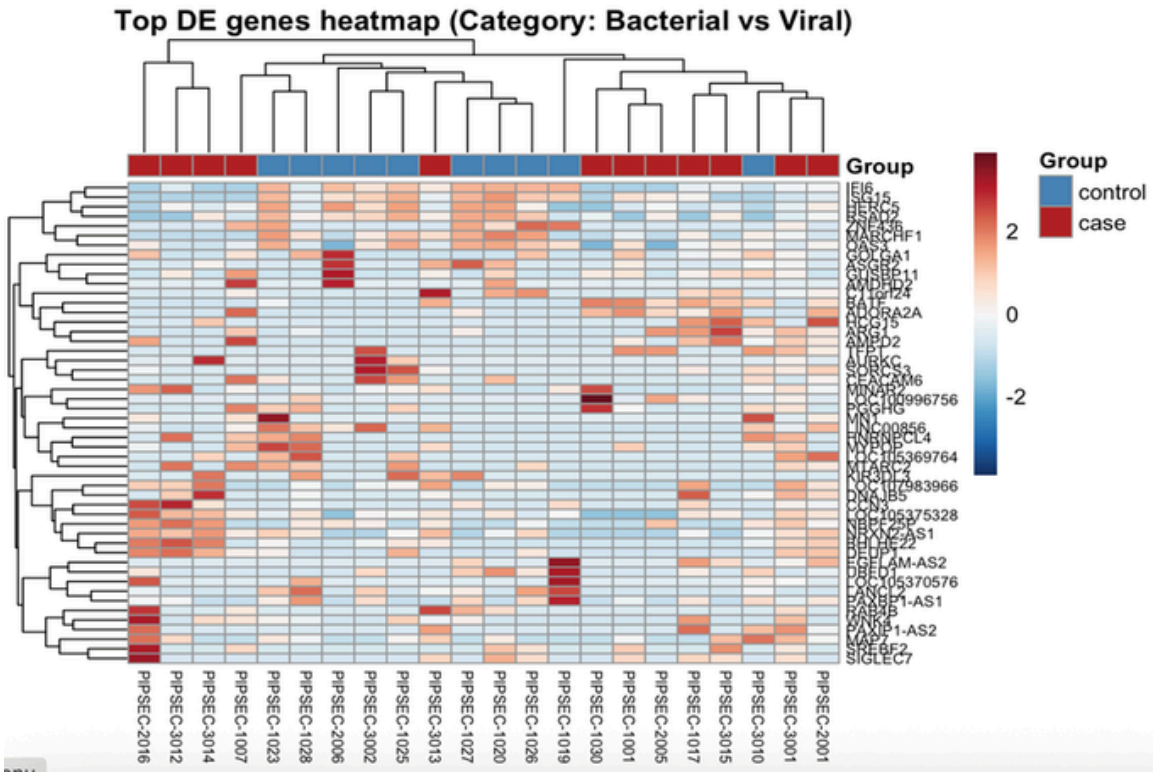

Figure 1b: Heatmap of DE genes. Cells shown in red are upregulated in bacterial meningitis, cells in blue are upregulated in viral meningitis. Greater log-fold change is represented by a deeper cell colour. (DE, differentially-expressed)

Results of DGE analysis

After adjustment for false discovery rate (FDR), 292 genes were differentially expressed (FDR-adjusted P<0.05) between bacterial and viral meningitis cases (figures 1a and 1b). Analysis of candidates from the RUVSeq analysis identified 10 candidate housekeeping genes with low variance and high expression across viral and bacterial samples.

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Machine learning classifier analysis

To rationalise the output from the DGE analysis and produce a parsimonious classifier, feasibly translated to PCR, a machine learning classifier (MLC) was developed in Python to test the predictive potential of DE genes singly and in combination. Logistic Regression, Random Forest, XGboost and Elastic Net MLCs were compared. These were chosen as they are not 'black box' classifiers, instead providing interpretable feature contributions.10 Of these, Random Forest (RF) provided the most consistency in feature rankings and AUCs compared to simpler linear models.

We performed a stratified train–test split (≈80/20), and within the training set conducted differential expression analysis on logCPM values using expression filtering, Welch t-tests, and BH-FDR correction to rank candidate genes. Top genes were evaluated as potential classifiers using logistic regression with stratified cross-validation to estimate training AUC, after which the selected gene set was fitted on the full training data and on the held-out test set. This process was repeated across 1000 bootstrap iterations to identify gene sets that were consistently selected, with stable classification performance (train and test AUC).

Due to limited cohort size, AUC values are unreliable: as such, final top-performing classifier genes and gene combinations were prioritised based on how frequently they were selected across bootstraps. Due to computational limitations, only 1-, 2- and 3- gene combinations were evaluated.

Genes used by the MLC to predict bacterial meningitis centre on roles in granulocyte activation and chemotaxis, whereas genes predicting viral meningitis centre on interferon pathways. Top-performing candidate genes for the PCR assay are shown in table 1 (overpage).

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Gene Name	Number of times selected by MLC	Mean test AUC across bootstraps	Mean SD across bootstraps
Single gene performance (predicts bacterial meningitis)			
IL1b	198	0.859	0.146
CXCL8	120	0.693	0.213
CCNG2	87	0.674	0.148
Single gene performance (predicts viral meningitis)			
IFI6	85	0.722	0.246
IFIT1	68	0.713	0.191
EPSTI	40	0.696	0.213
2-gene classifier performance			
IFI6+CXCL8	24	1	
CXCL8+IFIT1	15	1	
IL1b+HOOK1	12	1	

Table 1: Candidate genes for PCR assay. Number of times selected is out of 1000 bootstraps. (SD, standard deviation; MLC, machine learning classifier)

2026 aims and plan

Over the next 3 months primers to for candidate genes will be developed and assessed. Experiments using mock positive controls will be performed with the purchase of synthetically designed genes. Once the RT-qPCR assay is demonstrated to work, this will be then trialled on recruited clinical samples (test cohort). Furthermore, the same bioinformatic pipeline will be used with RNAseq data generated from clinically recruited patients add additional autoimmune genes will be identified. These experiments are expected to be completed by the middle of 2026. We aim to submit an abstract on this work to the American Academy of Neurology. After further validation work we plan to submit this manuscript for peer review and publication.

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