EPIGENETIC SIGNATURES AND EARLY DETECTION OF NEURODEGENERATIVE DISEASES: DEVELOPMENT OF STRATIFYING BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS IN ASIAN COHORTS

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Introduction

Emerging technologies enable the ability to detect aberrations in the systemic epigenetic control of gene expression, providing a promising approach to early diagnosis of neurodegenerative diseases. Varying symptoms of disease presentation and lack of definitive diagnostic tools pose challenges in the diagnosis of amyotrophic lateral sclerosis (ALS), hindering proper care management of patients. Using the EpiSwitch™ technology platform, epigenetic aberrations can be detected through the interrogation of chromosome conformation signature (CCS) as a crucial step towards personalised medicine, with a rapid, reliable and minimally invasive testing method to detect and monitor the disease.

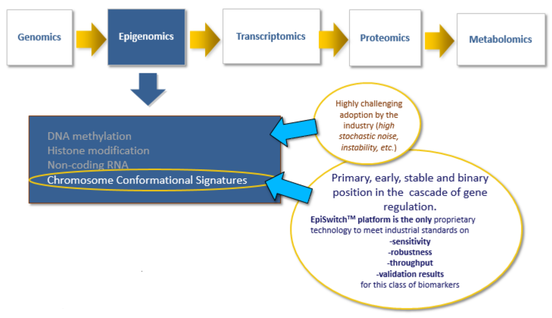
Objectives

1. To develop and evaluate epigenetic biomarker panels for the diagnosis and stratification of ALS by interrogating the structure-function of regulatory genome architecture through chromosome conformation signatures (CCS) .
2. To evaluate the prevalence of disseminating epigenetic deregulations associated with ALS within Caucasian and Asian cohorts.

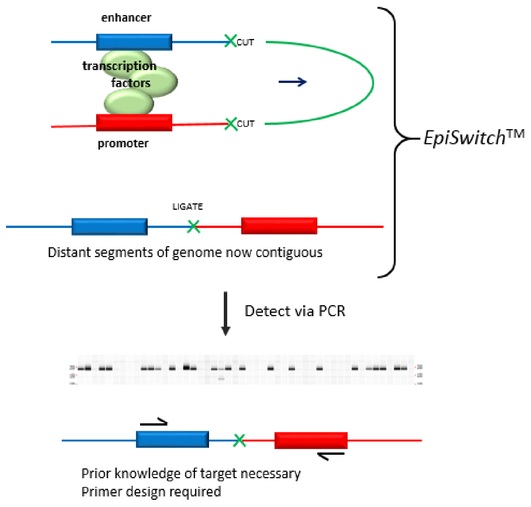
Methods

In this study, we present putative diagnostic biomarkers developed using EpiSwitch™ through the screening of ALS patients and healthy individuals amongst the Caucasian cohort (n=74) and Asian cohort (n=52). A microarray (8x60k gene array Agilent custom CGH) was designed to incorporate 309 genetic loci, 13880 potential interactions. Comparative microarray analysis and linear regression modeling were used to identify the top 153 biomarkers. Following that, multiple rounds of biomarker screening based on the Caucasian cohort were performed using nested PCR to identify the optimal markers with the ability to identify between ALS and control samples with a level of sensitivity and specificity needed for clinical application. The panel of top performing biomarkers were then tested on the Asian cohort to validate the top biomarkers. Bayesian Logistic modelling p-value (Pr(>|z|), Fisher-Exact test p-value and GLMNET were used to analyse the validation results.

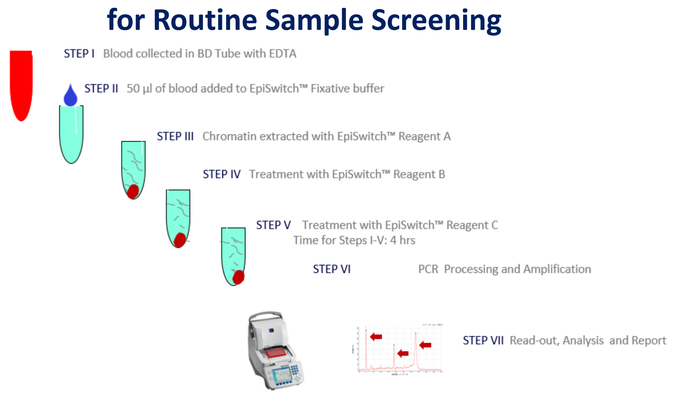
Chromosome Conformation Signatures as the Biomarkers for Monitoring Structure-Function of Regulatory Genome Architecture



Detection of High-Order Chromosomal Structures



The Workflow of the EpiSwitch™ Assay for Routine Sample Screening



Demography

For the Asian cohort, we have collected 5-ml whole blood from 26 clinically diagnosed ALS patients and defined mild, intermediate and severe according to ALSFRS score and 26 gender-matched and/or age-matched healthy individuals amongst the multi-ethnic patient cohort of University of Malaya Medical Center, Kuala Lumpur, Malaysia.

	Healthy Controls	ALS
Number of subjects	26	26
Gender		
Male, n (%)	7 (26%)	18 (69%)
Female, n (%)	19 (73%)	8 (31%)
Ethnicity		
Malay, n (%)	5 (19%)	5 (19%)
Chinese, n (%)	14 (54%)	15 (58%)
Indian, n (%)	7 (27%)	6 (23%)

Results & Discussion

The EpiSwitch™ platform identified the top **eight** blood-based biomarker for ALS based on a Caucasian population training set (n=74) and followed by a blinded Caucasian cohort (n=16) (Salter et al., 2016). Sensitivity and specificity for ALS detection in the 74 unblinded samples were **83.33%** and **76.92%**, respectively. In the blinded Caucasian cohort, sensitivity reached **87.50%** and specificity was **75%**. The top four Caucasian biomarkers were also tested on the Asian cohort (n=52), yielding a sensitivity at **69.23%** and specificity at **61.54%**. This supports disseminating powers of the selected markers across ethnic groups, but also indicates presence of additional deregulations prevalent in Asian cohort. Additional Asian-specific biomarkers are currently under evaluation.

Top 8 biomarkers in unblinded Caucasian cohort.

Statistics	Value	95% CI
Sensitivity	83.33%	51.59% to 97.91%
Specificity	76.92%	46.19% to 94.96%
Positive Likelihood Ratio	3.61	1.30 to 10.06
Negative Likelihood Ratio	0.22	0.06 to 0.79
Disease Prevalence	48% (*)	27.80% to 68.69%
Positive Predictive Value	76.92% (*)	46.19% to 94.96%
Negative Predictive Value	83.33% (*)	51.59% to 97.91%

Top 8 biomarkers in blinded Caucasian cohort

Statistics	Value	95% CI
Sensitivity	87.50%	47.35% to 99.68%
Specificity	75.00%	34.91% to 96.81%
Positive Likelihood Ratio	3.5	1.02 to 11.96
Negative Likelihood Ratio	0.17	0.03 to 1.09
Disease Prevalence	50% (*)	24.65% to 75.35%
Positive Predictive Value	77.78% (*)	39.99% to 97.19%
Negative Predictive Value	85.71% (*)	42.13% to 99.64%

Top 4 biomarkers in multi-ethnicity Asian cohort (work in progress)

Statistics	Value	95% CI
Sensitivity	69.23%	48.21% to 85.67%
Specificity	61.54%	40.57% to 79.77%
Positive Likelihood Ratio	1.80	1.04 to 3.12
Negative Likelihood Ratio	0.50	0.26 to 0.96
Disease Prevalence	50% (*)	35.81% to 64.19%
Positive Predictive Value	64.29% (*)	50.95% to 75.72%
Negative Predictive Value	66.67% (*)	51.03% to 79.33%

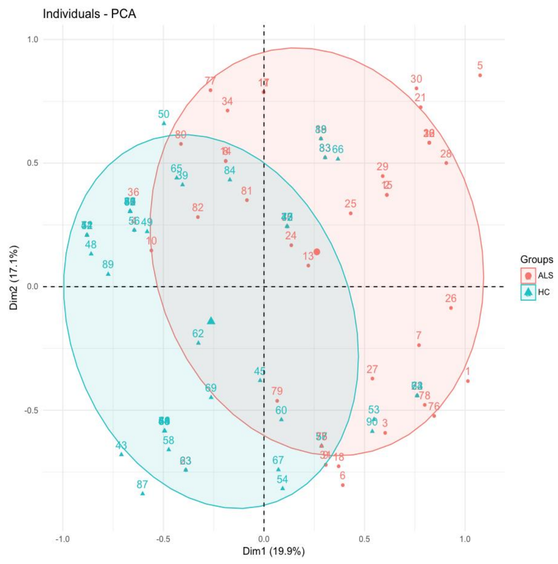


Figure 1. Principal component analysis of ALS-chromosome conformation signature applied to 74 known tissue samples, 16 unknown tissue samples (no annotation) from the Caucasian cohort, and 52 sample from the multi-ethnic Asian cohort.

The top eight significant biomarkers discovered in Caucasian cohorts are linked to neuroinflammatory mechanism in the pathogenesis of ALS. Our preliminary assessment demonstrated four (CD36, DNM3, FYN and NEALS17) out of the top eight biomarkers perform well in the Asian cohort. CD36 is revealed to be a promising powerful biomarker that is able to diagnose ALS across Caucasian and multi-ethnic Asian populations. CD36 expression is associated to fatty acid transport protein and mitochondrial dysfunction. Meanwhile, amongst the top 8 biomarkers identified in the Caucasian cohort, FYN, GRB2, IKBKB and PTPRC are linked to Major Histocompatibility complex (MHC) 2 and acquired immunity, while CD36, TAB2, IKBKB biomarker proteins are embedded in pathways associated with the innate immune system.

Conclusion

Early and rapid diagnostic tools for ALS is crucial to good patient management. In this study, EpiSwitch™ biomarkers show strong potential in assisting with robust ALS stratification and early clinical decisions. Importantly, early results indicate that epigenetic nature of CCS biomarkers discriminates between deregulations prevalent in Caucasian and Asian populations. Further analysis is in progress.

References

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