A PROSPECTIVE STUDY OF TWO DISTINCT EPIGENETIC SIGNATURES FOR ALS DIAGNOSIS AND PROGNOSIS

W. Westra[1], M. Salter[1], E. Hunter[1], W. Elvidge[1], A. Ramadass[1], F. Grand[1], J. Green[1], L. Ossher[2], A. Thompson[2], J. Scaber[2], E. Feneberg[2], M. Turner[2], K. Talbot[2], M. Cudkowicz[3], A. Akoulitchev[1].

[1]Oxford BioDynamics Plc, 26 Beaumont Street, Oxford UK, OX1 2NP. [2]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford UK, O3 9DU.
[3]Department of Neurology, ALS Clinic, Massachusetts General Hospital, Boston, MA 02114 USA.

Introduction

The ability to detect epigenetic perturbations provides a promising approach for the early diagnosis of ALS and establishing patient prognosis. A comparative interrogation of the genomic architecture from healthy and diseased-patient blood samples revealed two ALS-related epigenetic signatures: one with diagnostic potential and a second for prognosis prediction. For this prospective study, samples collected by the clinical-research group at the Oxford Motor Neuron Disorders Clinic at the Nuffield Department of Clinical Neurosciences (NDCN) at the University of Oxford were analyzed using EpiSwitch™, the high throughput epigenetic discovery platform developed by the research team at Oxford BioDynamics Plc. This prospective study compares clinical annotations of the ALSFRS-R (1) Forced vital capacity (FVC) and other clinical observations to assign ALS-progression subtypes with concomitant analysis of diagnostic- and prognostic-epigenetic signatures.

Objective

To identify determine the sensitivity and specificity of two epigenetic-based ALS disease signatures for prospective diagnosis of ALS and prediction of prognosis.

Methods

100 patients, presenting to the Oxford Motor Neuron Disorders Clinic, enrolled in the study and were asked to return at 3 and 6 months. Spousal controls (n=100) were also collected. During each visit, participants underwent the ALS-FRS-R and FVC tests, and provided a blood sample. The samples were analyzed to identify an ALS- disease-related prognostic signature (ALS patients at 3 and 6 months). Results of the clinical assessments were compared to the EpiSwitch™ analysis at 0, 3 and 6 months. A cut off of a 0.5-point decline per month of the ALSFRS-R score was used to cluster the ALS patients into progression-subtypes.

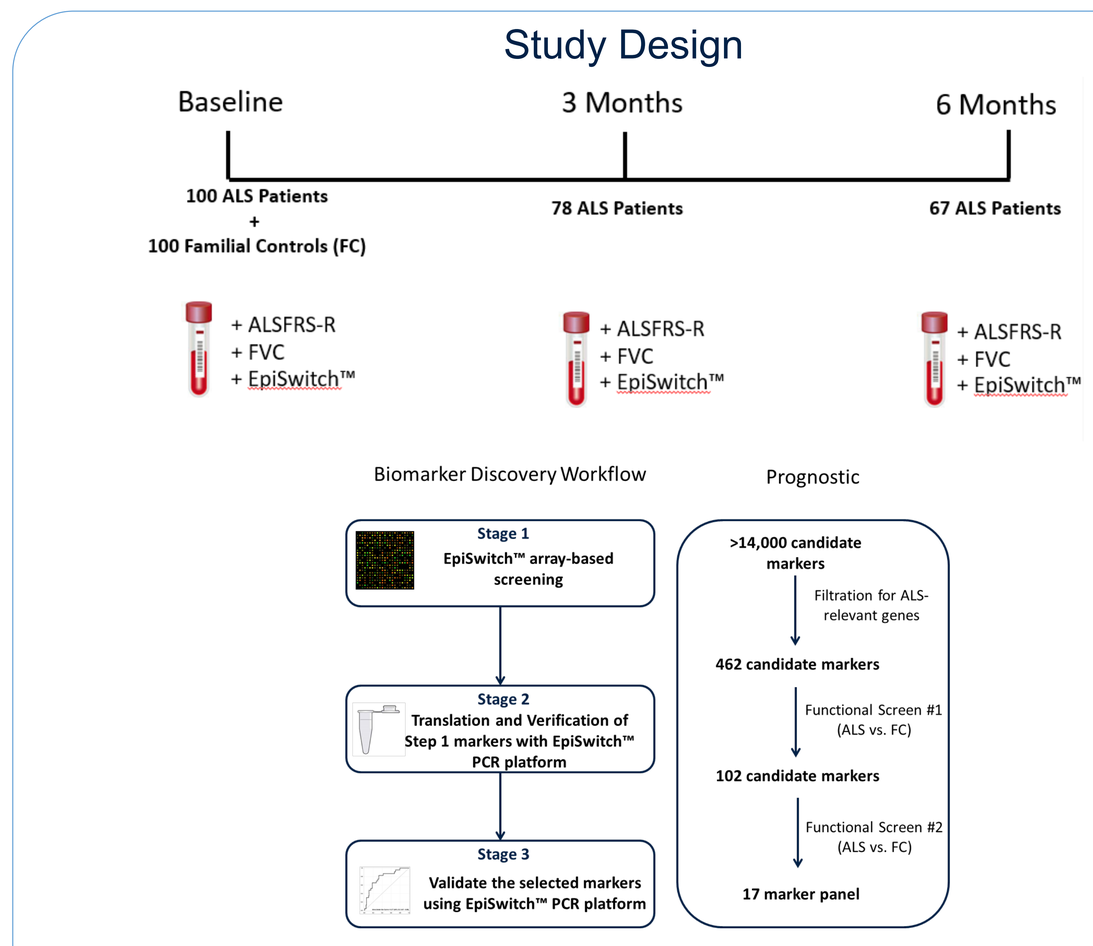


Figure 1. Study Design & Biomarker Discovery Workflow.

Demographics

For this study, 5-ml whole blood was collected from 101 clinically diagnosed ALS patients according to ALSFRS-R scoring and 100 age-matched familial controls. A cut off of a 0.5-point decline per month of the ALSFRS-R score was used to group the ALS patients into Faster (>0.5) and Slower (<0.5) progression-subtypes.

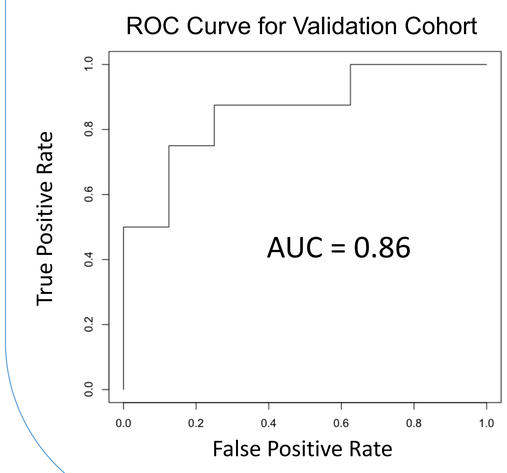
	Familial Controls	ALS
Number of subjects	100	100
ALSFRS-R score range	-	15-48
ALSFRS-R, Avg. (SD)	-	38 (8)
Age at onset, Avg. (SD)	-	60 (13)
Age at baseline, Avg. (SD)	58 (12)	61 (13)
Gender		
Male, n (%)	32 (32%)	61 (61%)
Female, n (%)	68 (68%)	39 (39%)
Progression		
Faster, n (%)	-	50 (50%)
Slower, n (%)	-	50 (50%)

Results

Diagnostic Biomarker

Biomarker Panel
CD36
TAB2
GLYCAM1
GRB2
FYN
PTPRC
DNM3
IKKB

The 8 gene loci making up the diagnostic biomarker panel for the diagnostic biomarker developed in a previous study are linked to biological mechanisms of the pathogenesis of ALS. CD36 is linked to a fatty acid transport protein and mitochondrial dysfunction in animal models of ALS[1]. Four biomarkers (FYN, GRB2, IKKB and CD45) included in the chromosome conformation signature can be linked to the Major Histocompatibility Complex (MHC) 2 and acquired immunity. Three (CD36, TAB2, IKKB) biomarker proteins are embedded in pathways associated with the innate immune system. Both sets hint at the involvement of neuroinflammatory mechanisms in the pathogenesis of the disease.



Statistic	Development Cohort (N=74)	Validation Cohort (N=16)
Sensitivity	83.3%	87.5%
Specificity	76.9%	75.0%
Positive Predictive Value	76.9%	77.8%
Negative Predictive Value	83.3%	85.7%

Figure 2. Diagnostic Biomarker. Receiver operating characteristic (ROC) curve and sensitivity and specificity of disease signature when used to classify a set of samples from two ALS clinical trials.

Prognostic Biomarker

Biomarker Panel
ZFPM2
VEGFA
ERBB4
ZFPM2
PASD1
GRM7
GRIK2
SORCS2
LINGO2

The 9 gene loci making up the diagnostic biomarker panel for the diagnostic biomarker are linked to biological mechanisms in the pathogenesis of ALS. Two markers (ZFPM2 and PASD1) encode transcription factors involved in regulating hematopoiesis and the T-cell response[2]. VEGFA is a growth factor that has been linked to prolonged survival in ALS[3]. Three of the markers, (GRM7, GRIK2 and SORCS2) encode neurotransmitter receptors. ERBB4 encodes a receptor for Epidermal Growth Factor (EGF) and mutations in ERBB4 have been shown to cause ALS[4]. LINGO2 is the least well functionally characterized, but has been shown to be involved in the development of essential tremor and linked to Parkinson's Disease[5].

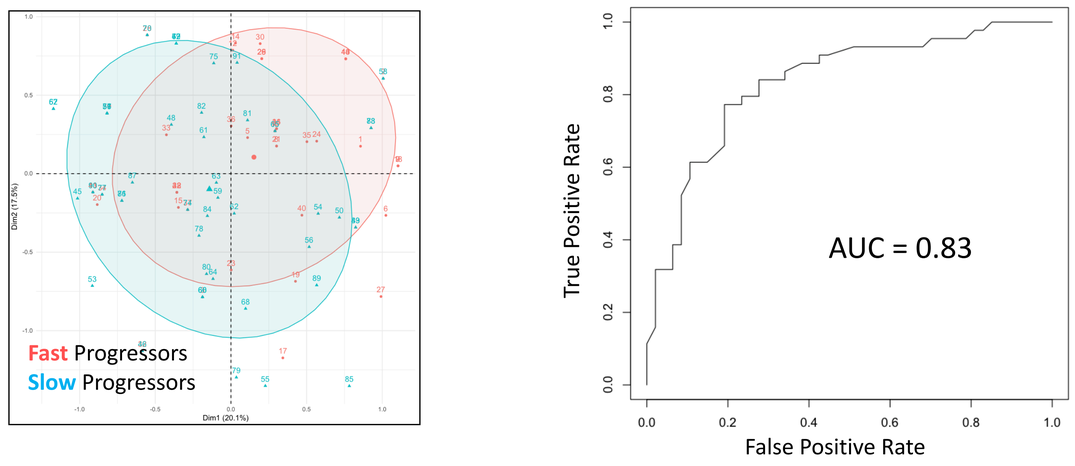


Figure 3. Prognostic Biomarker. Principle components analysis (PCA) and ROC curve for assessing the predictive ability of the 9-marker panel to differentiate between fast progressors (in red) and slow progressors (in blue) at baseline.

Based on the rate of decline in the ALS-FRS-R score, preliminary results from 48 samples at 3-month and 43 samples from 6-months demonstrated the epigenetic signature selected faster (>0.5) and slower (<0.5) progressing ALS patients with a sensitivity and specificity of 80%. The results indicate the prognostic signature is robust for selecting subtypes of ALS over time.

Conclusion

The application of ALS-disease related epigenetic biomarkers could improve the time to diagnosis for ALS patients and provide a predictive prognosis by identifying subtypes of ALS based on rate of disease progression. Further investigation is warranted.

References

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