Deviations from the natural history of delta power in Angelman syndrome reflect treatment effect size and correlate with UBE3A expression

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Background

- Angelman syndrome (AS) is a neurodevelopmental disorder caused by deficiency of the maternally inherited *Ube3a* gene in neurons.
- Antisense oligonucleotide (ASO) therapies targeting the *Ube3a* antisense transcript (*Ube3a-ATS*) are under development to reinstate UBE3A protein production.
- Non-invasive biomarkers to detect target engagement and treatment response are needed to support clinical trials.
- Abnormal delta power (2-4 Hz) measured in the scalp electroencephalogram (EEG) is a reliable biomarker for AS, but varies widely across individuals and throughout development, making detection of a treatment effect using single measurements challenging.
- Longitudinal measurements of delta power, accounting for each subject's age and elapsed time between measurements, are required to develop a more sensitive measure of target engagement in clinical trials.

Stability of delta power

- We assessed the stability of delta power estimates over shorter time intervals by comparing delta power estimates using increasing data sample sizes, randomly selected from one-second intervals in 5 AS patients with 24-hour continuous recordings.
- As the amount of data increases, the standard error decreases at a rate of approximately $1/\sqrt{x}$.
- When the sample size exceeds 8 minutes, the SEM plateaus and remains less than 0.009 (95% CI [0.008, 0.01]).
- Delta power estimates are stable over the course of a 24-hour period and can be reliably estimated from just 8 minutes of EEG data.

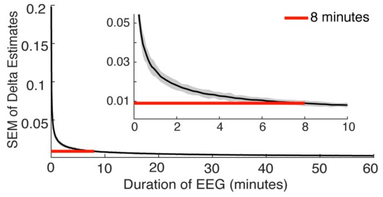

Figure 1. Only a few minutes of EEG data are needed to estimate delta power with high precision.

Longitudinal natural history model

- We utilized a dataset consisting of 204 longitudinal EEG recordings from 56 AS patients to develop a natural history model of delta power in AS to predict delta power at a future visit.

$$Delta_{Visit\ 2} \sim Delta_{Visit\ 1} + log_{10}(Age_{Visit\ 1}) : IVI + (1|Subject).$$

- The delta power at a second visit is given by the delta power at an initial visit, age at the initial visit, and elapsed time between visits.
- A random intercept is included to allow inter-subject variability in baseline delta power, for example genotype or disease severity.

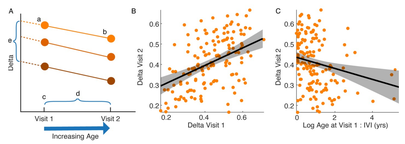

Figure 2. Natural history model overview. (A) Schematic of variables included in the natural history model: a. Delta power at visit 1; b. Delta power at visit 2; c. Age at visit 1; d. Inter-visit interval (IVI); e. Random intercept. (B,C) Model fit on longitudinal data.

Power to detect treatment effect

- Treatment effect: a deviation from the expected natural history due to treatment.
- A treatment effect that reduces delta power would result in significantly larger model residuals in the simulated treatment group compared to the simulated control group.
- Example simulation (n=50) where the effect size of the treatment group is a 0.1 reduction in delta power. (95% CI [0.08, Inf], p<1e-8, one-sided t-test).
- We can detect with 80% power a treatment effect size of 0.064 relative delta power in a sample of 25 subjects per group, 0.046 in a sample of 50 subjects per group, 0.033 for 100 subjects per group, and 0.027 for 150 subjects per group.

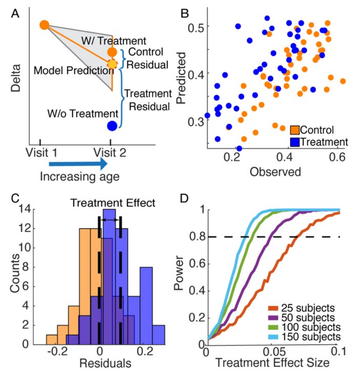

Figure 3. Simulation of model implementation. Example simulation (A-B) where the effect size is a 0.1 reduction in delta power. (B) Predicted versus the observed delta power at visit 2. (C) Histograms of the corresponding control and treatment residuals. (D) Power to detect a treatment effect versus treatment effect size at different sampling sizes.

The natural history model in AS mice

- We applied the natural history model to a preclinical mouse model of AS to detect ASO-mediated treatment effects on delta activity and *Ube3a* expression in ASO-treated (n=15) versus placebo-treated mice (n=26).
- To assess the impact of treatment, we compared model residuals for control ASO-treated and Ube3a-ATS ASO-treated mice using a resampling procedure.
- The effects at post-treatment weeks 2-8 were tested against a null distribution.

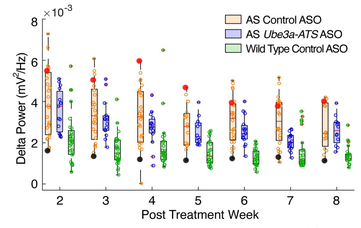

Figure 4. Longitudinal mouse LFP data. Boxplots of delta power across age per group at each time point.

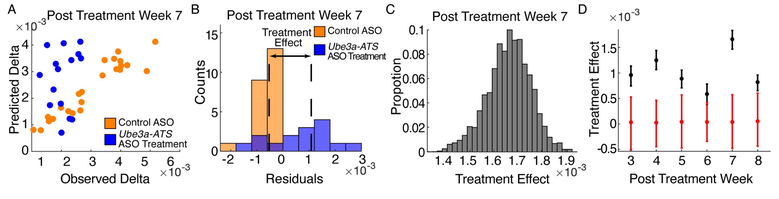

Figure 5. Application of natural history model to mouse model of AS. (A) Example iteration of predicted versus observed delta power (mV^2/Hz) values at visit 2. (B) Corresponding example histogram of model residuals and the treatment effect. (C) Histogram of estimated treatment effect at Week 7 post-treatment from 10,000 iterations. (D) Treatment effect size (black: actual; red: null hypothesis) versus post-treatment week.

Unsilencing of the *Ube3a* paternal allele

- We observed a ~70% reduction in *Ube3a-ATS* with the *Ube3a-ATS* ASO compared to control ASO treated mice at 8-weeks post-ASO administration.
- This level of *Ube3a-ATS* knockdown corresponded to ~2-fold increase in *Ube3a* mRNA.
- The relative *Ube3a* mRNA levels were fitted against the model residuals from the same mouse.
- Unsilencing of the *Ube3a* paternal allele with a *Ube3a-ATS* ASO in AS mice leads to increase *Ube3a* mRNA and UBE3A protein.
- *Ube3a* mRNA is positively corelated with model residuals. (p<0.001)

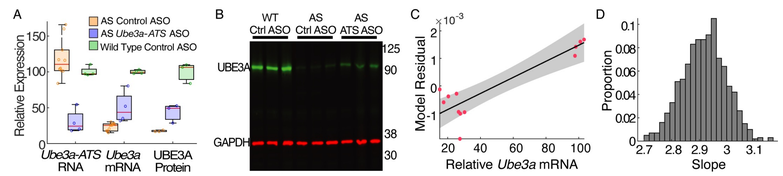

Figure 6. (A) *Ube3a-ATS* RNA levels, *Ube3a* mRNA levels, and UBE3A protein levels at 8-weeks post-ASO treatment. Mice were ICV dosed at P35. Each group was normalized to its own respective WT control group. (B) Western blot from WT and AS mouse cortical tissue. (C) Example model residuals (red dots) and *Ube3a* mRNA expression (normalized to WT control) and the linear fit to these data (mean, black line; gray shaded region, 95% confidence intervals). (D) Histogram of estimated slopes from all resamples.

Conclusions and Future Directions

- We utilized a large database of longitudinal EEG recordings from AS patients and developed a natural history model of delta power in this disorder.
- The model allows estimation of the populations required to detect treatment effects of various sizes on delta power for use in clinical trial planning.
- Deviations in delta power from a human natural history model in AS can detect ASO-mediated improvement in *Ube3a* expression in AS mice and may be relevant for human clinical trials.
- These results support utilizing non-invasive measures of delta power to demonstrate target engagement and potential treatment effect in human clinical trials in AS.
- Future work to validate the relationship between delta power and UBE3A expression after effective treatment in humans with AS would secure delta power as a mechanistic biomarker to gauge both target engagement and therapeutic response in clinical trials

Funding

ERS and WS were supported by an investigator-initiated sponsored research award to the Chu Lab from Biogen Inc. ERS is supported by National Science Foundation GRFP.