Feasibility of glycine-proline as a biomarker for antisense therapy in C9ORF72-mediated amyotrophic lateral sclerosis

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Outline

• Background
• Hypothesis
• Study population
• Study protocol
• Limitations
ALS and **C9ORF72**

- **Amyotrophic lateral sclerosis (ALS)** is a fatal neuromuscular disorder
  - Median survival = 2-5 years
  - No curative therapies to date
  - 90% sporadic, 10% familial

- **Leading known cause** is a hexanucleotide repeat expansion (GGGGGCC) in **C9ORF72**
  - 7% of sporadic cases, 34% of familial cases in the US
  - Not known why this mutation leads to ALS
  - Repeat size (700-1600 units) has prevented development of animal models

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Antisense oligonucleotide (ASO) therapy

- ASOs that bind the GGGGCC repeat are protective in patient-derived neurons *in vitro*\(^1\)
- ASOs do NOT alter C9ORF72 protein expression
- A biomarker is needed to monitor the efficacy of intrathecal ASO therapy in patients

1. Donnelly et al. (2013), *Neuron, 80*(2), 415–428
Repeat-associated non-ATG initiated (RAN) translation of C9ORF72

DNA

![DNA structure with repeats and corresponding amino acids]

Pre-mRNA

SENSE: ----GGGGGCCC-GGGGGCCC-GGGGGCCC-GGGGGCCC-GGGGGCCC-GGGGGCCC-GGGGGCCC----

ANTISENSE: ----CCCCCGG-CCCCCGG-CCCCCGG-CCCCCGG-CCCCCGG-CCCCCGG-CCCCCGG----

GGG-GCC = glycine-alanine (GA) = GAGA . . .

GGG-CCG = glycine-proline (GP) = GPGP . . .

GGC-CGG = glycine-arginine (GR) = GRGR . . .

GGC-CCC = glycine-proline (GP) = GPGP . . .

CGG-CCC = arginine-proline (AP) = APAP . . .

CCG-GCC = proline-alanine (PA) = GRGR . . .

Zu et al. (2013), PNAS, 110(51).
Stable isotope labeling tandem mass spectrometry (SILT) to assess *de novo* protein synthesis

- Stable isotope-labeled amino acid (\(^{13}\)C\(_6\)-leucine) is infused
- Serial CSF samples obtained via lumbar catheter
- Protein of interest is immunoprecipitated
- Labeled vs. unlabeled peptide is quantified using tandem mass spectrometry
- Fractional synthesis rate (FSR) and fractional clearance rate (FCR) are calculated:

![Graph depicting FSR and FCR](image)

Hypothesis

• The repeat-associated non-ATG initiated (RAN) peptide glycine-proline (GP) is synthesized and secreted into CSF at a rate of ≥ 5% per hour in ALS patients with the C9ORF72 hexanucleotide repeat expansion (GGGGGCC).
Study Population

• Four adult patients with C9ORF72 ALS, recruited from the C9ORF72 clinic at the NIH

• Must meet revised El Escorial criteria for ALS, according to the evaluation of the NIH neuromuscular specialist

• Exclusion criteria: (as determined by an independent physician on hospital admission).
  – INR > 1.5
  – platelet count < 100,000
  – current use of anticoagulation
  – signs/symptoms of intracranial space-occupying lesion
  – systemic or local (lumbar) infection
**Study protocol**

- **Phase 1: patient 1**
  - Admit to hospital, place lumbar catheter
  - Infuse $^{13}$C$_6$-leucine for nine hours (peripheral IV).
  - Collect 1 cc CSF per hour until labeled GP no longer detectable (up to 72 hours).
  - Determine time frame for synthesis, plateau, and clearance of labeled GP

- **Phase 2: patients 2-4**
  - Same labeling protocol
  - Collect 1 cc CSF per hour during synthesis, plateau, and clearance phases as determined in phase 1
  - Calculate FSR and FCR for each patient, determine mean and standard deviation
Significance

• If GP is actively synthesized and secreted into CSF, then GP can be used as a biomarker for an upcoming phase I safety trial of ASO therapy in \textit{C9ORF72} patients.
Limitations

• Small sample size
• Potential for significant variability
• No control group planned
• Invasive procedure required
• Expensive and time consuming assay
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