Establishing a baseline and drug-induced SMN expression profile in SMA disease-relevant human tissues in expedited autopsies

Spinal muscular atrophy (SMA) is a recessive neuromuscular disease that is caused by loss-of-function mutations in the survival motor neuron 1 (SMN1) gene and is one of the most common inheritable causes of infant death, where without immediate treatment, infants succumb to respiratory insufficiency within the first years of life. Disease penetrance can inversely correlate with copy number of the paralog gene SMN2 – which can vary from 2 to 5 copies in most individuals – depending on sufficiency in the levels of functional full-length SMN proteins being produced, which is a very small minority from SMN2 mRNA that make it as full-length transcripts that include exon 7.

SMA gene therapy is one of the areas of intense interest in therapeutics due to the fact that SMA is thought to be caused by a single monogenic mutation, which may help simplify the possible mechanisms and consequently, the target against which to build creative therapeutic solutions. Among the most discussed strategies, antisense oligonucleotides (ASOs) and small molecules to help modify the natural splicing behaviors of SMN2 pre-mRNAs or the delivery of exogenous SMN1 to supplement levels of functional SMN proteins using recombinant adeno-associated viruses – have gained great momentum for both their proof-of-concept findings and observable motor function improvements in various studies.

In fact, onasemnogene abeparvovec-zioi, a self-complementary rAAV 9 expressing SMN1 cDNA, was approved by the FDA mid-2019 for use in infants two years and younger with varying degrees of clinical benefits from recuperation of

motor milestones to no change in motor function. In an effort to shed additional light on understanding the variability behind these therapeutic efficiencies, Dr. Ramos and Dr. Sumner's team investigated baseline and drug-induced SMN levels in disease-relevant human tissues in control and SMA patients ranging in age from 15 weeks gestation to 14 years, including five SMA patients treated with nusinersen – an FDA approved splice-switch ASO delivered by lumbar intrathecal injection – in expedited autopsies.

They found that SMN protein levels are 2.3-fold and 2.6-fold higher in prenatal controls compared with postnatal controls < 3 months and 6.5-fold and 4.3-fold higher than postnatal controls > 3 months through 14 years in thoracic/lumbar spinal cord or cortex, respectively, using HTFR and validated by ECL and Western blot (Figure 1). These general patterns were also observed in iliopsoas muscle and diaphragm muscles, two tissues implicated in SMA disease progression.

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Spinal cord SMN mRNA levels were also measured using RT-qPCR assaying full-length (including exon 7) SMN1 (SMN1-FL), full length SMN2 (SMN2-FL), and SMN2 lacking exon 7 (SMN2-Δ7), and showed that the full length mRNA had a modest overall decrease between early and late postnatal samples, while changes in SMN2-Δ7 were minimal, indicating minor if any effect of age on splicing patterns. Additional correlation studies indicated that additional post-transcriptional mechanisms may more heavily contribute to decreases in SMN protein levels during perinatal development rather than a strict dependency of SMN protein levels to mRNA levels or SMN2 copy number.

The authors moved on to assess the impact of ASO treatment compared to un-treated, age-matched controls. ASO drug concentrations were variable in cervical spinal cord and brain tissues where available for analysis. Drug concentration patterns were associated with 3-fold increases in SMN2-FL mRNA at the spinal cord level in nusinersen-treated cases, though unchanged in brain samples (n=2 available who had received multiple doses); increases in whole-tissue protein levels were not observed, though case-by-case showed some interesting patterns (Figure 2).

Upon assessing the presence of ASO and SMN in the spinal cord via immunostaining between nusinersen-treated, untreated, or unaffected controls, ASO staining intensity appeared highest in the lumbar/sacral and thoracic spinal cord while less or modestly in the cervical spinal cord and upper brain regions. Using a user- trained paradigm in Neuron ID 2-RBD software, SMN expression was quantified and showed that the total percentage of SMN- positive or low-SMN-positive cells were increased with treatment compared to non-treated controls in the lumber and cervical spinal cords (similar trend observed in thoracic cords).

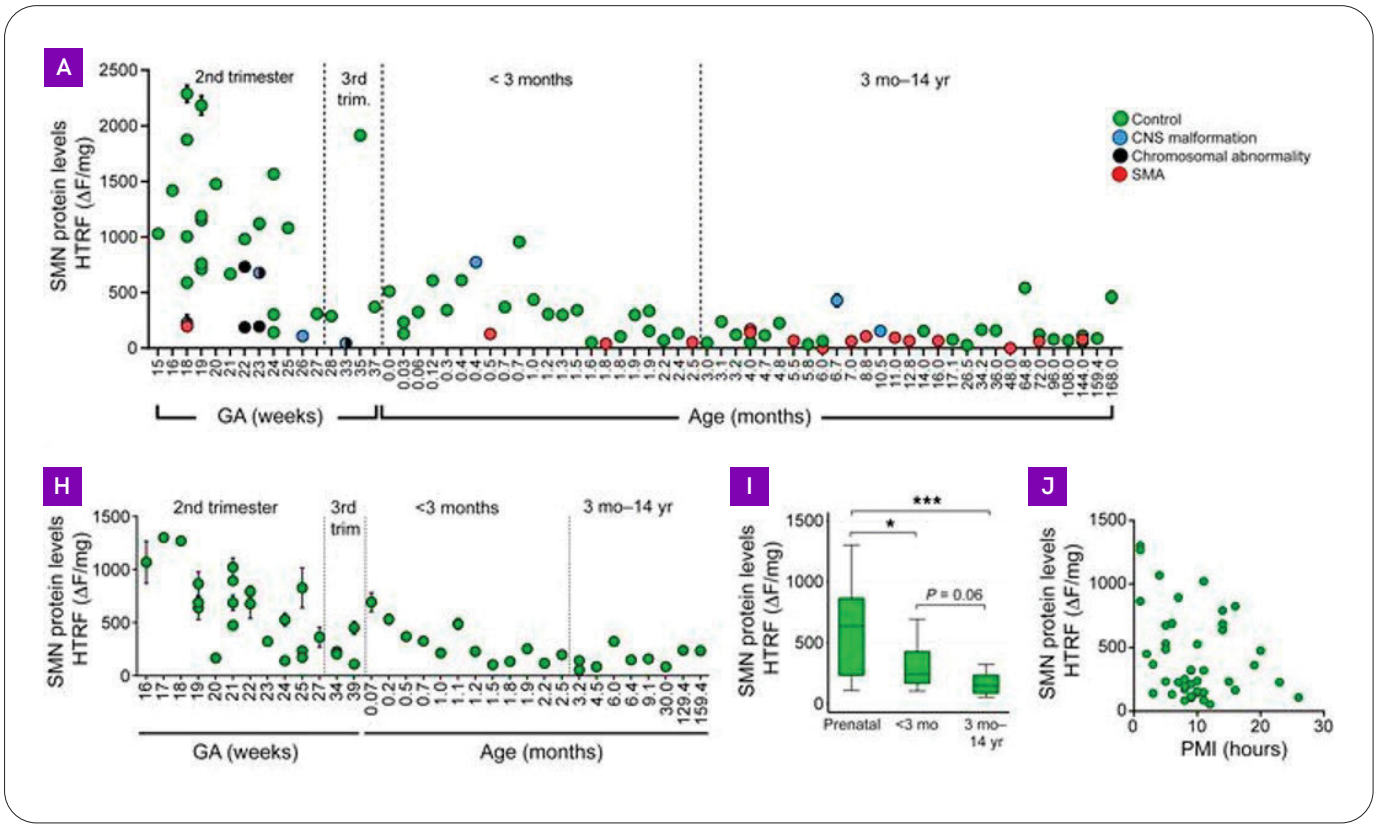

Figure 1: Characterizing SMN protein levels in spinal cord (A) and cortex (H, I) using HTRF. Additional method details can be found in the method and figure legend of https://doi.org/10.1172/JCI124120.

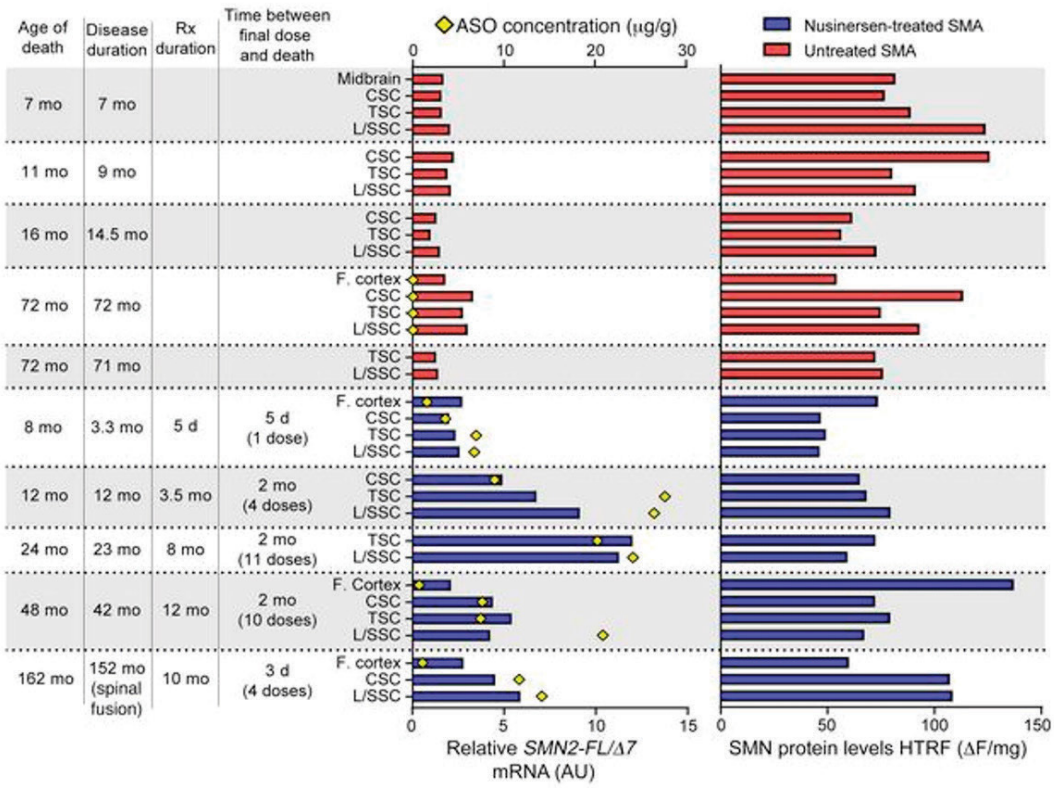

Figure 2: Case-by-case SMN relative SMN2FL/Δ7 mRNA and protein level expression in whole spinal cord of Rx-treated SMA cases compared to age-matched control (F). Rx, nusinersen treatment; CSC, cervical spinal cord, TSC, thoracic spinal cord, L/SSC, lumber/sacral spinal cord. Additional method details can be found in the method and figure legend of https://doi.org/10.1172/JCI124120.

The work from this lab in establishing SMN expression level baselines in disease-relevant human tissues across a broad age range was a result of the efforts behind a multistate, decade-long program of expedited autopsy tissue collection. In addition to establishing baselines, the research noted additional venues of research that were worth exploring, including mechanisms into epigenetic changes at the gene promoter, translation or protein in translation, protein stability as protein levels did not heavily associate with mRNA transcript levels. These results help shed light on considerations for optimization strategies that may improve clinical benefits for novel SMA therapies being developed, especially as therapeutic efficacy so strongly dependent on a short window of time to act.

References

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