**Title:** Meeting report on the 2nd Clinical and Scientific Conference on ADSS1 myopathy. 24 October 2025, Boston Children's Hospital, Brookline, Massachusetts, United States of America

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**Highlights**

- The 2nd ADSS1 myopathy conference was held in Boston on 24 October 2025.
- FDA clearance enabled the first Phase 1b trial of ASA in ADSS1 myopathy
- ADSS1 myopathy is emerging as a multisystem metabolic muscle disease
- Imaging, EIM, 31P-MRS and plasma biomarkers may support clinical trials
- New models are advancing ASA, gene therapy and AI-guided drug repurposing

**Keywords**

Adenylosuccinate synthase 1 myopathy, ADSS1 myopathy, inborn error of metabolism, purine disorder, ultra-rare neuromuscular disease, skeletal muscle, cardiac muscle, clinical presentation, pre-clinical models, therapeutics, biomarkers, consortium, guidelines

## 1. Introduction

Adenylosuccinate synthase 1 (ADSS1) myopathy is an ultra-rare, autosomal recessive metabolic myopathy caused by pathogenic variants in *ADSS1* [1, 2], which encodes the muscle-enriched adenylosuccinate synthase enzyme required for de novo purine nucleotide synthesis. Disruption of this pathway impairs the conversion of inosine monophosphate (IMP) to adenylosuccinate and ultimately adenosine monophosphate (AMP), limiting the ability of skeletal muscle to maintain adenine nucleotide pools during rest, activity and recovery. Clinically, ADSS1 myopathy is characterised by progressive muscle weakness and wasting, often with early lower-limb involvement, facial and masticatory muscle weakness, fatigue, respiratory insufficiency and, in some patients, cardiac involvement [2-4]. Increasing clinical and molecular data indicate that the disease is broader than a distal myopathy alone, with emerging evidence of mitochondrial dysfunction [5], neuromuscular junction instability [4, 6], inflammatory signalling [4], altered lipid and glucose metabolism [7], and variable connective tissue or systemic features [8].

The 2nd Clinical and Scientific Conference on ADSS1 Myopathy, held at Boston Children's Hospital in October 2025, brought together clinicians, scientists, patient advocates and therapeutic development partners to review progress across diagnosis, natural history, biomarker development, patient care and preclinical therapeutic pipelines since our inaugural 2024 meeting [9]. The meeting occurred at a pivotal moment for the field, following U.S. FDA Investigational New Drug (IND) clearance for the first experimental therapeutic trial in ADSS1 myopathy (IND 168876, NCT07412821 (https://clinicaltrials.gov/study/NCT07412821)) and substantial expansion of international collaborative research activity.

Across the program, presentations highlighted the rapid maturation of ADSS1 myopathy research from case discovery toward mechanism-led clinical translation. Clinical sessions refined understanding of disease progression, respiratory care, exercise management and non-invasive outcome measures. Molecular and imaging studies identified candidate

biomarkers suitable for longitudinal monitoring and clinical trial readiness. Preclinical sessions demonstrated convergent evidence from yeast, worm, cell and mouse models that ADSS1 deficiency disrupts purine homeostasis, energy metabolism, mitochondrial function and neuromuscular signalling. Collectively, the meeting highlighted that ADSS1 myopathy is a multisystem muscle energy disorder requiring integrated clinical surveillance, mechanistic biomarker development and coordinated international trial infrastructure.

### 1.1 Background

**Mr. Naveen Baweja** provided opening remarks on Cure ADSSL1's activities since the last meeting, including recent U.S. FDA IND clearance of the first experimental therapeutic for ADSS1 myopathy.

**Dr. Emma Rybalka**, Cure ADSSL1's Scientific Director, remarked on the ADSS1 Myopathy Research Consortium's publication and grant outcomes since the last meeting. Publications included comparative case studies of siblings from China and Japan, and detailed phenotyping studies describing the first animal models of ADSS1 myopathy (the mouse study was accepted for publication and in preprint at the time). Grants totalling >USD$1.7M were awarded in the United States and Australia to understand disease biology (PI Hanna-Rose, R21NS143788), translate the first experimental therapeutic to clinical trial (PI Rybalka, GA409776), and compose an extensive registry and biorepository from patients and carriers (PI Beggs, MDA1457288).

## 2. Clinical perspectives

### 2.1 Keynote

**Dr. Perry Shieh, M.D., Ph.D.** presented the design of the first-in-class Phase 1b clinical trial for adenylosuccinic acid (ASA) at UCLA (NCT07412821). ASA is the immediate product

of the ADSS enzyme and is being investigated as a therapeutic replacement. The study involves two adults with ADSS1 myopathy and utilizes a single and multiple ascending dose (SAD/MAD) escalation design. Primary objectives are safety and tolerability, with secondary measures including pharmacokinetics and preliminary efficacy tracked through motor function tests and needle core muscle biopsies. A unique aspect of the protocol is the use of repetitive nerve stimulation to monitor muscle decrement, alongside a neuromuscular function battery and patient-reported outcomes to capture quality-of-life changes.

*2.2 Session 1: Insights from the clinic*

**Dr. Kexin Jiao, M.D.,** shared a case study of a 22-year-old patient exhibiting mitochondrial abnormalities alongside classic myopathy symptoms. Genetic testing identified a homozygous *ADSS1* variant (c.781G>A; p.D261N), consistent with prior reports [8, 10]. Muscle biopsies revealed COX-negative and ragged red fibers, and swollen, disorganized mitochondria. These findings are notable, as mitochondrial dysfunction is not yet considered a universal hallmark of ADSS1 myopathy, raising important questions about disease heterogeneity and secondary metabolic stress responses. This case highlighted a broader clinical spectrum for ADSS1 myopathy than previously thought, noting that pathology can include metabolic, structural, mitochondrial, and neuromuscular features. The patient showed a partial response to pyridostigmine, suggesting that the disease involves neuromuscular junction dysfunction and is a potentially treatable component in some patients. The findings emphasize the need for a differential diagnosis from congenital myasthenic syndromes.

**Dr. Yoshihiko Saito, M.D., Ph.D.,** presented quantitative whole-body muscle imaging data from 57 Japanese patients, identifying distinct patterns of disease progression. Forty-three muscles were classified into six clusters based on the degree of fat replacement indicative of disease progression. Early-stage involvement typically begins in the lower leg (tibialis anterior and gastrocnemius), while the trunk muscles remain relatively spared even in late stages. The

masseter muscle is often the earliest affected, suggesting it could serve as a novel diagnostic marker and a rationale for early nutritional and rehabilitative support. Diaphragm thickness correlated with respiratory function. The study concluded that imaging indices accurately reflect disease duration and provide a practical tool for monitoring therapeutic interventions.

**Dr. Merve Yekedüz, M.D.,** presented a clinical evaluation identifying non-invasive structural and functional biomarkers using electrical impedance myography (EIM) and magnetic resonance imaging (MRI) (now published [4]). The study compared five ADSS1 myopathy patients to healthy controls, finding markedly reduced grip strength and significantly higher levels of fatigue. EIM results, which expanded upon prior work employing EIM in patients with ADSS1 myopathy [11], again showed a decrease in phase angle and reactance, alongside an increase in resistance in lower and upper extremities, which likely reflects muscle atrophy as well as fatty infiltration of muscle tissue. Additionally, speech tests revealed voice quality changes – such as hypernasality and breathiness – reflecting weakness in the laryngeal and soft palate muscles. These tools offer an alternative way to track disease progression without invasive biopsies.

**Dr. Hyder Jinnah, M.D.,** discussed the metabolic rationale for ADSS1 myopathy, focusing on the purine metabolic pathway's role in determining muscle adenine nucleotide levels. Using Phosphorus Magnetic Resonance Spectroscopy ($^{31}$P-MRS), baseline ATP levels were shown to be chronically low in muscle of ADSS1 patients, often at nearly half the concentration of healthy controls. The study monitored thigh muscles during and after exercise, revealing that ATP regenerates more slowly in affected individuals. While normal metabolic pathways usually compensate for ATP loss during exercise, the ADSS1 defect prevents the synthesis of new adenine nucleotides to replace what is lost. This technique provides a non-invasive window into real-time muscle energy dynamics.

**Dr. Emma Rybalka, Ph.D.,** presented integrated RNA sequencing from Korean and Chinese patient cohorts with plasma proteomics and metabolomics to map disease mechanisms. The analysis identified 290 overlapping genes that characterize a metabolic stress signature, including lipogenic rewiring and increased insulin sensitivity as the muscle attempts to uptake more glucose for purine synthesis. The molecular profile revealed endoplasmic reticulum stress, impaired calcium handling, and an inflammatory profile like idiopathic inflammatory myopathies. A neuromuscular junction stress signature suggestive of denervation–reinnervation dynamics and impaired synaptic stability was also identified, providing a mechanistic basis for the aberrant neuromuscular junction physiology previously identified in animal models. Plasma profiling identified candidate biomarkers that could indicate muscle fat accumulation and structural instability.

### *2.3 Session 2: Patient Care Strategies*

**Dr. John Bach, M.D.**, presented on pulmonary management of ADSS1 myopathy advocating for non-invasive ventilatory support such as mouthpiece ventilation and the Cough Assist machine, which uses positive and negative pressure to clear secretions. He emphasized that the Peak Cough Flow is the most critical parameter for these patients, rather than simple vital capacity. His approach has allowed other neuromuscular disease patients with zero vital capacity to remain stable and active for decades without tracheostomy.

**Dr. Eric Voorn, Ph.D.,** presented on exercise therapy for neuromuscular disease patients to circumvent the "vicious circle of inactivity" [12]. Based on experience including other metabolic myopathies [13], a polarized aerobic exercise model inspired by endurance athletes, where 70-80% of volume is performed at low intensity, with only a small portion at high intensity was proposed. This approach aims to improve aerobic capacity ($VO_2$ peak) without causing overexertion. Although resistance exercise may seem counterintuitive, it has proven to be safe and effective in McArdle’s disease patients (another metabolic disease) when adapted to short

duration, low repetition efforts with submaximal loads and long recovery periods, allowing phosphocreatine to serve as the primary energy substrate. It was stressed that patients must monitor their "readiness to train" and recovery times to avoid long-term muscle damage.

**Dr. Cara Timpani, Ph.D.,** from the Rybalka Lab, presented on AI driven drug repurposing for ADSS1 myopathy using Drugst.One (https://drugst.one/home) [14] to mine patient muscle transcriptomes for therapeutic targets. The tool identified 145 drug candidates hitting 25 ADSS1 disease-relevant genes. Key findings included the downregulation of the androgen receptor, suggesting that testosterone therapy could potentially improve muscle mass and neuromuscular junction integrity. Other hits included prednisolone for its anti-inflammatory effects and potential to boost glutamine synthetase, and levothyroxine to overcome thyroid hormone receptor dysregulation. It was emphasized that follow-up on AI generated drug hits requires human oversight and high-quality omics data to validate leads and avoid false positives.

*2.4 Clinical discussion and action points*

Focused discussion following Session 1 addressed the high variability of metabolomic data, noting that factors like diet, exercise, and sleep can skew results. Meeting participants recommended establishing multiple baseline measurements prior to clinical trials to ensure meaningful data based on patient-specific disease trajectory. A critical question regarding cancer risk was raised, as purine salvage enzymes are often upregulated in malignancies, e.g., breast cancer [15]. However, clinical participants reported no evidence of increased cancer incidence in their patient cohorts. The session also touched on the stability of $^{31}$P-MRS measurements compared to diet-sensitive plasma metabolites.

The Session 2 discussion focused heavily on the practical application of exercise, the limitations of artificial intelligence in rare disease research, and the challenges of global patient identification:

*Exercise and overexertion:* A primary concern for patients was whether exercise accelerates disease progression. Patients provided anecdotal evidence that strenuous activities — such as walking all day at a theme park — result in extreme fatigue and functional setbacks lasting up to a week. Clinicians suggested that for ADSS1 myopathy, exercise should focus on low-intensity, low-load activities while avoiding eccentric contractions, as these patients have difficulty rebuilding muscle after tearing. There was also a debate on whether the disease's "mitochondrial" characteristics might mean that some level of aerobic exercise could benefit mitophagy and mitochondrial remodelling.

*AI Platforms and Data Limitations:* The discussion addressed the utility of AI-driven drug repurposing tools like Drugst.One. Researchers noted that these platforms often "hallucinate" or provide generic leads related to cancer or broad muscular dystrophies because there is a lack of ADSS1-specific literature for AI to mine. Furthermore, current transcriptomic datasets are extremely small (some with N=2 or N=4), which limits the power of these computational tools.

*Global Patient Estimation:* A major barrier to attracting pharmaceutical interest is the lack of an accurate global patient count. This is particularly difficult for ADSS1 myopathy because most cases involve missense variants that cause a partial lack of enzyme activity rather than a total deficiency, making genetic interpretation and population estimates more complex. The potential of recently developed tools to estimate prevalence of recessive genetic conditions [https://genie.broadinstitute.org/] was discussed.

3. Preclinical perspectives

*3.1 Session 3: Scientific Horizons 1: ADSS1 deficient models*

**Dr. Linlin Wan, M.D.**, identified significant gender differences in an *Adss1* knock out (KO) mouse model, with male mice exhibiting more severe motor and cardiac impairment than females. Metabolic profiling revealed energy shortages, enhanced purine catabolism, and mitochondrial damage. The study explored three potential interventions: carnitine, which improves fatty acid oxidation; allopurinol, which inhibits purine loss; and

dehydroepiandrosterone sulfate (DHEA-S), a naturally occurring steroid hormone that peaks in puberty. Preliminary results showed that carnitine supplementation significantly improved performance on rotarod tests and showed a trend toward better cardiac output in male mice.

**Prof. Elena Stepchenkova, Ph.D.,** presented on ADSS encoded by the *ADE12* gene in yeast [16]. Deletion of this gene results in a conditionally lethal phenotype where the massive accumulation of IMP (detected as inosine) leads to an impaired cellular energy metabolism, characterized by depletion of ATP and deoxynucleoside triphosphate (dNTP) pools. This metabolic stress causes a cascading dysregulation of nitrogen exchange, significantly reducing levels of proteinogenic amino acids and pyrimidine precursors, such as 4,5-dihydroorotic acid, while triggering the accumulation of storage carbohydrates like trehalose and glycerol. These perturbations ultimately cause cell division arrest at the G1 phase, as the cell lacks the energy and building blocks required to progress through DNA replication.

ADSS also plays an essential role in maintaining genomic stability by regulating nucleotide pool balances and detoxifying mutagenic base analogs such as 6-N-hydroxylaminopurine. In *ade12* mutants, the combination of slowed replication and insufficient dNTP levels recruits the error-prone DNA polymerase ζ (Zetta), leading to a significant increase in spontaneous mutagenesis. While ADSS can bind to single-stranded DNA in vitro, research suggests its protective effect on the genome is primarily derived from its enzymatic role in purine metabolism rather than a direct structural role at replication origins. These findings in yeast parallel the pathology of human ADSS1 myopathy.

**Dr. Wendy Hanna-Rose Ph.D.**, presented on neuromuscular function disruption in ADSS deficient *C. elegans* models [6]. The team utilized a mutant line, *adss-1*(Δ), where the entire coding region is deleted. These worms exhibit severe phenotypes, including extreme developmental delays, sterility, and reduced body size. Interrogating the neuromuscular junction revealed a complex imbalance. The mutants showed suboptimal postsynaptic

function (resistance to levamisole) alongside enhanced presynaptic signalling (hypersensitivity to aldicarb).

The presentation also highlighted that muscle mitochondria in the mutants are fragmented, and affected worms possess significant mechanosensory defects, such as an impaired response to light touch and plate taps. Treatment with Febuxostat and adenine appeared to partially restore both mitochondrial morphology and mechanosensation. These findings were contrasted with adenylosuccinate lyase (ADSL) deficiency, noting that while both share features like slow speed and small size, they have distinct neuromuscular junction and sensory signatures. These worm models provide a high-throughput platform for understanding the diverse cellular impacts of purine metabolic disorders and testing potential therapeutic supplements.

**Dr. Kathryn Yammine, Ph.D.**, presented a comprehensive phenotypic characterization of three distinct ADSS1 mouse models used at the Beggs Laboratory: a constitutive *Adss1* KO, *Adss1*:p.D261N knock-in (KI), and a compound heterozygous KO/KI. The constitutive KO mice were generally healthy with no major motor defects, yet histological analysis revealed rimmed vacuoles specifically in glycolytic (Type IIB) fibers. They also showed *ex vivo* contractility defects and dysregulated purine homeostasis [17]. In contrast, the homozygous KI mice suffered from neonatal lethality at postnatal day 6, the cause of which remains under investigation with hypotheses including a potential dominant-negative protein interaction.

The compound heterozygous KO/KI mice emerged as the "best" phenotypic model for human disease. These mice exhibited significant reductions in body mass, grip strength, and treadmill endurance. They also displayed a unique "immediate rigor mortis" phenotype post-mortem, occurring within 1–2 minutes of death following exhaustive exercise and asphyxiation by $CO_2$. The KO/KI model provides a more robust and relevant platform for interrogating disease mechanisms and evaluating the efficacy of therapeutic interventions like gene therapy.

**Dr. Behzad Moghadaszadeh, Ph.D.**, outlined the development of adeno-associated virus (AAV)-based gene therapies for ADSS1 myopathy in the Beggs Laboratory, focusing on optimizing vector delivery to skeletal muscle. Initial pilot studies utilized AAV9 combined with a synthetic muscle-specific promoter (MHCK7) to deliver the human *ADSS1* transgene. While this approach showed transgene expression in muscles, the team transitioned to "MyoAAV," a next-generation vector designed for superior muscle tropism and the ability to de-target the liver.

The results with MyoAAV were promising, achieving higher transgene expression at a 10-fold lower dose (1E+13 Vg/kg) than the standard AAV9. In the *Adss1* KO/KI mouse model, this gene therapy successfully restored body weight and grip strength to wild-type levels. Furthermore, *ex vivo* physiology tests confirmed that treated mice had improved peak muscle force in the extensor digitorum longus, comparable to wild-type levels. Designing self-complementary AAV vectors, which skip the second-strand DNA synthesis step to provide faster and more robust expression, was also discussed. Because the human *ADSS1* cDNA is relatively small (1,374bp), it is an ideal candidate for this optimized vector format, potentially further enhancing the therapeutic efficacy for patients.

**Dr. Jeffrey Brault, Ph.D.**, presented a detailed analysis of adenine nucleotide metabolism within ADSS1 myopathy mouse models from the Beggs Laboratory. Purine nucleotides are regulated by a balance of synthesis, salvage, and degradation, with available cellular energy depending on the ATP/ADP ratio rather than the absolute amount of ATP. Using ultra-performance liquid chromatography, the team quantified nucleotides and their degradation products in the muscles of wild-type and *Adss1* KO mice subjected to fatiguing contractions.

The study found significant dysregulation in purine homeostasis within KO muscles. Under resting (non-contracted) conditions, *Adss1* KO muscles exhibited lower ATP and higher IMP levels compared to wild-type [17] and fatiguing contractions reduced IMP and uric acid levels in KO muscles. Gene therapy (ADSS1 overexpression) normalized nucleotide levels in both rested and contracted KO muscles. These data highlights that ADSS1 deficiency primarily

impairs the muscle's ability to maintain its nucleotide pool under metabolic stress, and that restoring enzyme activity through gene therapy is an effective strategy for correcting these underlying metabolic imbalances.

**Dr. Shushu Huang, M.D. Ph.D.**, presented on establishing human *ADSS1* KO cell lines to study disease mechanisms and facilitate drug screening in the laboratory of Monkol Lek. Utilizing CRISPR/Cas9 technology on a parental line of healthy MB135 myoblasts, the team targeted exon 2 of the main skeletal muscle isoform (NM_152328.5) to induce frameshift mutations. The strategy of targeting an early exon was chosen to prevent the production of truncated but partially functional proteins. The team successfully achieved a KO efficiency of 92-94% in pooled cells and established two clonal *ADSS1* KO lines: one with a homozygous 11bp deletion, and another with compound heterozygous mutations (1bp insertion and 8bp deletion). Characterization of these lines revealed markedly reduced *ADSS1* mRNA levels in both myoblasts and myotubes. However, confirming the loss of ADSS1 protein was technically difficult because the available antibody failed to produce a detectable signal even in wild-type lysates. Functional assays, such as the MTT assay, showed only subtle changes in myoblast viability and were not sensitive enough to reveal a robust loss-of-function phenotype in myotubes. Notably, the KO myoblasts were able to differentiate into myotubes without significant defects. The lack of a strong phenotype might be due to species differences, as previous mouse C2C12 models showed more severe effects, or potential genetic compensation by *ADSS2*. Future research will explore *ADSS1/ADSS2* double KO's and more sensitive metabolic flux assays.

### 3.2 Session 4: Scientific Horizons 2: Disease and treatment insights

**Dr. Manoj Pandey, Ph.D.**, explored a novel pathological mechanism in ADSL deficiency, specifically the role of the complement system in inducing neuroinflammation [18]. *ADSL* downregulation is reported in ADSS1 myopathy patient transcriptomes [7]. It was hypothesized that the accumulation of toxic metabolites, particularly SAICAR, triggers the

alternative complement pathway, leading to cellular injury. To test this, his team developed a human cell model using iPSC-derived neurons and microglia, where ADSL-targeted monoclonal antibodies were used to induce a disease state.

The model successfully recapitulated neuronal cell loss and revealed a central role for complement activation. ADSL-deficient cells showed significantly increased mRNA expression of C5 and C5a, as well as higher levels of the C5a receptor 1 (C5aR1). This activation led to microglial activation and the production of pro-inflammatory cytokines such as TNF-$\alpha$, IL-1$\beta$, and IL-6. This neuroinflammatory environment may contribute to both the neurological symptoms and the muscle wasting (atrophy) seen in ADSL deficiency. He suggested that repurposing existing drugs targeting the C5a–C5aR1 axis, such as Avacopan or Eculizumab, could represent a viable therapeutic strategy for ADSS1 myopathy patients.

**Dr. Matteo Bordi, Ph. D.**, framed ADSL deficiency as a secondary mitochondrial disease, showing that mutations lead to significant mitochondrial fragmentation and reduced activity [19]. Using various models of ADSL deficiency, including primary fibroblasts, IPS-derived neurons, and Drosophila, the team demonstrated that ADSL protein levels are downregulated in correlation with disease severity. A defect in the activation of ERK2, and to a lesser extent, AKT signalling was identified in patient-derived cells. Under normal conditions, these kinases normally promote de novo purine synthesis via phosphorylation of PFAS and TKT. Overexpression of constitutively active ERK but not AKT, partially rescued mitochondrial morphology, membrane potential, and mtDNA copy number in both the Drosophila model and patient-derived EBV-LCLs, indicating ERK as a critical molecular link downstream of ADSL. Notably, the ADSL protein itself proved stable, with an extended half-life uniform across pathogenic variants; the reduction in ADSL levels in patients was therefore attributed to concordant transcriptional/translational downregulation rather than increased protein degradation. Metabolomic analysis did not show the expected decrease in fumarate, suggesting metabolic reprogramming. Supplementation with adenine or guanine rescued

mitochondrial morphology and oxidative phosphorylation activity and increased mitochondrial mass (48 h) and mtDNA copy number (24 h), in mild-form patient fibroblasts and EBV-LCLs.

**Dr. Frances Evesson, Ph.D.**, presented on PYROXD1 myopathy which shares common symptoms with ADSS1 myopathy. *PYROXD1* gene variants lead to generalized muscle weakness, respiratory dysfunction, and unique connective tissue issues such as osteopenia and joint laxity [20, 21]. Joint laxity has also been reported in cases of ADSS1 myopathy [8], in addition to muscle weakness/wasting [1, 4, 22] and respiratory decline [3]. PYROXD1 functions as an oxidoreductase enzyme with critical roles in mitochondrial health and tRNA ligase regulation, though the exact disease mechanism is still being investigated. To better understand the disorder, a mouse model was generated that successfully mirrors the physical and cellular traits observed in human patients [21]. Current efforts leverage proteomic data to identify new roles for the protein in RNA processing. It would be interesting to cross-compare ADSS1 myopathy proteomes to identify overlapping signatures.

### *3.3 Preclinical discussion and action points*

The final discussion expanded on the secondary nature of mitochondrial defects in ADSS1 myopathy. The conference participants considered if ADSS1 myopathy might be a secondary ADSL-driven effect, as ADSL is often downregulated in other muscle disease models. There was a proposal to measure mitochondrial nucleotide pools specifically, as they are separate from cytosolic pools. The possibility of carrier studies was raised; although carriers are typically asymptomatic, they show intermediate metabolite levels that could provide larger cohorts for study. The conference concluded with a call for integrated panels of biomarkers – including ADSS, ADSL, and AMPD1 – to better track disease progression across these related metabolic myopathies.

4. Summary and Strategic Next Steps

Strategic next steps emerging from the meeting centred on consolidating ADSS1 myopathy as a trial-ready, mechanism-defined rare disease program. A priority is to expand and harmonise international natural history data, with standardised clinical assessments spanning strength, endurance, fatigue, bulbar function, respiratory status, cardiac surveillance and patient-reported outcomes. Whole-body imaging, diaphragm assessment, EIM, speech analysis and $^{31}$P-MRS should be further evaluated as non-invasive measures capable of tracking progression and therapeutic response. Given the variability of plasma metabolomics, repeated baseline sampling before intervention studies will be important to distinguish disease signal from effects of diet, exercise, sleep and acute physiological state.

The development of clinical care guidelines is necessary to support patient care. Respiratory and rehabilitative care require practical, patient-facing guidance. Peak cough flow, early recognition of hypoventilation, non-invasive ventilatory support and mechanical insufflation–exsufflation should be incorporated into clinical care pathways. Exercise guidance should be conservative and individualised, emphasising low-intensity, low-load activity, avoidance of excessive eccentric loading, careful recovery monitoring and exploration of whether controlled aerobic conditioning can support mitochondrial adaptation without precipitating prolonged functional setbacks.

At the mechanistic level, integrated multi-omic analyses should be expanded across larger patient cohorts and linked to genotype, phenotype, imaging and functional data. Attention should be given to purine pathway flux, ADSS1–ADSL–AMPD1 interactions, mitochondrial nucleotide pools, neuromuscular junction biology, inflammatory/complement signatures, lipid handling, calcium homeostasis and connective tissue features. Carrier studies may provide a useful complementary strategy, as intermediate biochemical changes could help increase cohort size and clarify metabolic dose effects.

Preclinical priorities include continued characterization of the *Adss1* KO/KI mouse as the most clinically informative mammalian model, alongside orthogonal yeast, worm and human cell systems for rapid mechanistic testing. Therapeutic development should proceed through

parallel tracks, including ASA replacement, AAV-mediated *ADSS1* restoration, metabolic support strategies such as carnitine or purine catabolism modulation, and carefully curated drug-repurposing candidates. AI-supported discovery may be useful, but only when grounded in high-quality ADSS1-specific datasets and expert biological validation.

Overall, the meeting defined the next phase as one of integration: linking clinical phenotyping, biomarkers, mechanistic models and therapeutic studies into a coordinated international framework capable of supporting robust trials and accelerating treatment development.

## 5. Conference participants

### *5.1 In-person presenters (in presenter order)*

- Naveen Baweja, Cure ADSSL1, Glendale, USA
- Emma Rybalka, Ph.D., Director of Science, Cure ADSSL1, Glendale, USA; and Professor, Institute for Health and Sport and Australian Institute for Musculoskeletal Science, Inherited and Acquired Myopathies Program, Victoria University, Melbourne, AUS
- Perry Shieh, M.D., Ph.D., Professor of Neurology and Pediatrics, University of California Los Angeles, Los Angeles, USA
- Merve Koç Yekedüz, M.D., Boston Children's Hospital, Harvard Medical School, Boston, USA
- Hyder Jinnah, M.D., Ph.D., Professor, Department of Neurology, Human Genetics and Pediatrics, Emory University, Atlanta, USA
- John Bach, M.D., Departments of Physical Medicine and Rehabilitation and Neurology, Rutgers University – New Jersey Medical School; and Center for Ventilator Management Alternatives, University Hospital, Newark, USA.
- Cara Timpani, Ph.D., Senior Research Fellow, Institute for Health and Sport and Australian Institute for Musculoskeletal Science, Inherited and Acquired Myopathies Program, Victoria University, Melbourne, AUS

- Wendy Hanna-Rose, Ph.D., Professor of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, USA
- Kathryn Yammine, Ph.D., Postdoctoral Research Fellow, Manton Centre for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, Boston, USA
- Behzad Moghadaszadeh, Ph.D., Senior Research Fellow, Manton Centre for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, Boston, USA
- Jeff Brault, Ph.D., Associate Professor of Anatomy, Cell Biology & Physiology, Indiana University School of Medicine, Indianapolis, USA
- Shushu Huang, M.D., Ph.D., Associate Research Scientist, Department of Genetics, Yale University School of Medicine, New Haven, USA
- Manoj Pandey, Ph.D., Assistant Professor, Division of Human Genetics, Cincinnati Children's Hospital Medical Center; and Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA.

*5.2 Remote presenters (in presenter order)*

- Jiao Kexin, M.D., Ph.D., Department of Neurology and Rare Disease Center, Huashan Hospital, Fudan University, Shanghai, China
- Yoshihiko Saito, M.D., Ph.D., Department of Neuromuscular Research, National Institute of Neuroscience, Kodaira, Tokyo, Japan
- Eric Voorn, Ph.D., Research Associate, Rehabilitation Medicine, University of Amsterdam, Amsterdam, The Netherlands
- Elena Stepchenkova, Ph.D., Research Fellow, Department of Genetics and Biotechnology, Saint Petersburg State University, Saint Petersburg, Russia
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- Indumathi Mohanchandra
- Fabrizio Pertusati

- Julie Shulman
- Inder Singh
- Keith Sutton
- Ankita Tulangekar

**List of abbreviations**

$^{31}$P-MRS: phosphorus magnetic resonance spectroscopy

AAV: Adeno-associated virus

ADP: adenosine diphosphate

ADSS: adenylosuccinate synthase

ADSS1/ADSSL1: adenylosuccinate synthase isozyme (like) 1

ADSS2: adenylosuccinate synthase isozyme 2

ADSL: adenylosuccinate lyase

AI: artificial intelligence

AMP: adenosine monophosphate

AMPD1: adenosine monophosphate deaminase 1

ASA: adenylosuccinic acid

ATP: adenosine triphosphate

C5/5a: complement component 5/5a

C5aR1: complement component 5a receptor 1

cDNA: complementary deoxyribonucleic acid

COX: cytochrome c oxidase

DHEA-S: dehydroepiandrosterone sulfate

DNA: deoxyribonucleic acid

dNTP: deoxynucleoside triphosphate

EIM: electrical impedance myography

ERK2: extracellular signal-regulated kinase 2

FDA IND: Food and Drug Administration Investigational New Drug

IL-1β: interleukin-1 beta

IL-6: interleukin-6

iPSC: induced pluripotent stem cells

KO: knock-out

KI: knock-in

MRI: magnetic resonance imaging

mRNA: messenger ribonucleic acid

Myo-AAV: muscle directed-adenoviral associated vector

PFAS: phosphoribosylformylglycinamidine synthase

PI: principal investigator

PYROXD1: pyridine nucleotide-disulphide oxidoreductase domain-containing protein 1

RNA: ribonucleic acid

SAD/MAD: single and multiple ascending dose

SAICAR: N-succinyl-5-aminoimidazole-4-carboxamide ribotide

TKT: transketolase

TNF-α: tumour necrosis factor alpha

tRNA: transfer ribonucleic acid

$VO_2$: volume of oxygen

**Declarations**

*Ethics approval and consent to participate*

Human and animal studies were approved by the relevant institutional ethics boards and informed consent was given by all human participants.

*Consent for publication*

Not applicable.

*Availability of data and materials*

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*Authors contributions*

ER drafted the manuscript. All authors proofread the final version.

*Declaration of Interest*

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*Declaration of generative AI and AI-assisted technologies in the manuscript preparation process*

During the preparation of this work, ER used NotebookLM to transcribe the audio recordings of each speaker's presentation. ER edited the outputs and drafted the manuscript before each author reviewed the final version. All authors take full responsibility for the content of the published article.

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