A novel drug Sapropterin (Kuvan) ameliorates the disease phenotype in a mouse model of multisystem smooth muscle dysfunction syndrome.

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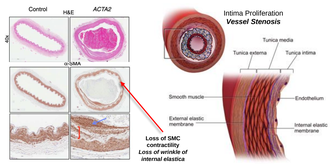
BACKGROUND & SIGNIFICANCE

Studying an ultra rare form of cerebral vascular disease can benefit the field of stroke

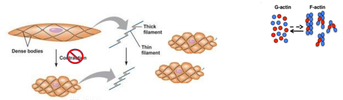
- Ultra rare disease affecting acta2 gene can be diagnosed soon after birth
- Diagnosis can be soon after birth
- Strokes occur after kids start walking
- Aortic Dissections follow in the second decade of life
- No medical or surgical treatments
- MGH main referral center for the disease

ACTA2 Vasculopathy Pathomechanism

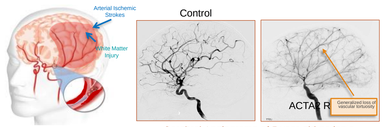


Cerebral Arteries Histology of ACTA2 R179H Patient

ACTA2 R179H alters actin polymerization

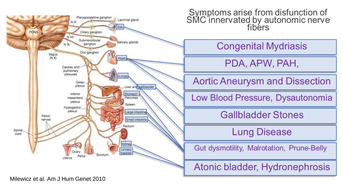


Cerebral Manifestation of MSMDS

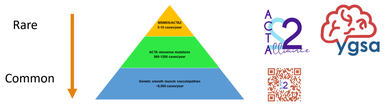


Cerebral Angiograms of 5 year-old patients

Systemic Manifestation of MSMDS



Milewicz et al. Am J Hum Genet 2010



INTRODUCTION

- MSMDS is a rare genetic disorder caused by a specific missense mutation at the Arginine 179 position within the ACTA2 gene.
- The ACTA2 gene is responsible for encoding alpha smooth muscle actin (α-SMA).
- MSMDS leads to severe health complications, including aortic dissection, strokes, and even childhood mortality.

Current Treatment Challenges

- Despite the devastating consequences of MSMDS, there are currently no effective and readily accessible treatments available to manage this debilitating disease.

Insight from Molecular Interactions:

- Recent advancements in computational biology have allowed us to delve into the molecular interactions involving ACTA2 with possible small molecule candidates
- Particular interest is the process of protein dimerization, a critical step in maintaining the structural integrity of fibrillary actin and the cytoskeleton.

A Potential Breakthrough: Sapropterin (Kuvan)

- In-silico modeling of ACTA2 interactions (in collaboration with QR genetics) has yielded a promising candidate for MSMDS treatment—Sapropterin. Kuvan, a medication previously used to treat phenylketonuria (PKU), presents an intriguing opportunity for repurposing in our exploration of MSMDS treatment.
- This modeling suggests that Sapropterin has the potential to restore normal ACTA2 dimerization, which is crucial for cellular function.

Investigating Kuvan's Impact

- In this study, our primary goal is to explore the effects of Kuvan, a pharmaceutical agent, on enhancing phenotypic function in a murine model of MSMDS. We aim to shed light on the potential therapeutic benefits of Kuvan in mitigating the effects of MSMDS using a multimodal approach. Our findings hold the promise of improved treatment options for individuals affected by this devastating condition.

METHODS

A cre-inducible knock in murine model of MSMDS was generated. The mutation was activated in whole body smooth muscle with Myh11-cre (Myh11-Cre:Acta2R179fl/+). Ad-libitum access to water containing Sapropterin (10mg/kg) was provided to pregnant moms to ensure maternal fetal drug access. Eight weeks post treatment, animals were subject to a battery of physiology, behavior and immunohistochemistry modalities, to assess systemic improvements in disease.

Generation of ACTA2 mutant mouse model

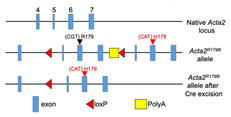
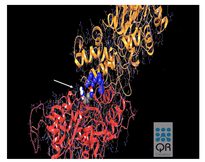
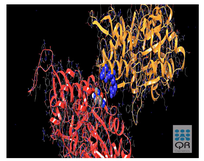


Figure 1 ACTA2R179fli mouse model allele structure. Cre excision results in the replacement of normal coding sequence with a new exon 6 coding for Histidine substitution at position 179. The result is a mouse with the ability to inducibly express the ACTA2 R179H mutation systemically throughout the mouse. This novel mutant one of a kind mouse is the Myh11-Cre:ACTA2R179Hfl/+ mouse that is the model for MSMDS disease.



Acta2 Native behavior

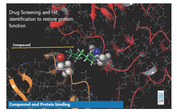
The R179 (arrow) binds to 193 and 196 residues marked in Blue (Orange chain). The binding distance between one chain to another is less than 5A, and it stays like this throughout the simulation.
This means that the bond is very strong which enables the proper function of the actin fiber.



Acta2 Mutation effect

The 179H binds to 193 and 196 residues marked in Blue (Orange chain). A gap is observed between the two chains (circle) affecting their binding and the function of the actin fiber.

Rationale for Drug Trial - (Kuvan) Sapropterin



Molecular interactions of Sapropterin with mutant histidine residues

- Sapropterin (Kuvan) has a good prediction binding score with bonds formed between monomers.
- Drug forms a stable bond filling the gap created by the mutation.
- Sapropterin (Kuvan) is therapeutically approved for treatment of Phenylketonuria.

RESULTS

Saropterin (Kuvan treated) R179H mutant fibroblasts significantly improve on G- and F-actin levels vs untreated mutant and controls.

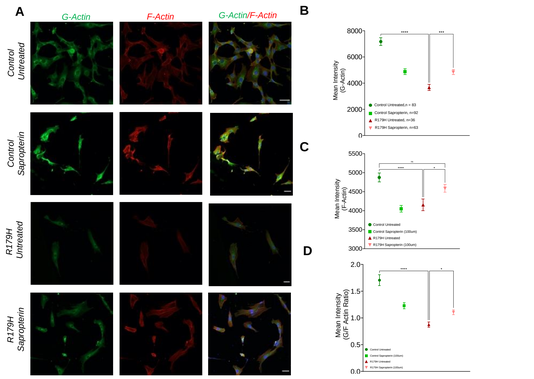


Figure 2 | G- and F actin expression in untreated vs sapropterin treated fibroblasts. (A) Control and R179H mutant fibroblasts (untreated and Sapropterin treated) were paraformaldehyde fixed and stained with phalloidin (F-actin stain) and DNASE1 (G-actin stain). Mutant R179H fibroblasts showed aberrant cellular morphology with phenotype reversal with Sapropterin treatment. B) & C) Show reduced G- and F-actin fluorescent intensity levels in mutant R179H fibroblasts compared to controls. Sapropterin treatment (100um) reverses phenotype by significantly increasing G- and F- actin intensity levels. D) G/F actin ratio show significant increase in treated mutant fibroblast vs untreated.

Saropterin treatment (R179H) show significant rescue of G/F actin ratio in treated R179H mutant fibroblasts vs untreated mutants

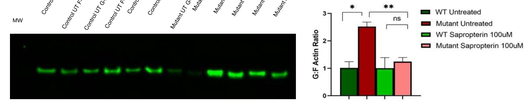
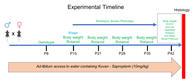


Figure 3 | G- and F actin protein expression in untreated vs sapropterin treated fibroblasts and quantification by western blot (A) WT and R179H mutant fibroblasts (untreated and Saropterin treated) were harvested, with G and F actin components isolated. Western blot shows protein expression bands for both G- and F actin fractions for each condition. **(B)** Quantification of G/F actin fragment show significantly higher ratio in mutant R179H fibroblasts vs WT. Treatment of mutant R179H fibroblasts with Saropterin restores G/F actin ratio.

Oral Saropterin treatment in MSMDS mouse disease model



Saropterin treated mice had better survival and normal body weight measurements indicating phenotypic normalcy

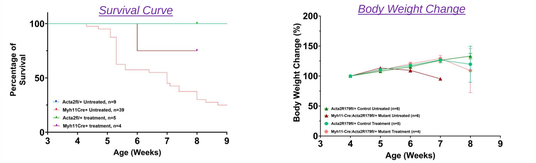


Figure 4 | Survival curve and body weight measurements in untreated and Saropterin treated MSMDS mouse model. (A) Myh11Cre+ mutant show decreased survival with mortality starting as early as 4 weeks. Sapropterin treated Myh11Cre+ mutant show better survival rates comparable to controls. (B) Myh11Cre+ mutant mice show lowered body weights compared to control Actafl/+ mice. Sapropterin treated Myh11Cre+ mutant mice show physiologically comparable body weights vs controls showing treatment efficacy.

RESULTS

Sapropterin treated mice had improved sensory and motor recovery/performance in behavior assays

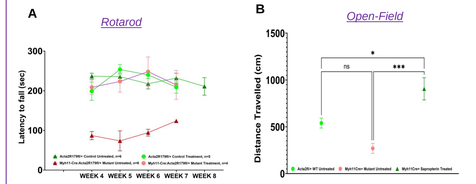


Figure 5 | Behavioral assessments in untreated and Sapropterin treated MSMDS mouse model. (A) Myh11Cre+ mutant show decreased motor performance throughout week 4 to week 8 compared to control mice. Sapropterin treated Myh11Cre+ mutant mice had restored motor activity comparable to control Acfl-/+ mice. (B) Sensory performance measured by open field test show significantly increased response in Sapropterin treated Myh11Cre+ mutant mice showing restored sensory performance.

Gross gut anatomy show restoration of gut length in treated animals comparable to controls

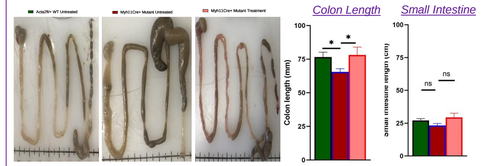


Figure 6 | Colon and small intestine measurement in control vs mutant vs Sapropterin treated mutant mice. (A) Representative image of intestinal morphology with Myh11Cre+ mutant showing fecal obstruction/impaction due to reduced intestinal motility. (B) Sapropterin treated Myh11Cre+ mutant show restored colon and small intestine length.

Cholinergic system in the gut is restored in Sapropterin-treated ACTA2-Mutant mice

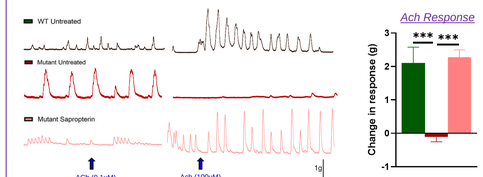


Figure 7 | Gut physiology experiments of cholinergic system. A) Representative traces showing cholinergic activity measured in control vs mutant vs Sapropterin treated mutant mice. (B) Sapropterin treated Myh11Cre+ mutant show significantly restored response to acetylcholine compared to mutant Myhcre+ mice.

Global depolarization of smooth muscle cells is restored in Saropterin-treated ACTA2-Mutant mice

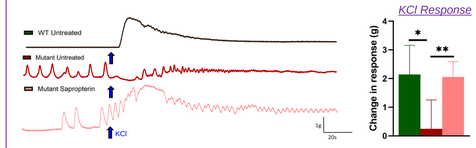


Figure 8 | Gut physiology experiments of depolarizing potential. A) Representative traces showing depolarization activity measured in control vs mutant vs Sapropterin treated mutant mice. (B) Sapropterin treated Myh11Cre+ mutant show significantly restored response to KCl compared to untreated mutant Myhcre+ mice.

RESULTS

EFS-induced muscle contraction (neuromuscular communication) is restored in Sapropterin-treated ACTA2--mutant mice

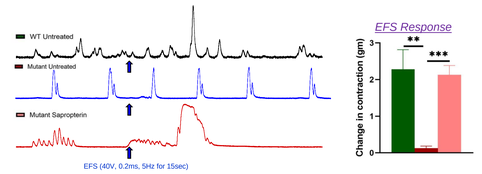


Figure 9. Smooth muscle contractility in colon. (A) Representative traces of colonic contractile force in healthy, mutant and treatment mice in response to electrical field stimulation (EFS). Arrows indicate the time of stimulation. (B) Quantification of EFS-induced contraction (maximum effects are shown as absolute change from basal values). Mutant colon showed no contraction in response to EFS, while EFS induced muscle contraction in healthy and treatment mice.

Improved myelination by luxol fast staining in Sapropterin-treated ACTA2--Mutant mice

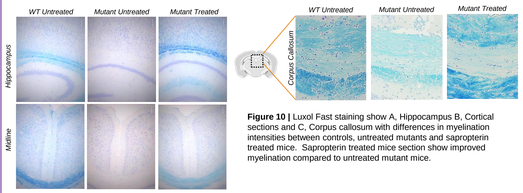


Figure 10 | Luxol Fast staining show A, Hippocampus B, Cortical sections and C, Corpus callosum with differences in myelination intensities between controls, untreated mutants and sapropterin treated mice. Sapropterin treated mice section show improved myelination compared to untreated mutant mice.

SUMMARY

- Kuvan (Sapropterin) demonstrates promise as a treatment for Multisystem Smooth Muscle Dysfunction Syndrome (MSMDS).-MSMDS arises from a pathogenic mutation in the ACTA2 gene, causing severe systemic smooth muscle cell dysfunction.
- The characterization of MSMDS remains limited, highlighting the need for effective treatments.
- Molecular studies suggest that Kuvan can restore protein integrity by binding to mutant ACTA protein monomers.
- In a murine MSMDS model, Kuvan-treated mice exhibited higher survival rates, improved systemic blood pressure, and enhanced sensorimotor recovery compared to untreated mice.
- These findings suggest that Kuvan may effectively restore phenotypic characteristics in MSMDS, emphasizing the potential for further research and clinical applications.

REFERENCES

1. Milewicz, D. M.et al.De novo ACTA2 mutation causes a novel syndrome of multisystemic smoothmuscle dysfunction.American journal of medical genetics. Part A152A, 2437-2443,doi:10.1002/ajmg.a.33657 (2010).
2. Regalado, E. S.et al.Aortic Disease Presentation and Outcome Associated with ACTA2 Mutations.Circulation. Cardiovascular genetics8, 457-464, doi:10.1161/CIRCGENETICS.114.000943 (2015).
3. Lauer, A.et al.Cerebrovascular Disease Progression in PatientsWith ACTA2 Arg179 PathogenicVariants.Neurology96, e538-e552, doi:10.1212/WNL.0000000000011210 (2021).
4. Levy, Harvey L., et al. "Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a phase III randomised placebo-controlled study." *The Lancet* 370.9586 (2007)
5. Engineer, Anish, et al. "Sapropterin reduces coronary artery malformation in offspring of pregestational diabetes mice." *Nitric Oxide* 94 (2020): 9-18.