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Impaired polyamine metabolism causes behavioral and neuromuscular deficits in a novel mouse model of Snyder-Robinson Syndrome

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Abstract

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Polyamines (putrescine, spermidine, and spermine) are essential molecules for normal cellular functions and are subject to strict metabolic regulation. Mutations in the gene encoding spermine synthase (SMS) lead to accumulation of spermidine in an X-linked recessive disorder known as Snyder-Robinson syndrome (SRS). Clinical manifestations of SRS include intellectual disability, hypotonia, and skeletal abnormalities. Although the biochemical hallmarks of SRS are well-established, the mechanisms underlying the development of the SRS phenotype. Mice harboring this mutation exhibit a complete loss of SMS protein and elevated spermidine/spermine ratio in skeletal muscles and the brain. In addition, the CNS mice demonstrate increased anxiety, impaired learning, and decreased exploratory behavior in four conditioning. Motor water maze, and open field tests, respectively. Furthermore, these mice failed to gain weight over time and exhibit abnormalities in brain structure and bone density. Transcriptomic analysis of the cerebral cortex revealed significant changes in gene expression patterns that might contribute to these observed abnormalities. Overall, our findings suggest that impaired mitochondrial bioenergetics in fibroblasts derived from SRS mice, indicating a correlation between these processes in the affected mice. Collectively, our findings establish the first in depth characterization of an SRS preclinical mouse model that exhibits cellular processes that could be targeted for future therapeutic development.

Keywords: Polyamines, Spermine synthase, spermine, neurological, disease, pathogenesis

Introduction

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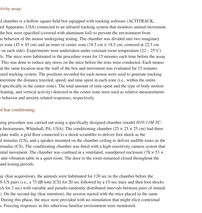
The polyamines putrescine, spermidine, and spermine are ubiquitous molecules essential for normal cellular functions. As a group, these polycationic molecules are responsible for maintaining chromatin structure, regulating gene expression, metabolic pathways, and specific ion channels, as well as cell growth and death. Polyamines are also critical contributors to the many processes underlying immune cell activation, wound healing, and tissue and cell-type growth and development[1–7]. Given their central role in cellular metabolism, the levels of intracellular polyamines are tightly regulated via de novo synthesis, interconversion, and transport into and out of the cell (Figure 1).


Ornithine
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The decreased body fat composition might be attributed to the increase in tissue spermidine, as this polyamine has been implicated in promoting lipolysis ([21]). However, it is also possible that the absence of SMS can result in impaired mitochondrial functions ([10-20]). In this case, the mice will depend more heavily on glycolysis as a means of energy generation; resulting in the utilization of greater amounts of food with less available to be converted to and stored as body fat. The decreased body weight seen in these mice supports the general idea that disturbances in polyamine homeostasis impair cell growth and tissue development ([5]) which may lead to general growth failure.

Similar to what has been reported in many SRS patients, the G56S mice display signs of cognitive impairment and exhibit significant reduction in exploratory behavior when evaluated in an open field test. These findings suggest that these mice exhibit amplified anxiety-related behaviors. This hypothesis was further studied using the fear-conditioning test. Mutant mice displayed significantly heightened fear responses when presented with an auditory conditioned stimulus (CS) followed by unconditioned stimulus (US; foot shock). The increase in fear responses in both the contextual and cued tests further confirm the observed heightened anxiety-related behavior in the G56S mouse strain. Overall, this data provides some evidence suggesting the existence of neurological abnormalities in the mutant mice. Since polyamines are important contributors to the development of the nervous system ([22]), several regions of the brain might be contributing to these behavioral defects. Both the amygdala and the hippocampus in the G56S mice, which are brain regions involved in fear-associated memory and learning showed decrease volume compared to the WT, similar to that reported in SRS patients ([22]). Thus, these results suggest that impaired polyamine metabolism and excess spermidine accumulation may lead to atrophy and neuronal loss in these regions ([23]) as well as the defects in behavioral and learning outcomes observed in these mice. The possibility that disrupted polyamine metabolism might lead to brain atrophy was further confirmed by the decrease in the fa value found in G56S mice. Although we did not measure the polyamine content in specific regions of the brain, we believe that the total brain polyamine content most likely reflects the overall content in the different regions, given that G56S mice lack SMS in all tissues and that the mutation is not tissue or brain-region specific.

Another potential mechanism that might explain the behavioral defects observed in G56S mice is spermidine-mediated disruption of receptor signaling. In an earlier study, Rubin *et al.* ([13]) reported that intra-amygdala administration of spermidine in an experimental rat model resulted in a dose-dependent increase in freezing responses. These results suggested that the excess accumulation of spermidine in the brains of G56S mice might contribute to the observed increase in anxiety-related behaviors. The mechanisms underlying spermidine-mediated increases in fear responses have not yet been clarified.

Spermidine may regulate the function of the amygdala via interactions with and modulation of the ion channel receptor for N-methyl-D-aspartate (NMDA); an earlier report detailed polyamine-mediated negative regulation of this receptor ([24]). Administration of arcaine, a putative competitive antagonist at the polyamine binding site of the NMDA receptor, resulted in a decrease in spermidine-induced fear responses in rats ([25]). Collectively, these results suggest that spermidine levels may have an impact on amygdala function and that excess accumulation of spermidine may induce a fear response as well as other behavioral abnormalities seen in the G56S mice. It is important to note that anxiety has been identified as one of the main symptoms of numerous neurological disorders ([26]). Although there are no clinical reports that document this specific behavior in SRS patients, family members have confirmed anxiety and the prevalence of fear-related behaviors in some patients (personal communication, Snyder-Robinson Foundation Conference 2022).

In addition to the neuroanatomic defects, other potential mechanisms contributing to the phenotypic abnormalities observed in the G56S mice were revealed by the transcriptomic analysis. These include impaired mitochondrial function, alterations in ribosomal protein synthesis signaling pathways, and upregulation of genes implicated in the pathogenesis of Huntington's disease. The mitochondria are important energy-generating cellular organelles; mitochondrial dysfunction has been implicated in a variety of different neurological or neurodegenerative diseases ([27]). Results from previous studies have suggested that impaired mitochondrial function might contribute to the pathogenesis of SRS ([10-20]) via one of several potential mechanisms:

i. Increased spermidine levels that accumulate in cells that lack SMS may promote the synthesis and release of reactive oxygen species (ROS) secondary to increased catabolism. Elevated ROS results in mitochondrial oxidative stress and impaired mitochondrial function ([19]).
ii. Polyamines play essential roles in modulating gene expression. Earlier reports suggest that spermine modulates mammalian mitochondrial translation initiation processes ([17,20]). Thus, the lack of SMS or spermine could inhibit synthesis of mitochondrial proteins and result in impaired mitochondrial functions.
iii. Normal mitochondrial metabolism can result in the accumulation of potentially damaging levels of by-products including ROS and Ca^{2+} ([7]). As a polycationic molecule, spermine can scavenge mitochondrial ROS ([10,11]), and reduce the levels of mitochondrial permeability transition pore (mPTP) generated in response to Ca^{2+} accumulation ([12]). Thus, the lack of SMS or an observed decrease in cellular spermine content may result in mitochondrial damage.
iv. Another potential mechanism of mitochondrial impairment in SRS may relate to the decreased expression of nuclear genes encoding mitochondrial proteins reported in this study. Although we do not yet understand how *Sms* mutations and/or decrease spermine content result in the changes in gene expression pattern observed in this study, either factor may be involved in direct or indirect interactions with critical transcription factors. It will be important to identify relevant transcription factors as this may improve our understanding of how spermine and/or SMS modulate mitochondrial function.

In conclusion, efforts to develop effective therapies for SRS will require a better understanding of the disease pathophysiology as well as suitable disease-specific animal models that recapitulate many of the critical abnormalities diagnosed in these patients. The findings presented in this study suggest that the G56S mouse is a good model that can be used to study SRS pathogenesis and may be an important tool for therapeutic development. This study also provides parameters that may be used to assess the effectiveness of therapy for SRS in a murine model. Finally, the data shown here offers insight that could be used to improve the current clinical management of SRS.

Supplementary Material

Go to:

Supplement 1

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Supplement 2

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References:

Go to:

1. Bachrach U, Wang YC, Tabib A. Polyamines: New cues in cellular signal transduction. *News in Physiological Sciences*. 2001;16(3). [PubMed] [Google Scholar]

2. Landau G, Bercovich Z, Park MH, Kahana C. The role of polyamines in supporting growth of mammalian cells is mediated through their requirement for translation initiation and elongation. *Journal of Biological Chemistry*. 2010;285(7). [PMC free article] [PubMed] [Google Scholar]

3. Hesterberg R, Cleveland J, Epling-Burnette P. Role of Polyamines in Immune Cell Functions. *Medical Sciences*. 2018;6(1). [PMC free article] [PubMed] [Google Scholar]

4. Kemaladewi DU, Benjamin JS, Hyatt E, Ivakine EA, Cohn RD. Increased polyamines as protective disease modifiers in congenital muscular dystrophy. *Hum Mol Genet*. 2018;27(11). [PubMed] [Google Scholar]

5. Akinyele O, Wallace HM. Understanding the Polyamine and mTOR Pathway Interaction in Breast Cancer Cell Growth. *Medical Sciences*. 2022. Sep 10;10(3):51. [PMC free article] [PubMed] [Google Scholar]

6. Arruabarrena-Aristorena A, Zabala-Letona A, Carracedo A. Oil for the cancer engine: The cross-talk between oncogenic signaling and polyamine metabolism. Vol. 4, *Science Advances*. 2018. [PMC free article] [PubMed] [Google Scholar]

7. Holbert CE, Cullen MT, Casero RA, Stewart TM. Polyamines in cancer: integrating organismal metabolism and antitumor immunity. *Nat Rev Cancer*. 2022. Aug 27;22(8):467–80. [PMC free article] [PubMed] [Google Scholar]

8. Lewandowski NM, Ju S, Verbinsky M, Ross B, Geddie ML, Rockenstein E, et al. Polyamine pathway contributes to the pathogenesis of Parkinson disease. *Proc Natl Acad Sci U S A*. 2020;107(39). [PMC free article] [PubMed] [Google Scholar]

9. Soda K. The mechanisms by which polyamines accelerate tumor spread. *Journal of Experimental and Clinical Cancer Research*. 2011;30(1). [PMC free article] [PubMed] [Google Scholar]

10. Milovic V, Tuechanova L. Polyamines and colon cancer. In: *Biochemical Society Transactions*. 2003. [PubMed] [Google Scholar]

11. Schwartz CE, Peron A, Kutler MJ. Syndrome Snyder-Robinson ; Adam MP, Ardinger HH, et al. *Snyder-Robinson Syndrome Synonym: Spermine Synthase Deficiency*. 2013.

12. de Alencastro G, McCloskey DE, Kliemann SE, Marandola CMC, Pegg AE, Wang X, et al. New SMS mutation leads to a striking reduction in spermine synthase protein function and a severe form of Snyder-Robinson X-linked recessive mental retardation syndrome. *J Med Genet*. 2008;45(8). [PubMed] [Google Scholar]

13. Larcher L, Norris JW, Lejeune E, Buratti J, Mignot C, Garel C, et al. The complete loss of function of the SMS gene results in a severe form of Snyder-Robinson syndrome. *Eur J Med Genet*. 2020;63(4). [PubMed] [Google Scholar]

14. Valera Ribera C, Martinez-Ferrer, Flores Fernández E, Vázquez Gómez I, Orenes Vera A, Valls Pascual E, et al. Snyder-Robinson syndrome: differential diagnosis of osteogenesis imperfecta. *Osteoporosis International*. 2022;33(5). [PubMed] [Google Scholar]

15. Cason AL, Ikeguchi Y, Skinner C, Wood TC, Holden KR, Lubs HA, et al. X-linked spermine synthase gene (SMS) defect: The first polyamine deficiency syndrome. *European Journal of Human Genetics*. 2003;11(12). [PubMed] [Google Scholar]

16. Li C, Brazill JM, Liu S, Bello C, Zhu Y, Morimoto M, et al. Spermine synthase deficiency causes lysosomal dysfunction and oxidative stress in models of Snyder-Robinson syndrome. *Nat Commun*. 2017;8(1). [PMC free article] [PubMed] [Google Scholar]

17. Tao X, Zhu Y, Díaz-Perez Z, Yu SH, Foley JR, Stewart TM, et al. *Phenylbutyrate modulates polyamine acetylase and ameliorates Snyder-Robinson syndrome in a Drosophila model and patient cells*. 2022; Available from: 10.1172/jci. [PMC free article] [PubMed] [CrossRef]

18. MacKintosh CA, Pegg AE. Effect of spermine synthase deficiency on polyamine biosynthesis and content in mice and embryonic fibroblasts, and the sensitivity of fibroblasts to 1,3-bis-(2-chloroethyl)-N-nitrosourea. *Biochemical Journal*. 2000;351(2). [PMC free article] [PubMed] [Google Scholar]

19. Wang X, Ikeguchi Y, McCloskey DE, Nelson P, Pegg AE. Spermine synthesis is required for normal viability, growth, and fertility in the mouse. *Journal of Biological Chemistry*. 2004;279(49). [PubMed] [Google Scholar]

20. Meyer RA, Henley CM, Meyer MH, Morgan PL, McDonald AG, Mills C, et al. Partial deletion of both the spermine synthase gene and the Pex gene in the X-linked hypophosphatemia, Gyro (Gy) mouse. *Genomics*. 1998;48(3). [PubMed] [Google Scholar]

21. Lyon MF, Scriver CR, Baker LR, Tenenhouse HS, Kronick J, Mandla S. The Gy mutation: Another cause of X-linked hypophosphatemia. *Proc Natl Acad Sci U S A*. 1986;83(13). [PMC free article] [PubMed] [Google Scholar]

22. Laskowski S. *THE JACKSON LABORATORY AND SNYDER-ROBINSON FOUNDATION ANNOUNCE NEW RESEARCH MODELS FOR SNYDER-ROBINSON SYNDROME*.

23. Kesler SR, Schwartz C, Stevenson RE, Reiss AL. The impact of spermine synthase (SMS) mutations on brain morphology. *Neurogenetics*. 2009;10(4). [PMC free article] [PubMed] [Google Scholar]

24. Starks RD, Kirby PA, Ciliberto MA, Hefti MM. Snyder-Robinson Syndrome: An Autopsy Case Report. *Autops Case Rep*. 2018;8(3). [PMC free article] [PubMed] [Google Scholar]

25. Tsien JZ, Huerta PT, Tonegawa S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell*. 1996;87(7). [PubMed] [Google Scholar]

26. Khan M, Gasser S. Generating primary fibroblast cultures from mouse ear and tail tissues. *Journal of Visualized Experiments*. 2016;2016(107). [PMC free article] [PubMed] [Google Scholar]

27. Kabra PM, Lee HK, Lubich WP, Marton LJ. Solid-phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phase liquid chromatography: Improved separation systems for polyamines in cerebrospinal fluid, urine and tissue. *J Chromatogr B Biomed Sci Appl*. 1986;380(C). [PubMed] [Google Scholar]

28. Zhang Z, Teng S, Wang L, Schwartz CE, Alexov E. Computational analysis of missense mutations causing Snyder-Robinson syndrome. *Hum Mutat*. 2010. Sep;31(9):1043–9. [PMC free article] [PubMed] [Google Scholar]

29. Clarkson AN, Lin H, Pearson L, Kapoor M, Harrison JC, Sammut IA, et al. Neuroprotective effects of spermine following hypoxia-ischemia-induced brain damage: A mechanistic study. *The FASEB Journal*. 2004. Jul 7;18(10):1114–6. [PubMed] [Google Scholar]

30. Ramsay AL, Alonso-Garcia V, Chaboya C, Radut B, Le B, Florez J, et al. Modeling Snyder-Robinson Syndrome in multipotent stromal cells reveals impaired mitochondrial function as a potential cause for deficient osteogenesis. *Sci Rep*. 2019;9(1). [PMC free article] [PubMed] [Google Scholar]

31. Monelli E, Villacampa P, Zabala-Letona A, Martinez-Romero A, Llena J, Beirou D, et al. Angiocrine polyamine production regulates adiposity. *Nat Metab*. 2022;4(3). [PubMed] [Google Scholar]

32. Stotkin TA, Bartolome J. Role of ornithine decarboxylase and the polyamines in nervous system development: A review. *Brain Res Bull*. 1986;17(3). [PubMed] [Google Scholar]

33. Rubin MA, Berlese DB, Stiegemeier JA, Volkwein MA, Oliveira DM, dos Santos TLB, et al. Intra-Amygdala Administration of Polyamines Modulates Fear Conditioning in Rats. *Journal of Neuroscience*. 2004;24(9). [PMC free article] [PubMed] [Google Scholar]

34. Subramaniam S, O'connor MI, Musukawa LM, McGonigle P. Polyamine effects on the NMDA receptor in human brain. *Exp Neurol*. 1994;130(2). [PubMed] [Google Scholar]

35. Davies RD, Gabbert SL, Riggs PD. Anxiety disorders in neurologic illness. *Curr Treat Options Neurol*. 2001;3(4). [PubMed] [Google Scholar]

36. Guo CY, Sun L, Chen XP, Zhang DS. Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen Res*. 2013;8(2). [PMC free article] [PubMed] [Google Scholar]

37. Lee M, Matsunaga N, Akabane S, Yasuda I, Ueda T, Takeuchi-Tomita N. Reconstitution of mammalian mitochondrial translation system capable of correct initiation and long polypeptide synthesis from leaderless mRNA. *Nucleic Acids Res*. 2021;49(1). [PMC free article] [PubMed] [Google Scholar]

38. Christian BE, Haque ME, Spremulli LL. The effect of spermine on the initiation of mitochondrial protein synthesis. *Biochem Biophys Res Commun*. 2010;393(1). [PMC free article] [PubMed] [Google Scholar]

39. Zoroz DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Vol. 94, *Physiological Reviews*. 2014. [PMC free article] [PubMed] [Google Scholar]

40. Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA. The natural polyamine spermine functions directly as a free radical scavenger. *Proc Natl Acad Sci U S A*. 1998;95(19). [PMC free article] [PubMed] [Google Scholar]

41. Sava IG, Battaglia V, Rossi CA, Salvi M, Toninello A. Free radical scavenging action of the natural polyamine spermine in rat liver mitochondria. *Free Radic Biol Med*. 2006;41(8). [PubMed] [Google Scholar]

42. Elustondo PA, Negoda A, Kane CL, Kane DA, Pavlov E v. Spermine selectively inhibits high-conductance, but not low-conductance calcium-induced permeability transition pore. *Biochim Biophys Acta Bioenerg*. 2015;1847(2). [PubMed] [Google Scholar]

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