Riboflavin Transporter Deficiency: Effectiveness of High Dose Riboflavin Therapy and Clinical Trial Readiness

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A thesis submitted in fulfilment of the requirement for the degree of

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Candidate's Statement

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

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Jack Richard Fennessy

21st September 2024

Supervisor's Statement

This is to certify that the thesis titled '*Riboflavin Transporter Deficiency Effectiveness of High Dose Riboflavin Therapy and Clinical Trial Readiness*' submitted by *Jack Fennessy* is in fulfilment of the requirements for the degree of Masters of Philosophy, it is sufficiently well presented to be examined, and does not exceed the prescribed word limit or any extended word limit for which prior approval has been granted.

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Abstract

Riboflavin Transporter Deficiency (RTD) is a progressive inherited neuropathy of childhood onset characterised clinically by bulbar palsy, limb muscle weakness, sensorineural hearing loss, visual impairment, sensory ataxia and respiratory compromise. Without treatment, the condition progresses relentlessly resulting in early death due to respiratory compromise. High dose oral riboflavin supplementation has been shown to be life saving and to improve functional outcomes. RTD is a rare condition with approximately one hundred genetically confirmed cases in the literature. There is limited data describing the quantum of benefit from riboflavin supplementation and the resulting long term outcome in individuals with RTD. The aims of this thesis are to quantify the benefit from riboflavin supplementation in RTD and to standardise the ongoing monitoring of affected individuals is standardised in preparation for clinical trials of emerging therapies to treat the condition. To achieve this aim, the objectives of this thesis are to review the literature to quantify the benefit of riboflavin supplementation in published reports, analyse the long-term data collected from a cohort of individuals treated with riboflavin at a tertiary peripheral neuropathy management clinic, and develop and trial a functional outcome measure specific for children with RTD.

In Chapter Two, a literature search was conducted which identified 94 genetically confirmed cases of RTD who received riboflavin supplementation and had follow-up assessments. Information on the clinical and functional status before and after riboflavin supplementation was collected and analysed. Seventy-six of the 94 individuals (80.9%) showed an overall improvement after riboflavin supplementation, and the remaining (19.1%) were stable, though some individuals had deterioration in individual domains, with no reported deaths. The domains that had the highest rates of response to riboflavin supplementation were gross motor function (93.3% improved), bulbar palsy (91.3%), and ataxia (90.0%). Improvements were also seen in limb muscle weakness, audiology, facial nerve palsy and respiratory function. Despite treatment, affected individuals were left with residual disability in multiple domains. Many individuals required assistance to ambulate and had severe or profound hearing loss, and some remained gastrostomy or tracheostomy dependent.

In Chapter Three, a longitudinal case series study was conducted during the period 1991 to 2022 at the Children's Hospital at Westmead. 11 individuals with RTD were assessed at 12-month intervals for monitoring of disease progression. Affected individuals had commenced high-dose oral riboflavin supplementation from the time of genetic diagnosis. Individuals for whom riboflavin supplementation was initiated early after disease onset had better outcomes compared to those in whom diagnosis was delayed. Despite ongoing riboflavin supplementation, the Charcot-Marie-Tooth disease Pediatric Scale (CMTPedS) total score and the subitems of balance and the 6-minute walk test distance as well as respiratory function worsened, while grip strength improved. There was evidence of improvement in hearing loss and optic atrophy limited to the first 12 months of treatment. While treatment with riboflavin supplementation slowed disease progression, affected individuals were left with residual disability.

In Chapter Four, an RTD-specific functional outcome measure, the Riboflavin Transporter Deficiency Pediatric Scale (RTDPedS), was developed by modifying the CMTPedS to account for phenotypic differences between children with CMT and RTD identified during the prior studies. The CMTPedS data collected over the last 10 years in individuals with RTD attending the Peripheral Neuropathy Management Clinic at the Children's Hospital at Westmead (Sydney, Australia) were reviewed to evaluate each item within the CMTPedS. A literature review of articles published until September 2021 for functional outcome measures generated an item pool for pilot testing. The results of this pilot testing, along with analysis of existing CMTPedS item scores in the RTD cohort, informed the modification of the CMTPedS. 'Shoulder internal rotation' and the '30-second sit to stand test' were added as proximal measures of strength and function. The composite balance item comprising nine tasks in the CMTPedS showed a ceiling effect and was replaced with the single 'Feet apart on a line eyes open' balance item. 'Pinprick sensation' was removed due to a floor effect. This study provides preliminary evidence that the RTDPedS is a functional outcome measure covering strength, upper and lower limb function, balance, and mobility for individuals with RTD to assess disease severity and progression in clinical trials and cohort studies.

Overall, Chapter Two is a larger, worldwide review study but is limited by mostly short term follow up. Chapter Three is a longitudinal and comprehensive review of a smaller cohort of affected individuals, but highlighted the deficiencies in the existing outcome measures. As a response to Chapter Three, Chapter Four developed a functional outcome measure specific for

use in children with RTD and made recommendations for ongoing monitoring for these individuals. This will allow for standardised and comprehensive assessment in future cohort studies and provides an outcome measure for clinical trials assessing the effectiveness of emerging therapies.

Table of Contents

Thesis Outline

This thesis examines the response to high-dose oral riboflavin supplementation in riboflavin transporter deficiency (RTD), and details the development of a functional outcome measure for ongoing assessment to ensure clinical trial readiness. This thesis contains five chapters.

Chapter One introduces RTD and describes what is known about its pathogenesis, phenotype, treatment, outcome, and explores gaps in our knowledge of this disorder.

Chapter Two presents a literature review of the response to high-dose oral riboflavin supplementation in individuals with RTD.

Chapter Three presents a case series of individuals with RTD who attended our institution for regular assessment, examines their long-term response to riboflavin supplementation, and makes recommendations for ongoing clinical and functional assessment.

Chapter Four presents the development of the riboflavin transporter deficiency pediatric scale (RTDPedS), including a pilot study of new the outcome measure in a group of individuals with RTD.

Chapter Five presents a summary of the findings of Chapters Two through Four and outlines the future directions for the field.

Dissemination of Research

Parts of the work presented in this thesis were presented at the following conferences and published in the following journals.

Conference presentations (poster):

Jack R. Fennessy, Kayla M.D. Cornett, Joshua Burns, Manoj P. Menezes. Benefit of High-Dose Oral Riboflavin Therapy in Riboflavin Transporter Deficiency. Peripheral Nerve Society Meeting. Copenhagen, Denmark 17 – 20th June 2023.

Jack R. Fennessy, Gabrielle Donlevy, Kayla M.D. Cornett, Marnee McKay, Joshua Burns, Manoj P. Menezes. Development of a Disability Scale for Riboflavin Transporter Deficiency. Peripheral Nerve Society Meeting. Copenhagen, Denmark 17 – 20th June 2023.

Jack R. Fennessy, Gabrielle Donlevy, Kayla M.D. Cornett, Marnee McKay, Joshua Burns, Manoj P. Menezes. Development of a Functional Outcome Measure for Patients with Riboflavin Transporter Deficiency. Peripheral Nerve Society Meeting. Montreal, Canada 22 – 25th June 2024.

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Fennessy JR, Donlevy GA, McKay MJ, Burns J, Cornett KMD, Menezes MP. Development of a functional outcome measure for riboflavin transporter deficiency. *J Peripher Nerv Syst.* Jun 2024;29(2):185-192. doi:10.1111/jns.12619

Fennessy JR, Cornett KMD, Donlevy GA, Mckay MJ, Burns J, Menezes MP. Long-term outcomes in children with riboflavin transporter deficiency and surveillance recommendations. Dev Med Child Neurol. Published online September 9, 2024. doi:10.1111/dmcn.16083

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Chapter 1

Introduction

1.1 Background and Early Innovations

Riboflavin Transporter Deficiency (RTD) (previously eponymously known as Brown-Vialetto-Van Laere syndrome) is a progressive inherited neuropathy of childhood onset. It is a rare condition with an estimated incidence of one in one million.[1] It was first described by Brown in 1894 as an infantile form of amyotrophic lateral sclerosis[2], followed by reports by Vialetto in 1936[4] and Van Laere in 1966[5]. The condition was re-named Riboflavin Transporter Deficiency after the discovery of the causative genetic mutations in *SLC52A3* encoding the riboflavin transporter RFVT3 in 2010[6], and *SLC52A2* encoding the riboflavin transporter RFVT2 in 2012[7]. Individuals with mutations in *SLC52A2* are designated as having RTD2, and those with mutations in *SLC52A3* are designated as to having RTD3. Oral riboflavin supplementation was successfully trialled in genetically confirmed cases of RTD from 2010 onwards, proving to be a life saving treatment.[6-12] Since 2010, there have been approximately one-hundred genetically confirmed cases of RTD treated with high-dose oral riboflavin supplementation reported in the literature.

1.2 Impairments in Children with Riboflavin Transporter Deficiency

RTD is clinically characterised by bulbar palsy (including impairments to cranial nerves IX through XII, dysarthria and dysphagia), sensorineural deafness, sensory ataxia, muscle weakness, optic atrophy and respiratory failure.[13,14] A sensorimotor peripheral neuropathy is caused by the degeneration of anterior horn cells and sensory ganglia resulting in limb muscle weakness, ataxia and diaphragmatic paralysis. The age of onset of the condition is variable with first symptoms presenting anywhere from infancy to adulthood, though the initial presentation for the majority of individuals is in the first decade of life.[14] Sensorineural

hearing loss is the most commonly observed initial impairment in RTD though a minority of affected individuals may not develop hearing loss until later in the disease course.[15] Limb muscle weakness is characterised by both proximal and distal limb weakness and muscle atrophy. Optic nerve atrophy causing visual impairment is more common in RTD2 compared to RTD3. Many affected individuals experience bulbar symptoms such as dysphagia and dysarthria, and progression of bulbar palsy results in some individuals becoming gastrostomy dependent. Respiratory symptoms are present in 62% of individuals, with some requiring artificial respiratory devices such as continuous positive airway pressure masks to treat with obstructive sleep apnoea.[13] Some individuals are ventilator-dependent, and death is predominantly due to respiratory failure.

1.3 Pathophysiology

RTD is caused by biallelic mutations in *SLC52A2* (RTD2) or *SLC52A3* (RTD3) which encode the riboflavin transporters RFVT2 and RFVT3 respectively. The disorder has an autosomal recessive pattern of inheritance.[6,8] Riboflavin (vitamin B_2) cannot be produced endogenously in the body and is an essential nutrient in the diet. Riboflavin is an integral component of the flavoenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), molecules that are important for the metabolism of amino acids, carbohydrates and lipids. Riboflavin transporters are needed to get dietary riboflavin to the tissues where it is needed. The RFVT2 transporter is expressed mainly in the brain and spinal cord, while RFVT3 is expressed in high levels in the small intestine. *SLC52A3* and *SLC52A2* mutations reduce riboflavin transporter protein expression and therefore riboflavin uptake, through the gut and into neurons respectively.[12,16] While the metabolic function of the flavoenzymes, including mitochondrial energy production, β-oxidation of fatty acids, and

branched-chain amino acid catabolism, is well understood, the specific reason why riboflavin transporter deficiency causes the specific pattern of neuronal dysfunction seen in RTD is not yet clearly elucidated, though work in iPSC-derived motor neurons and in the fruit fly have suggested that mitochondrial dysfunction, oxidative stress and cytoskeletal abnormalities play a role.[17-21]

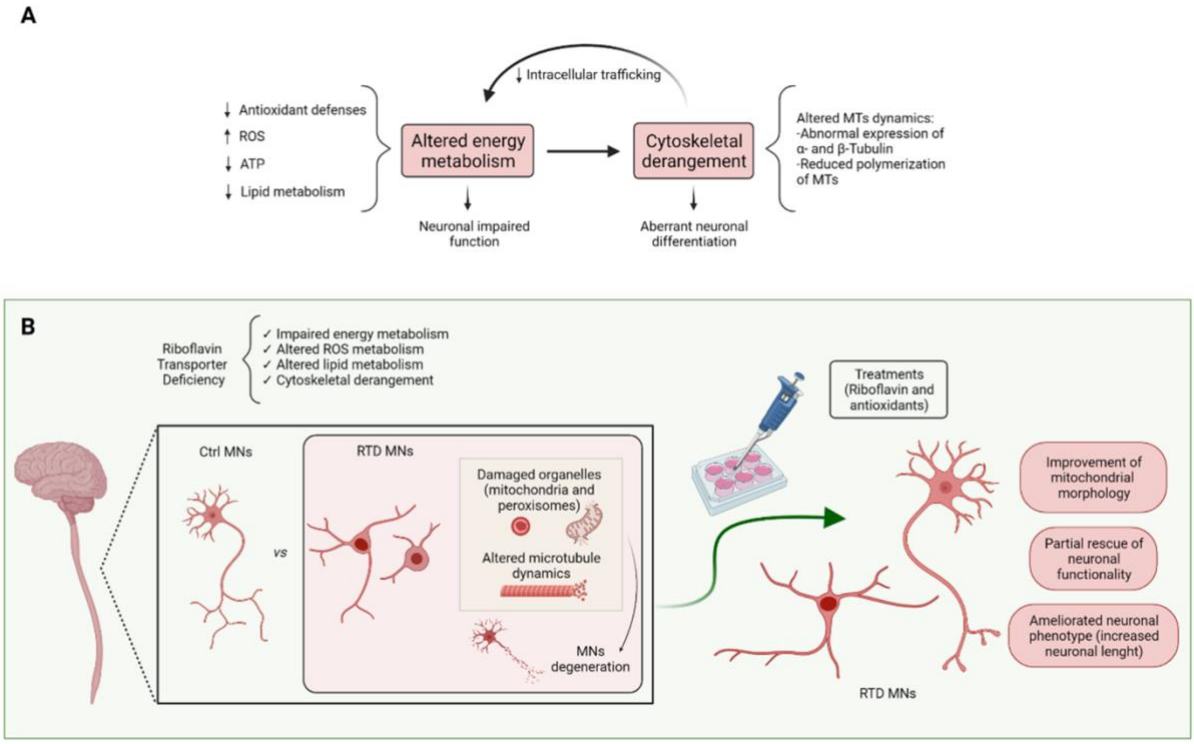

Figure 1. (Obtained from Colasuonno et al., 2022)[18]

(**A**). As a precursor of FMN and FAD, RF plays a crucial role in energy metabolism. Deficiency of RF transporters leads to mitochondrial and peroxisomal energy dysmetabolism and altered redox state which also affects cytoskeletal arrangements (e.g., altered MTs dynamics). Arrows indicate increased or decreased concentration of the specific molecules. (**B**). RTD-patient-derived MNs exhibit altered morphology associated with ROS overproduction. Diseased cells also show damaged organelles and MN degeneration. RF treatments alone or combined with other antioxidants result in a partial rescue of the altered phenotype. MTs, microtubules; MNs, motor neurons; ↑ and ↓ indicate increased or decreased levels.

1.4 Treatment

The only known effective treatment for RTD is high-dose oral riboflavin therapy, shown in short-term studies to slow the disease course and improve motor function.[14,16] In a review of published cases, 71% of 39 individuals treated with high-dose oral riboflavin, after a follow

up ranging from days to months, showed clinical improvement, while the remaining 29% had a stable clinical course.[22] Over half of untreated individuals died, while no deaths were reported in individuals supplemented with riboflavin.[22] It is thought that the best outcomes are achieved when supplementation is commenced early in the disease course as prolonged time left untreated may result in irreparable loss of motor and sensory neurons.[16] Other supplements such as Flavin Mononucleotide, Coenzyme Q, L-Carnitine, and fish oil have been taken by some individuals at their own discretion, however there is currently no evidence to demonstrate they improve outcomes. Genetic therapies have not been trialled in individuals with RTD. We should consider the information that preclinical studies in iPSC neurons derived from RTD subjects have shown that riboflavin supplementation in combination with other antioxidants such as coenzyme Q10, vitamin C, idebenone, and N-acetyl cysteine (NAC) can improve the cellular phenotype in vitro and may contribute to slowing disease progression.[19]

1.5 Outcomes

Without treatment, affected individuals have a poor outcome with significant motor disability and an early death due to respiratory failure. In a subset of previously studied affected individuals (n = 61) who did not receive riboflavin supplementation, 46% died with a mean age of death of 8.2 years (SD = 5.2) and a mean time between first presentation and death of 5.0 years (SD = 7.9).[14]

71% of individuals treated with high-dose oral riboflavin supplementation show overall clinical improvement while the remaining have a stable clinical course, though some individuals are left with residual disability despite early treatment.[22] No deaths have been reported to date in those treated with high dose oral riboflavin supplementation.

1.6 Functional Outcome Measures

Given the significant motor disability and residual disease burden following treatment, it is imperative to measure the disease severity and functional limitations of individuals with RTD. Functional outcome measures provide a standardised and consistent method of tracking disease progression over time, and provide comparisons against age- and gender-matched individuals using functional assessments. Functional outcome measures need to be disease-specific so they are sensitive to impairments of the condition they are assessing. The Charcot-Marie-Tooth Disease Pediatric Scale (CMTPedS) is a validated functional outcome measure for use in children with Charcot-Marie-Tooth disease, a peripheral neuropathy with impairments that overlap with those in children with RTD.

1.7 Summary and Gaps in Knowledge

RTD is a rare inherited neuropathy of childhood that responds to high dose oral riboflavin supplementation and is life saving when given early, however this therapy is not curative as affected individuals are left with residual disability.

There is clear benefit from treatment with high dose riboflavin, but the relative benefit for each of the specific impairments seen in RTD is difficult to quantify as published case reports do not have a uniform way of assessing change in each of these domains. Quantifying the benefit more accurately will allow for newly diagnosed individuals to be provided with a more accurate prognosis, and the need for additional disease-modifying therapies can be considered.

Additionally, most case reports of RTD in the literature provide detailed phenotypic descriptions at a single point in time but only a few consistently and uniformly track the

response to riboflavin supplementation over time. This is partially due to the lack of a functional outcome measure validated for RTD. A robust and responsive clinical outcome measure is also essential for future clinical trials of disease-modifying therapies including genetic therapies.

1.8 Aims of the Thesis

The first aim of this thesis is to review the published cases of RTD, alongside historical data collected from our own cohort of affected individuals, to determine the response to riboflavin supplementation in multiple domains of function. The second aim of this thesis is to make recommendations on the modalities that should be monitored as part of regular clinical surveillance for individuals with RTD. The third aim of this thesis is to develop and trial a functional outcome measure for children with RTD to ensure readiness for clinical trials evaluating the efficacy of emerging therapies.

1.9 References

1. Cali E, Dominik N, Manole A, Houlden H. Riboflavin Transporter Deficiency . 2021;
2. Brown CH. Infantile Amyotrophic Lateral Sclerosis of the Family Type. *The Journal of Nervous and Mental Disease*. 1894;21(2):707-716. doi:10.1097/00005053-189411000-00003
3. Burns J, Ouvrier R, Estilow T, et al. Validation of the Charcot-Marie-Tooth disease pediatric scale as an outcome measure of disability. *Ann Neurol*. May 2012;71(5):642-52. doi:10.1002/ana.23572
4. Vialetto E. Contributo alla forma ereditaria della paralisi bulbare progressive. *Riv Sper Freniat*. 1936:1-24.
5. Van Laere J. Paralysie bulbo-pontine chronique progressive familiale avec surdité. Un cas de syndrome de Klippel-Trenaunay dans la même fratrie. Problèmes diagnostiques et génétiques [Familial progressive chronic bulbo-pontine paralysis with deafness. A case of Klippel-Trenaunay syndrome in siblings of the same family. Diagnostic and genetic problems]. *Rev Neurol (Paris)*. 1966;115(2):289-295.

6. Green P, Wiseman M, Crow YJ, et al. Brown-Vialetto-Van Laere syndrome, a ponto-bulbar palsy with deafness, is caused by mutations in c20orf54. *Am J Hum Genet*. Mar 12 2010;86(3):485-9. doi:10.1016/j.ajhg.2010.02.006
7. Johnson JO, Gibbs JR, Megarbane A, et al. Exome sequencing reveals riboflavin transporter mutations as a cause of motor neuron disease. *Brain*. Sep 2012;135(Pt 9):2875-82. doi:10.1093/brain/aws161
8. Bosch AM, Abeling NG, Ijlst L, et al. Brown-Vialetto-Van Laere and Fazio Londe syndrome is associated with a riboflavin transporter defect mimicking mild MADD: a new inborn error of metabolism with potential treatment. *J Inherit Metab Dis*. Feb 2011;34(1):159-64. doi:10.1007/s10545-010-9242-z
9. Anand G, Hasan N, Jayapal S, et al. Early use of high-dose riboflavin in a case of Brown-Vialetto-Van Laere syndrome. *Developmental Medicine & Child Neurology*. 2012;54(2):187-189. doi:10.1111/j.1469-8749.2011.04142.x
10. Ciccolella M, Catteruccia M, Benedetti S, et al. Brown-Vialetto-van Laere and Fazio-Londe overlap syndromes: a clinical, biochemical and genetic study. *Neuromuscul Disord*. Dec 2012;22(12):1075-82. doi:10.1016/j.nmd.2012.05.007
11. Koy A, Pillekamp F, Hoehn T, et al. Brown-Vialetto-Van Laere Syndrome: A Riboflavin-Unresponsive Patient With a Novel Mutation in the C20orf54 Gene. *Pediatric Neurology*. 2012;46(6):407-409. doi:10.1016/j.pediatrneurol.2012.03.008
12. Haack TB, Makowski C, Yao Y, et al. Impaired riboflavin transport due to missense mutations in SLC52A2 causes Brown-Vialetto-Van Laere syndrome. *J Inherit Metab Dis*. Nov 2012;35(6):943-8. doi:10.1007/s10545-012-9513-y
13. Sathasivam S. Brown-Vialetto-Van Laere syndrome. *Orphanet J Rare Dis*. Apr 17 2008;3:9. doi:10.1186/1750-1172-3-9
14. Bosch AM, Stroek K, Abeling NG, Waterham HR, Ijlst L, Wanders RJ. The Brown-Vialetto-Van Laere and Fazio Londe syndrome revisited: natural history, genetics, treatment and future perspectives. *Orphanet J Rare Dis*. Oct 29 2012;7:83. doi:10.1186/1750-1172-7-83
15. O'Callaghan B, Bosch AM, Houlden H. An update on the genetics, clinical presentation, and pathomechanisms of human riboflavin transporter deficiency. *J Inherit Metab Dis*. Jul 2019;42(4):598-607. doi:10.1002/jimd.12053
16. Foley AR, Menezes MP, Pandraud A, et al. Treatable childhood neuronopathy caused by mutations in riboflavin transporter RFVT2. *Brain*. Jan 2014;137(Pt 1):44-56. doi:10.1093/brain/awt315
17. Colasuonno F, Niceforo A, Marioli C, et al. Mitochondrial and Peroxisomal Alterations Contribute to Energy Dysmetabolism in Riboflavin Transporter Deficiency. *Oxid Med Cell Longev*. 2020;2020:6821247. doi:10.1155/2020/6821247
18. Colasuonno F, Bertini E, Tartaglia M, Compagnucci C, Moreno S. Mitochondrial Abnormalities in Induced Pluripotent Stem Cells-Derived Motor Neurons from Patients with Riboflavin Transporter Deficiency. *Antioxidants (Basel)*. Dec 9 2020;9(12)doi:10.3390/antiox9121252
19. Colasuonno F, Marioli C, Tartaglia M, Bertini E, Compagnucci C, Moreno S. New Insights into the Neurodegeneration Mechanisms Underlying Riboflavin Transporter Deficiency (RTD): Involvement of Energy Dysmetabolism and Cytoskeletal Derangement. *Biomedicines*. Jun 6 2022;10(6)doi:10.3390/biomedicines10061329
20. Rizzo F, Ramirez A, Compagnucci C, et al. Genome-wide RNA-seq of iPSC-derived motor neurons indicates selective cytoskeletal perturbation in Brown-Vialetto disease that is partially rescued by riboflavin. *Sci Rep*. Apr 6 2017;7:46271. doi:10.1038/srep46271
21. Niceforo A, Marioli C, Colasuonno F, et al. Altered cytoskeletal arrangement in induced pluripotent stem cells (iPSCs) and motor neurons from patients with riboflavin transporter deficiency. *Dis Model Mech*. Jan 19 2021;doi:10.1242/dmm.046391

22. Jaeger B, Bosch AM. Clinical presentation and outcome of riboflavin transporter deficiency: mini review after five years of experience. *J Inherit Metab Dis*. Jul 2016;39(4):559-64. doi:10.1007/s10545-016-9924-2

Chapter 2

Literature Review

Preface

High dose oral riboflavin supplementation is life saving for individuals with riboflavin transporter deficiency (RTD) and has been shown to improve outcomes. However, the quantum of benefit in different domains of function is not comprehensively understood. This chapter presents a review of the literature concerning the benefit of oral riboflavin supplementation in RTD on multiple domains of function. Case reports and reviews were reviewed and data collated to analyse the overall benefit of riboflavin supplementation for 94 published and genetically-confirmed cases of RTD.

The research presented in this chapter has been published in the *Journal of the Peripheral Nervous System* as the following:

> Fennessy JR, Cornett KMD, Burns J, Menezes MP. Benefit of high-dose oral riboflavin therapy in riboflavin transporter deficiency. *J Peripher Nerv Syst.* Sep 2023;28(3):308-316. doi:10.1111/jns.12587

The research presented in this chapter was presented at the following conference as a poster presentation:

> Jack R. Fennessy, Kayla M.D. Cornett, Joshua Burns, Manoj P. Menezes. Benefit of High-Dose Oral Riboflavin Therapy in Riboflavin Transporter Deficiency. Peripheral Nerve Society Meeting. Copenhagen, Denmark 17 – 20th June 2023.

Supplementary Table 1 is presented in the 'Appendices' section at the end of this thesis. A table containing supplementary data can be found online in the 'Supporting Information' section at the end of the article on the publisher's website.

Authorship Statement

The co-authors of the paper '*Benefit of high-dose oral riboflavin therapy in riboflavin transporter deficiency*' confirm that *Jack Fennessy* has made the following contributions:

- Collection and screening of papers
- Collection of data
- Analysis and interpretation of data
- Drafting and revising of the manuscript and critical appraisal of content

As the primary supervisor of the candidate and this paper, I can confirm that the above authorship attribution statement is correct.

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REVIEW

WILEY

Benefit of high-dose oral riboflavin therapy in riboflavin transporter deficiency

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Abstract

Riboflavin transporter deficiency (RTD) is a progressive inherited neuropathy of childhood onset, characterised by pontobulbar palsy, sensorineural deafness, sensory ataxia, muscle weakness, optic atrophy and respiratory failure. Riboflavin supplementation is beneficial in short-term reports, but the quantum of benefit in various clinical domains is not well understood. A PubMed search was conducted, which identified 94 genetically confirmed cases of RTD who received riboflavin supplementation and had follow-up assessments. Information on the clinical and functional status before and after riboflavin supplementation was collected and analysed. Seventy-six of the 94 patients (80.9%) showed an overall improvement after riboflavin supplementation, and the remaining (19.1%) were stable, though some patients had deteriorations in individual domains with no reported deaths. The domains that had the highest rates of response to riboflavin supplementation were gross motor function (93.3% improved), bulbar palsy (91.3%) and ataxia (90.0%). Improvements were also seen in limb muscle weakness, audiology, facial nerve palsy and respiratory function. Despite treatment, many patients required assistance to ambulate and had severe or profound hearing loss and some remained gastrostomy or tracheostomy dependent. Riboflavin supplementation is a lifesaving intervention for patients with RTD and results in a profound improvement in several functional domains, with early diagnosis and treatment further improving outcomes. Despite treatment, patients are left with residual disability. There is a need to accurately measure functional outcomes in children with RTD and develop additional disease-modifying therapies.

KEYWORDS
ataxia, neuropathy, paediatrics, pontobulbar palsy, riboflavin, RTD

1 | INTRODUCTION

Riboflavin transporter deficiency (RTD; previously known as Brown–Vialetto–Van Laere syndrome) is a progressive inherited neuropathy of childhood onset, caused by biallelic mutations in *SLC52A2* (RTD2) or *SLC52A3* (RTD3), which encode the riboflavin transporters RFVT2 and RFVT3 respectively. RTD is clinically characterised by pontobulbar palsy, sensorineural deafness, sensory ataxia, muscle weakness, optic atrophy and respiratory failure.[1,2] Without treatment, patients have a poor outcome with significant motor disability and early death

due to respiratory failure. Riboflavin (vitamin B_2) cannot be produced endogenously in the body and is an essential part of the diet. Riboflavin is an integral component of the flavoenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) molecules that are important for the metabolism of amino acids, carbohydrates and lipids. Riboflavin transporters are needed to get dietary riboflavin to the tissues where it is needed. The RFVT2 transporter is expressed mainly in the brain and spinal cord, while RFVT3 is expressed in high levels in the small intestine. *SLC52A2* and *SLC52A3* mutations reduce riboflavin transporter protein expression and therefore riboflavin uptake.[3,4] While the metabolic function of the flavoenzymes, including mitochondrial energy production, β-oxidation of fatty acids and branched-chain amino acid catabolism, is well understood, the specific reason why RTD causes the specific pattern of neuronal dysfunction seen in RTD is not yet clearly elucidated, though work in induced pluripotent stem cell-derived motor neurons and the fruit fly has suggested that mitochondrial dysfunction, oxidative stress and cytoskeletal abnormalities play a role.[5-9]

The only known effective treatment for RTD is high-dose oral riboflavin therapy, shown in short-term studies to slow the disease course and improve motor function.[4,10] In a review of published cases, 71% of 39 individuals treated with high-dose oral riboflavin, after a follow-up ranging from days to months, showed clinical improvement, while the remaining 29% had a stable clinical course.[11] Over half of the untreated individuals died, while no deaths were reported in individuals supplemented with riboflavin.[11] It is thought that the best outcomes are achieved when supplementation is commenced early in the disease course as prolonged time left untreated may result in irreparable loss of motor and sensory neurons.[4] Riboflavin therapy has also been shown to reverse the electrophysiological abnormalities of myelin permeability in RTD2.[12]

There is a clear benefit from treatment with high-dose riboflavin, but the relative benefit for each of the specific impairments seen in RTD is difficult to quantify as published case reports and cohorts all report only short-term outcomes and do not have a uniform way of assessing change in each of these domains. Quantifying the benefit more accurately will allow for newly diagnosed individuals to be provided with a more accurate prognosis, and the need for additional disease-modifying therapies can be considered. This review aimed to assess the response to riboflavin supplementation in individuals with RTD reported in the literature.

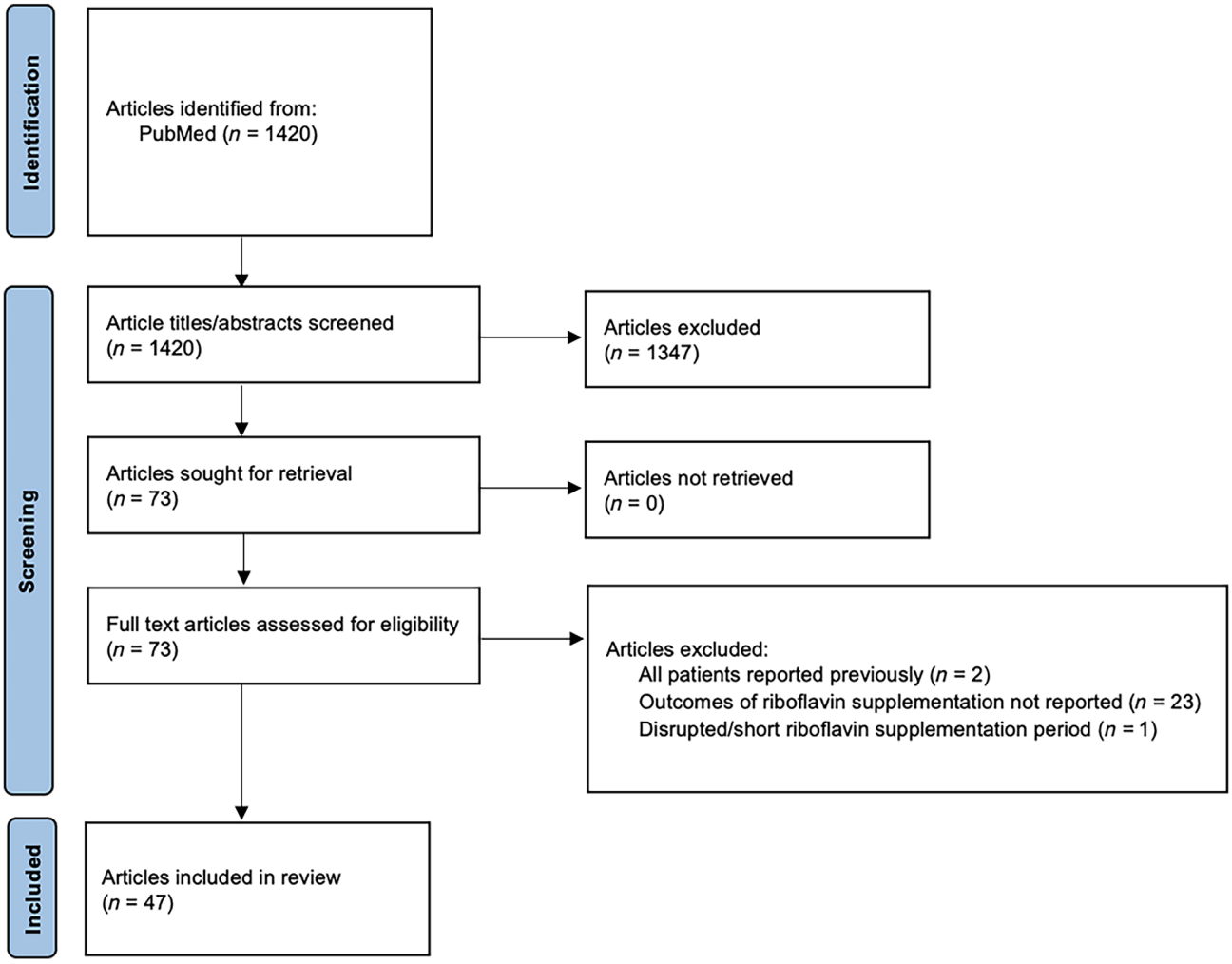

FIGURE 1 Literature search flow diagram.

2 | METHODS AND MATERIALS

A Medline, PubMed search was performed using the search terms 'Riboflavin Transporter Deficiency', 'RTD', 'Brown-Vialetto-Van Laere' and 'Fazio Londe' to find English language case reports and cohort studies of genetically confirmed RTD. Articles were only included if they reported patients' follow-up outcomes after riboflavin supplementation. We excluded patients without genetically confirmed disease, very short periods of supplementation (<2 weeks) and nonadherence to riboflavin supplementation. Individual patients who were reported in more than one article were identified to ensure their data was not duplicated in the analysis. Cases were also identified from published literature reviews.[10,11,13] All articles published before and including 1 March 2023 were included. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram is shown in Figure 1.

Data concerning mutation type, riboflavin supplementation dose, age at first treatment, time to follow up and detailed information regarding the response to riboflavin therapy in various clinical and biochemical domains were collected. The clinical characteristics of patients at follow-up (after a period of riboflavin supplementation) were compared to the same characteristics reported pretreatment and described as either 'improved', 'stable' or 'deteriorated'. This categorisation of overall response and individual domain response was based on our evaluation of the outcomes of the overall condition or specific disabilities described in individual reports or based on our evaluation of the assessments detailed in the report, as these specific terms were not universally used.

The complete dataset can be found in the online supplementary material. Statistical analyses were performed in the statistical software jamovi (Version 2.2.5). All statistical tests were independent samples T-tests or chi-square tests with $p < .05$ deemed significant.

3 | RESULTS

3.1 | Overall response

We identified 94 patients with mutations in *SLC52A2* or *SLC52A3* whose outcome after treatment with riboflavin was reported. A summary of the articles that reported follow-up data in each of the domains is shown in Table S1. Forty-seven of those patients had RTD2 due to biallelic mutations in *SLC52A2*,[2–4,12,14–33] 46 had RTD3 due to biallelic mutations in *SLC52A3*[18,20,24,27,31,34–57] and one patient had a mutation in both *SLC52A2* and *SLC52A3*.[58] Seventy-six of the 94 patients (80.9%) showed an overall improvement after riboflavin supplementation, and the remaining (19.1%) were stable after riboflavin supplementation. Some patients had deterioration in one domain but improvement in others, with the overall impression being one of stability or improvement. There were no reported deaths in patients who received riboflavin. There was a significant difference in the response to riboflavin supplementation between patients with RTD2 (72.3% improved) compared to patients with RTD3 (89.1% improved; $\chi^2 = 4.199$, $p = .040$). The frequency of individual impairments at baseline is shown in Table 1.

The median age at the commencement of treatment was 10.0 years (range: birth to 52.0 years). The median age at the commencement of treatment in patients with RTD2 was 8.5 years and in patients with RTD3 was 11.0 years. The median difference between the age at first symptom onset to the age at treatment was 2.0 years (3.0 years in RTD2 and 0.7 years in RTD3), and the median time to the latest reported follow-up was 1.0 year (range: 2.0 weeks to 11.0 years). Riboflavin supplementation doses were reported as either mg/day or mg/kg/day. The median riboflavin dose was 25 mg/kg/day (range: 0.6–80.0 mg/kg) or 775 mg/day (range: 200–1800 mg). There was no significant difference in the dose of riboflavin between patients who improved (median: 25 mg/kg/day or 750 mg/day) and patients who were stable (median: 25 mg/kg/day or 800 mg/day; mg/kg/day: $t = -.127$, $p = .899$; mg/day: $t = -.610$, $p = .548$). The minimum dose at which improvement was noted was 0.6 mg/kg/day or 200 mg/day. Acylcarnitine profiles were reported in 28 patients, of which 20 (71.4%) had an initially abnormal profile. Fourteen of 17 (82.4%) patients with RTD2 had an initially abnormal profile, compared to 6 of 11 (54.5%) with RTD3. Of the 12 patients who had an abnormal acylcarnitine profile before riboflavin treatment and had their profile recorded after treatment, all 12 had a normalised profile post-riboflavin.

3.2 | Limb muscle weakness

'Weakness' here includes assessments of upper and/or lower limb weakness and are mostly manual assessments as only one study

TABLE 1 Frequency of impairment in individual domains in riboflavin transporter deficiency (RTD).

	Overall Frequency ($n = 94$)	RTD2 ($n = 47$)	RTD3 ($n = 46$)
Limb Muscle Weakness	66 (70.2%)	35 (74.5%)	32 (70.0%)
Sensorineural Hearing Loss	80 (85.1%)	42 (89.4%)	37 (80.4%)
Ataxia*	44 (46.8%)	30 (63.8%)*	14 (30.4%)
Facial Nerve Palsy*	38 (40.4%)	3 (6.4%)	34 (73.9%)*
Bulbar Palsy*	59 (62.8%)	18 (38.3%)	40 (87.0%)*
Optic Atrophy and Visual Impairment*	36 (38.3%)	31 (66.0%)*	5 (10.9%)
Respiratory Impairment*	46 (48.9%)	18 (38.3%)	27 (58.7%)*

Note: Domains where there was a statistically significant ($p < .05$) difference in frequency are indicated with an asterisk.

specified the use of a dynamometer. Limb muscle weakness was reported in 66 of the 94 patients (70.2%). The median age of onset of upper and/or lower limb weakness was 4.5 years (range: birth to 35.0 years). The median age of onset of weakness in patients with RTD2 was 3.0 years, compared to 10.5 years in patients with RTD3. Of the 66 patients who had weakness, 33 had follow-up assessments after commencing riboflavin treatment. Twenty-seven of the 33 patients (81.8%) had improved strength after riboflavin, and 6 of the 33 had stable strength (18.2%). There were no patients in whom deterioration of strength was reported after commencing riboflavin supplementation. The median time between the onset of weakness and treatment was 2.0 years (4.5 years in patients with RTD2 and 6.0 months in patients with RTD3). There was no association between time untreated and response to riboflavin supplementation ($\chi^2 = .917$, $p = .338$).

Of the patients with RTD2, 25 of 33 individuals (75.8%) had predominantly upper limb weakness, and 7 (21.2%) had a generalised weakness. Of the patients with RTD3, 3 of 23 (13.0%) had an upper limb predominant weakness, and 18 of 23 (78.3%) had a generalised weakness.

3.3 | Gross motor function

The ability of affected patients to ambulate was reported in some publications. We extrapolated the reported motor ability to the Gross Motor Function Classification System (GMFCS)[59] to standardise these reports. While the GMFCS is a scale validated for measuring motor function in cerebral palsy, we have used this scale in patients with RTD due to the lack of alternative scales currently available for functional classification in this cohort. The classification system gives patients a score from I to V, with a score of I representing an ability to walk with minimal limitations and a score of V representing an individual who requires transport in a manual wheelchair in all settings. Wheelchair use may have been due to limb muscle weakness or severe ataxia. Thirty-six patients had sufficient data to extrapolate a GMFCS score. At their initial assessment prior to riboflavin supplementation, six patients had a GMFCS score of I (16.7%), one had a score of II (2.8%), 16 had a score of III (44.4%), 12 had a score of IV (33.3%) and one had a score of V (2.8%). GMFCS score was similar between patients with RTD2 and RTD3. Of the 30 patients who had their ambulatory ability reported before and after commencing riboflavin supplementation, 28 (93.3%) improved their ambulation and two patients were stable (Figure 2).

3.4 | Ataxia

Clinician-assessed ataxia was reported in 44 of the 94 patients (46.8%). The median age of onset of ataxia was 3.0 years (range: 11.0 months to 35.0 years). The median age of onset in patients with RTD2 was 3.0 years, compared to 14 years in patients with RTD3. Of the 44 patients who had ataxia, 20 had follow-up assessments after

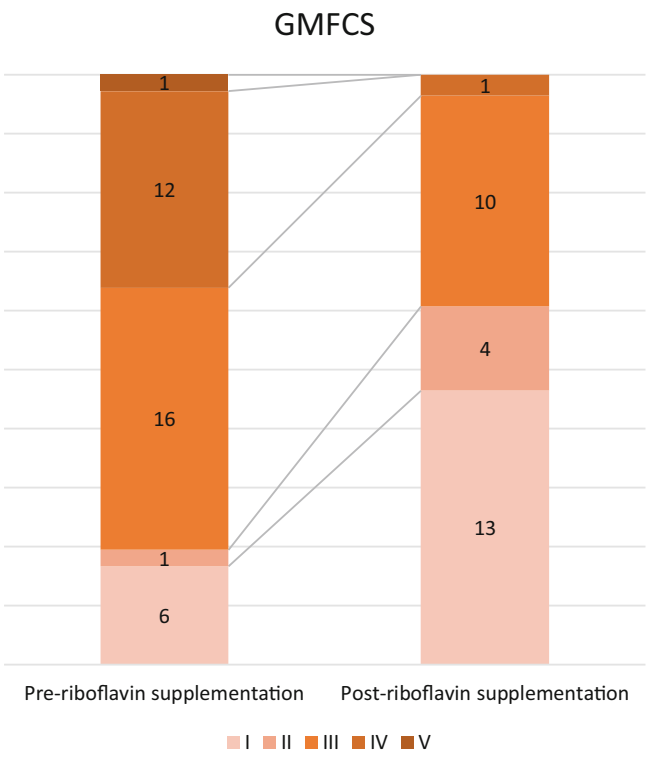

FIGURE 2 Comparison of Gross Motor Function Classification System (GMFCS) grade before and after treatment with riboflavin in riboflavin transporter deficiency (RTD). Numbers in the bar diagram indicates number of patients.

commencing riboflavin supplementation. Eighteen of the 20 (90.0%) showed improvement in ataxia, one patient's ataxia was stable and one patient's ataxia worsened.

3.5 | Audiology

Sensorineural hearing loss (SNHL) was reported in 80 of the 94 patients (85.1%). The median age of onset of SNHL was 5.0 years (range: 3.0 months to 34.0 years). The median age of onset of SNHL in patients with RTD2 was 3.0 years, compared to a median of 10.5 years in patients with RTD3. Of the 80 patients who had SNHL, 33 had a follow-up assessment after commencing treatment. Sixteen of the 33 (48.5%) had improvement in hearing, 15 (45.5%) had stable hearing and the hearing of two patients worsened. The median time from SNHL onset to treatment was 2.3 years, however, there was no difference in the post-riboflavin severity of hearing loss between those who were treated within 2 years of hearing loss onset and those who were treated beyond 2 years of onset. Outcomes of treatment in those treated within 2 years of onset (7/16 improved) were similar to those who were treated after 2 years of onset (6/12 improved). The severity of hearing loss was assessed before and after riboflavin supplementation in 16 patients (Figure 3). Of the 13 patients with severe or profound hearing loss prior to riboflavin supplementation, four (25%) improved, eight had stable hearing and

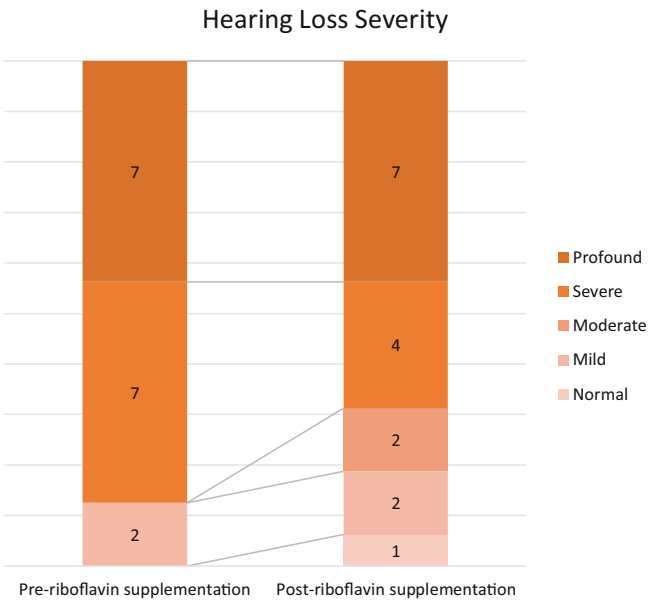

FIGURE 3 Comparison of severity of hearing loss before and after treatment with riboflavin in riboflavin transporter deficiency (RTD). Numbers in the bar diagram indicates number of patients.

one progressed from severe to profound. There were two patients with mild hearing loss; one remained mild and the other improved to normal hearing. Speech perception tests were performed before and after riboflavin supplementation in five patients with an improvement in speech perception seen in one.

3.6 | Optic atrophy and visual impairment

Optic atrophy and visual impairment were reported in 36 of the 94 patients (38.3%). The median age of onset of optic atrophy and visual impairment was 4.5 years (range: 1.0 year to 35.0 years). The median age of onset in patients with RTD2 was 3.3 years, compared to a median of 30.5 years in patients with RTD3. Of the 36 patients with optic atrophy and visual impairment, seven had follow-up assessments after riboflavin supplementation. Five of the seven (71.4%) had an improvement in their visual impairment, one patient was stable and one patient deteriorated.

3.7 | Facial nerve palsy and ptosis

Facial nerve palsy was reported in 38 of the 94 patients (40.4%). The median age of onset of facial nerve palsy was 4.5 years (range: 7.0 months to 37.0 years). The median age of onset in patients with RTD2 was 1.1 years, compared to a median age of onset of 5.0 years in patients with RTD3. Of the 38 patients who had facial nerve palsy, 14 had follow-up assessments after commencing riboflavin supplementation. Eleven of the 14 (78.6%) showed improvement in facial nerve palsy and three patients' facial nerve palsy was stable. There were no patients who received riboflavin supplementation and had a worsening of their facial nerve palsy.

Ptosis was reported in 14 of the 94 patients (14.9%), of which six had follow-up assessments after commencing riboflavin supplementation. Five of the six had an improvement in their ptosis and one patient's ptosis was stable.

3.8 | Bulbar palsy

Reports of dysarthria, dysphagia and excessive salivation were grouped under the term 'bulbar palsy' in this analysis. Bulbar palsy was reported in 59 of the 94 patients (62.8%). The median age of onset of bulbar palsy was 9.0 years (range: 10.0 weeks to 35.0 years). The median age of onset in patients with RTD2 was 1.9 years, compared to a median of 10.8 years in patients with RTD3. Of the 59 patients that had bulbar palsy, 23 had follow-up assessments after riboflavin supplementation. Twenty-one of the 23 (91.3%) showed improvements in their bulbar palsy and two patients remained stable after riboflavin supplementation. There were no patients who received riboflavin and had a worsening of their bulbar palsy.

Fourteen patients had a gastrostomy at some stage of their disease course. Three of those had the gastrostomy removed after riboflavin supplementation and one remained gastrostomy dependent after treatment.

3.9 | Respiratory dysfunction

Respiratory dysfunction was reported in 46 of the 94 individuals (48.9%). The median age of onset of respiratory dysfunction was 2.5 years (range: birth to 35.0 years). The median age of onset in patients with RTD2 was 2.3 years, compared to a median onset of 3.3 years in patients with RTD3. Of the 46 individuals that had respiratory dysfunction, 33 had follow-up assessments of their respiratory dysfunction after riboflavin supplementation. Twenty-seven of the 33 patients (81.8%) showed improvements in their respiratory function after riboflavin therapy, and 6 of the 33 (18.2%) had stable respiratory function. There were no reported cases of deterioration in respiratory function after riboflavin supplementation.

The maximal respiratory support requirement before riboflavin therapy was reported in 41 of the 46 patients with respiratory dysfunction. Invasive ventilation was required in 26 of the 41 (63.4%), and 10 of these individuals had a tracheostomy. Eight individuals had their tracheostomy removed after riboflavin therapy.

3.10 | Outcomes in children with the G306R mutation in *SLC52A2*

There were 19 patients identified who had the recurrent G306R mutation in *SLC52A2* (RTD2), a founder mutation in the Lebanese population. The median age at treatment for those with this mutation

was 10.0 years, compared to 8.0 years in those with other mutations in *SLC52A2*. Twelve of the 19 (63.2%) clinically improved after riboflavin supplementation, compared to 64 of the 75 (85.3%) who had other mutations. Thirteen (68.4%) had ataxia with a median age of onset of 3.0 years, and 15 (78.9%) reported limb weakness with a median age of onset of 6.0 years. All 19 patients reported hearing loss with a median age of onset of 3.0 years. Thirteen (68.4%) had optic atrophy and visual impairment with a median age of onset of 4.4 years. Nine (47.4%) had respiratory dysfunction with a median age of onset of 2.3 years. No patients had a facial nerve palsy, and one patient had a ptosis.

Patients with the G306R mutation were more likely to have a less-impaired GMFCS rating, with seven patients (41.7%) having a GMFCS of less than III, compared to 8.3% of individuals with other mutations. There was no clear difference in the severity of hearing loss between those with the mutation and those without. The age onset appears to be relatively consistent with those with G306R mutations (3.0 years with an interquartile range of 1.25 years), but there is a wide variability between and even within families. Family 3 reported by Foley et al.[4] had two siblings who were non-ambulant, had gastrostomies and required invasive/noninvasive ventilation, while the eldest sibling was ambulant and had no identified respiratory or bulbar dysfunction.

Overall, those with the G306R mutation had a later presentation and milder gross motor functional impairment compared to those with other mutations in *SLC52A2*. However, those with the mutation had a higher incidence of deficits in most other domains and were less likely to show clinical improvement after riboflavin supplementation compared to those with other mutations.

3.11 | Residual disability

Despite riboflavin supplementation, patients with RTD were left with residual disability in multiple domains. The residual GMFCS of the 28 patients with follow-up data was II in four individuals (14.3%), III in 10 (35.7%), and IV in one (3.6%). Five of the 44 (11.4%) patients with ataxia required a wheelchair or mobility device (GMFCS III or more severe) in follow-up assessments. Ten out of 16 individuals (62.5%) with SNHL had severe or profound hearing loss at follow-up. One individual remained gastrostomy dependent, and two remained tracheostomy dependent.

4 | DISCUSSION

This study has summarised the data from previous case reports and reviews and highlighted the benefit of high-dose riboflavin in different domains of impairment in a large cohort of children with RTD. In patients with RTD, riboflavin supplementation is an effective treatment with well-defined and significant improvement in multiple domains in most of the individuals treated compared to progressive deterioration in historical controls, supporting the need for considering this diagnosis and commencing high-dose riboflavin early in the disease course, possibly even prior to genetic confirmation, in individuals who may have only some of the classical features of the disease.[1] The proportion of individuals who improved after riboflavin in our study (80.9%) was similar to previous reviews.[11,13] The domains that had the highest rates of response to riboflavin supplementation were ataxia, motor function and bulbar palsy. Eight out of 10 patients with a tracheostomy were decannulated, and one patient with a gastrostomy could have this removed after riboflavin supplementation. There was a wide variability in the dose of riboflavin used with a median dose of 25 mg/kg/day or 775 mg/day.

We gathered data on the prevalence of specific deficits in patients with RTD2 or RTD3 who received treatment. The rates of limb muscle weakness and SNHL were similar between the two groups, though limb weakness was usually upper limb predominant in RTD2 and generalised in RTD3. Those with RTD2 were more likely to have ataxia (63.8% compared to 30.4%) and optic atrophy (66.0% compared to 10.9%). Limb muscle weakness, SNHL, ataxia and optic atrophy were of much earlier onset in RTD2 (median of around 3.0 years of age) compared with RTD3 (median of beyond 10.0 years of age). Patients with RTD3 were more likely to have bulbar palsy (87.0% compared to 38.3%) and facial nerve palsy (73.9% compared to 6.4%). The frequency of these impairments is consistent with the findings of previous reviews.[4,11,13] However, our findings are different to a previous review, which reported no difference in the response to riboflavin supplementation between patients with RTD2 and RTD3.[13] We found that those with RTD2, despite being treated earlier (8.5 years compared to 11.0 years for RTD3), showed less response to riboflavin supplementation (89.1% improved for RTD3 vs. 72.3% for RTD2). Some of the difference in this outcome may be related to the diagnostic delay and later initiation of high-dose riboflavin therapy in RTD2 (median delay of 3.0 years between first symptom onset and treatment in RTD2 vs. 0.7 years in RTD3), supporting the need for early diagnosis and treatment.

Patients with the G306R mutation may represent a milder form of the disease, consistent with functional analyses showing that riboflavin transport is reduced in this mutation but not completely abolished.[4] They were less likely to have a high GMFCS rating, but the mutation did not influence SNHL severity. We were not able to assess the severity of impairments in other domains because of the inconsistent reporting of severity data.

Even after riboflavin supplementation, patients were still left with significant residual disability. Despite treatment, 39.3% of individuals still had a GMFCS of greater than III, 37.5% had severe or profound hearing loss, two individuals remained tracheostomy dependent and one individual remained gastrostomy dependent. Complete recovery of function in any domain was very rare likely because of irreparable loss of motor neurons and sensory ganglia. This indicates the need to both diagnose individuals early before the establishment of irreversible disability and the need to identify additional therapies that further improve outcomes for patients with RTD.

Limitations of this study include the relatively short median follow-up time of 1 year and the bias that comes with summarising and analysing retrospective case reports and cohort studies. This bias may include a publication bias, as studies indicating improvement are

more likely to be published, and any children with poor long-term outcomes are not being reported in the literature.

Understandably for a severe and progressive disorder, there were no studies that had control groups or randomised patients. Many publications reported the initial presentation in great detail and present a variety of clinical deficits. However, there is a distinct paucity in the reporting of follow-up assessments of previously described deficits. Additionally, case studies were more inclined to report improvement or deterioration after treatment, as opposed to quantifying the severity of impairment before and after treatment. Therefore, the severity of the disease was only able to be assessed or extrapolated for motor disability and SNHL. The lack of a uniform process of measuring and reporting outcomes at baseline and after treatment stresses the importance of standardisation in the way disability is reported in individuals with RTD. We advocate for a standardised, sensitive and reliable outcome measurement of function to determine the severity of impairments and track patients' response to riboflavin supplementation. A robust outcome measure is also essential for the evaluation of potential future therapies for RTD in clinical trials.

This study highlights the benefit of riboflavin supplementation across multiple domains. However, significant residual disability after supplementation shows the need for early diagnosis and treatment. The inconsistent reporting of outcome data indicates the need for a robust outcome measure to accurately measure disease progression and the additional benefit from novel therapies to address residual disability.

AUTHOR CONTRIBUTIONS

Conceptualisation: Manoj P. Menezes and Jack R. Fennessy; data curation and analysis: Jack R. Fennessy, Kayla M. D. Cornett and Manoj P. Menezes; writing (original draft): Jack R. Fennessy; review and editing: Manoj P. Menezes, Kayla M. D. Cornett, Joshua Burns and Jack R. Fennessy.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

INFORMED CONSENT AND ANIMAL RIGHTS

This article does not contain any studies with human or animal subjects performed by any of the authors.

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REFERENCES

1. Sathasivam S. Brown-Vialetto-Van Laere syndrome. *Orphanet J Rare Dis*. 2008;3:9. doi:10.1186/1750-1172-3-9
2. Johnson JO, Gibbs JR, Megarbane A, et al. Exome sequencing reveals riboflavin transporter mutations as a cause of motor neuron disease. *Brain*. 2012;135(Pt 9):2875-2882. doi:10.1093/brain/aws161
3. Haack TB, Makowski C, Yao Y, et al. Impaired riboflavin transport due to missense mutations in SLC52A2 causes Brown-Vialetto-Van Laere syndrome. *J Inherit Metab Dis*. 2012;35(6):943-948. doi:10.1007/s10545-012-9513-y
4. Foley AR, Menezes MP, Pandraud A, et al. Treatable childhood neuronopathy caused by mutations in riboflavin transporter RFVT2. *Brain*. 2014;137(Pt 1):44-56. doi:10.1093/brain/awt315
5. Colasuonno F, Marioli C, Tartaglia M, Bertini E, Compagnucci C, Moreno S. New insights into the neurodegeneration mechanisms underlying riboflavin transporter deficiency (RTD): involvement of energy dysmetabolism and cytoskeletal derangement. *Biomedicine*. 2022;10(6):1329. doi:10.3390/biomedicines10061329
6. Colasuonno F, Bertini E, Tartaglia M, Compagnucci C, Moreno S. Mitochondrial abnormalities in induced pluripotent stem cells-derived motor neurons from patients with riboflavin transporter deficiency. *Antioxidants (Basel)*. 2020;9(12):1252. doi:10.3390/antiox9121252
7. Colasuonno F, Niceforo A, Marioli C, et al. Mitochondrial and peroxisomal alterations contribute to energy dysmetabolism in riboflavin transporter deficiency. *Oxid Med Cell Longev*. 2020;2020:6821247. doi:10.1155/2020/6821247
8. Niceforo A, Marioli C, Colasuonno F, et al. Altered cytoskeletal arrangement in induced pluripotent stem cells (iPSCs) and motor neurons from patients with riboflavin transporter deficiency. *Dis Model Mech*. 2021;14:dmm046391. doi:10.1242/dmm.046391
9. Rizzo F, Ramirez A, Compagnucci C, et al. Genome-wide RNA-seq of iPSC-derived motor neurons indicates selective cytoskeletal perturbation in Brown-Vialetto disease that is partially rescued by riboflavin. *Sci Rep*. 2017;7:46271. doi:10.1038/srep46271
10. Bosch AM, Stroek K, Abeling NG, Waterham HR, Ijlst L, Wanders RJ. The Brown-Vialetto-Van Laere and Fazio Londe syndrome revisited: natural history, genetics, treatment and future perspectives. *Orphanet J Rare Dis*. 2012;7:83. doi:10.1186/1750-1172-7-83
11. Jaeger B, Bosch AM. Clinical presentation and outcome of riboflavin transporter deficiency: mini review after five years of experience. *J Inherit Metab Dis*. 2016;39(4):559-564. doi:10.1007/s10545-016-9924-2
12. Menezes MP, Farrar MA, Webster R, et al. Pathophysiology of motor dysfunction in a childhood motor neuron disease caused by mutations in the riboflavin transporter. *Clin Neurophysiol*. 2016;127(1):911-918. doi:10.1016/j.clinph.2015.05.012
13. O'Callaghan B, Bosch AM, Houlden H. An update on the genetics, clinical presentation, and pathomechanisms of human riboflavin transporter deficiency. *J Inherit Metab Dis*. 2019;42(4):598-607. doi:10.1002/jimd.12053
14. Menezes MP, O'Brien K, Hill M, et al. Auditory neuropathy in Brown-Vialetto-Van Laere syndrome due to riboflavin transporter RFVT2 deficiency. *Dev Med Child Neurol*. 2016;58(8):848-854. doi:10.1111/dmcn.13084
15. Cirali C, Ozdemir O, Yis U, et al. P385 hypotonic infant with riboflavin transporter deficiency due to slc52a2 mutations. *Arch Dis Child*. 2017;102:A181-A182. doi:10.1136/archdischild-2017-313273.473

16. Petrovski S, Shashi V, Petrou S, et al. Exome sequencing results in successful riboflavin treatment of a rapidly progressive neurological condition. *Cold Spring Harb mol Case Stud*. 2015;1(1):a000257. doi:10.1101/mcs.a000257
17. Guissart C, Drouot N, Oncel I, et al. Genes for spinocerebellar ataxia with blindness and deafness (SCABD/SCAR3, MIM# 271250 and SCABD2). *Eur J Hum Genet*. 2016;24(8):1154-1159. doi:10.1038/ejhg.2015.259
18. Allison T, Roncero I, Forsyth R, Coffman K, Pichon JL. Brown-Vialetto-Van Laere syndrome as a mimic of neuroimmune disorders: 3 cases from the clinic and review of the literature. *J Child Neurol*. 2017;32(6):528-532. doi:10.1177/0883073816689517
19. Set KK, Weber ARB, Serajee FJ, Huq AM. Clinical reasoning: siblings with progressive weakness, hypotonia, nystagmus, and hearing loss. *Neurology*. 2018;90(7):e625-e631. doi:10.1212/WNL.0000000000004973
20. Woodcock IR, Menezes MP, Coleman L, et al. Genetic, radiologic, and clinical variability in Brown-Vialetto-van Laere syndrome. *Semin Pediatr Neurol*. 2018;26:2-9. doi:10.1016/j.spen.2017.03.001
21. Bamaga AK, Maamari RN, Culican SM, Shinawi M, Golumbek PT. Child neurology: Brown-Vialetto-Van Laere syndrome: dramatic visual recovery after delayed riboflavin therapy. *Neurology*. 2018;91(20):938-941. doi:10.1212/wnl.0000000000006498
22. Fan J, Fogel BL. Successful treatment of a genetic childhood ataxia due to riboflavin transporter deficiency. *Cerebellum Ataxias*. 2018;5(1):12. doi:10.1186/s40673-018-0091-0
23. Forman EB, Foley AR, King MD. Dramatic improvement of a rare syndrome with high dose riboflavin treatment. *Pediatr Neurol*. 2018;86:77-78. doi:10.1016/j.pediatrneurol.2018.05.005
24. Anderson P, Schaefer S, Henderson L, Bruce IA. Cochlear implantation in children with auditory neuropathy: lessons from Brown-Vialetto-Van Laere syndrome. *Cochlear Implants Int*. 2019;20(1):31-38. doi:10.1080/14670100.2018.1534035
25. Gorcenco S, Vaz FM, Tracewska-Siemiatkowska A, et al. Oral therapy for riboflavin transporter deficiency—what is the regimen of choice? *Parkinsonism Relat Disord*. 2019;61:245-247. doi:10.1016/j.parkreldis.2018.10.017
26. Shi K, Shi Z, Yan H, et al. A Chinese pedigree with Brown-Vialetto-Van Laere syndrome due to two novel mutations of SLC52A2 gene: clinical course and response to riboflavin. *BMC Med Genet*. 2019;20(1):76. doi:10.1186/s12881-019-0811-1
27. Carreau C, Benoit C, Ahle G, et al. Late-onset riboflavin transporter deficiency: a treatable mimic of various motor neuropathy aetiologies. *J Neurol Neurosurg Psychiatry*. 2020;92:27-35. doi:10.1136/jnnp-2020-323304
28. Kranthi P, Garuda BR, Gopi S, Kumar TS. Brown-Vialetto-Van Laere syndrome: a rare case report of MND mimic. *Neurol India*. 2020;68(5):1217-1219. doi:10.4103/0028-3886.299175
29. Pillai NR, Amin H, Gijavanekar C, et al. Hematologic presentation and the role of untargeted metabolomics analysis in monitoring treatment for riboflavin transporter deficiency. *Am J Med Genet A*. 2020;182(11):2781-2787. doi:10.1002/ajmg.a.61851
30. Liu Z, Peng Q, Li J, Rao C, Lu X. BVVLS2 overlooked for 3 years in a pediatric patient caused by novel compound heterozygous mutations in SLC52A2 gene. *Clin Chim Acta*. 2021;523:402-406. doi:10.1016/j.cca.2021.10.031
31. Omar R, Rajput K, Sirimanna T, Rajput S, Pagarkar W. The audiovestibular profile of Brown-Vialetto-Van Laere syndrome. *J Laryngol Otol*. 2021;135(11):1000-1009. doi:10.1017/S0022215121002395
32. Yilmaz BS, Ceylaner S, Mungan NO. Brown Vialetto Van Laere syndrome: presenting with left ventricular non-compaction and mimicking mitochondrial disorders. *Turk J Pediatr*. 2021;63(2):314-318. doi:10.24953/turkjped.2021.02.016
33. Naami N, Borkhardt A, Yoshimi A, Grinstein L, Escherich G. Thirteen-month-old girl with hyporegenerative macrocytic anemia due to Brown-Vialetto-Van Laere syndrome 2. *Am J Hematol*. 2022;97:1495-1496. doi:10.1002/ajh.26573
34. Bosch AM, Abeling NG, Ijlst L, et al. Brown-Vialetto-Van Laere and Fazio Londe syndrome is associated with a riboflavin transporter defect mimicking mild MADD: a new inborn error of metabolism with potential treatment. *J Inherit Metab Dis*. 2011;34(1):159-164. doi:10.1007/s10545-010-9242-z
35. Ciccolella M, Catteruccia M, Benedetti S, et al. Brown-Vialetto-van Laere and Fazio-Londe overlap syndromes: a clinical, biochemical and genetic study. *Neuromuscul Disord*. 2012;22(12):1075-1082. doi:10.1016/j.nmd.2012.05.007
36. Anand G, Hasan N, Jayapal S, et al. Early use of high-dose riboflavin in a case of Brown-Vialetto-Van Laere syndrome. *Dev Med Child Neurol*. 2012;54(2):187-189. doi:10.1111/j.1469-8749.2011.04142.x
37. Spagnoli C, Pitt MC, Rahman S, de Sousa C. Brown-Vialetto-van Laere syndrome: a riboflavin responsive neuronopathy of infancy with singular features. *Eur J Paediatr Neurol*. 2014;18(2):231-234. doi:10.1016/j.ejpn.2013.09.006
38. Cosgrove J, Datta S, Busby M. Adult onset Brown-Vialetto-Van Laere syndrome with opsoclonus and a novel heterozygous mutation: a case report. *Clin Neurol Neurosurg*. 2015;128:1-3. doi:10.1016/j.clineuro.2014.10.016
39. Davis A, Josifova D, Lloyd-Owen S, Radunovic A, Swash M. Brown-Vialetto-Van Laere syndrome: a 28-year follow-up. *J Neurol Neurosurg Psychiatry*. 2016;87(6):681-682. doi:10.1136/jnnp-2014-310088
40. Horoz OO, Mungan NO, Yildizdas D, et al. Brown-Vialetto-Van Laere syndrome: two siblings with a new mutation and dramatic therapeutic effect of high-dose riboflavin. *J Pediatr Endocrinol Metab*. 2016;29(2):227-231. doi:10.1515/jpem-2015-0198
41. Bashford JA, Chowdhury FA, Shaw CE. Remarkable motor recovery after riboflavin therapy in adult-onset Brown-Vialetto-Van Laere syndrome. *Pract Neurol*. 2017;17(1):53-56. doi:10.1136/practneurol-2016-001488
42. Khadilkar SV, Faldu HD, Udani V, Patil SB, Malvadkar S. Reversible posterior column dysfunction in Brown-Vialetto-Von Laere syndrome. *Muscle Nerve*. 2017;56(4):E28-E31. doi:10.1002/mus.25694
43. Thulasi V, Veerapandiyan A, Pletcher BA, Tong CM, Ming X. A Case of Brown-Vialetto-Van Laere syndrome due to a novel mutation in SLC52A3 gene: clinical course and response to riboflavin. *Child Neurol Open*. 2017;4:2329048X17725610. doi:10.1177/2329048X17725610
44. Camargos S, Guerreiro R, Bras J, Mageste LS. Late-onset and acute presentation of Brown-Vialetto-Van Laere syndrome in a Brazilian family. *Neurol Genet*. 2018;4(1):e215. doi:10.1212/NXG.0000000000000215
45. Chaya S, Zampoli M, Gray D, et al. The first Case of riboflavin transporter deficiency in sub-Saharan Africa. *Semin Pediatr Neurol*. 2018;26:10-14. doi:10.1016/j.spen.2017.03.002
46. Gowda VK, Udhayabanu T, Varalakshmi P, Srinivasan VM, Ashokkumar B. Fazio-Londe syndrome in siblings from India with different phenotypes. *Brain Dev*. 2018;40(7):582-586. doi:10.1016/j.braindev.2018.02.010
47. Nimmo GAM, Ejaz R, Cordeiro D, Kannu P, Mercimek-Andrews S. Riboflavin transporter deficiency mimicking mitochondrial myopathy caused by complex II deficiency. *Am J Med Genet A*. 2018;176(2):399-403. doi:10.1002/ajmg.a.38530
48. Abbas Q, Jafri SK, Ishaque S, Rahman AJ. Brown-Vialetto-Van Laere syndrome: a novel diagnosis to a common presentation. *BMJ Case Rep*. 2018;2018:bcr-2018-224958. doi:10.1136/bcr-2018-224958
49. Garg M, Kulkarni SD, Hegde AU, Shah KN. Riboflavin treatment in genetically proven Brown-Vialetto-Van Laere syndrome. *J Pediatr Neurosci*. 2018;13(4):471-473. doi:10.4103/JPN.JPN_131_17
50. Mutlu B, Torun Topcu M, Ciprut A. A Case with Brown-Vialetto-Van Laere syndrome: a sudden onset auditory neuropathy Spectrum

disorder. *Turk Arch Otorhinolaryngol*. 2019;57(4):201-205. doi:10.5152/tao.2019.4639
51. Carey G, Kuchcinski G, Gauvrit F, et al. Three cases of adult-onset Brown-Vialetto-Van Laere syndrome: novel variants in SLC52A3 gene and MRI abnormalities. *Neuromuscul Disord*. 2021;31(8):752-755. doi:10.1016/j.nmd.2021.06.009
52. Frederick AL, Yang JH, Schneider S, et al. To Be or No B2: a rare cause of stridor and weakness in a toddler. *Child Neurol Open*. 2021;8:2329048X211030723. doi:10.1177/2329048X211030723
53. Gayathri S, Gowda VK, Udhayabanu T, et al. Brown-Vialetto-Van Laere and Fazio-Londe syndromes: SLC52A3 mutations with puzzling phenotypes and inheritance. *Eur J Neurol*. 2021;28(3):945-954. doi:10.1111/ene.14682
54. Rabbani B, Bakhshandeh MK, Navaeifar MR, et al. Brown-Vialetto-Van Laere syndrome and Fazio-Londe syndrome: a novel mutation and in silico analyses. *J Clin Neurosci*. 2020;72:342-349. doi:10.1016/j.jocn.2020.01.040
55. do Amaral MSA, Massuda ET, Mitikami Fenolio GH, Barbosa Reis ACM, Angelo Hyppolito M. Cochlear implant in Brown-Vialetto-Van Laere syndrome patient. *J Int Adv Otol*. 2022;18(2):192-195. doi:10.5152/iao.2022.21159
56. Gedik Soyuyuce O, Ayanoglu Aksoy E, Yapici Z. A case report of sudden-onset auditory neuropathy spectrum disorder associated with Brown-Vialetto-Van Laere syndrome (riboflavin transporter deficiency). *Int J Audiol*. 2022;61(3):258-264. doi:10.1080/14992027.2021.1921291
57. Khani M, Shamshiri H, Taheri H, et al. BVVL/ FL: features caused by SLC52A3 mutations; WDFY4 and TNFSF13B may be novel causative genes. *Neurobiol Aging*. 2021;99(102):e1-e10. doi:10.1016/j.neurobiolaging.2020.09.021
58. Udhayabanu T, Subramanian VS, Teafatiller T, et al. SLC52A2 [p.P141T] and SLC52A3 [p.N21S] causing Brown-Vialetto-Van Laere syndrome in an Indian patient: first genetically proven case with mutations in two riboflavin transporters. *Clin Chim Acta*. 2016;462:210-214. doi:10.1016/j.cca.2016.09.022
59. Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised gross motor function classification system. *Dev Med Child Neurol*. 2008;50(10):744-750. doi:10.1111/j.1469-8749.2008.03089.x

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Chapter 3

Long Term Outcomes in Children with Riboflavin Transporter Deficiency and Surveillance Recommendations

Preface

The previous chapter has shown that high dose oral riboflavin therapy improves outcomes for individuals with riboflavin transporter deficiency (RTD) in multiple domains, however individuals are left with residual disability despite treatment. The conclusions of the previous chapter were limited due to the fact that many of the case reports and reviews had relatively short follow up periods, contained predominantly qualitative data without standardised assessments, and were subject to publication bias. It is necessary to gather comprehensive long term data using standardised assessments to gain a comprehensive understanding of the benefit of riboflavin supplementation in RTD.

This chapter presents a case series of eleven individuals with RTD who presented for assessment at the Peripheral Neuropathy Management Clinic at the Children's Hospital at Westmead over the period 1991 to 2022. The aim of this chapter is to present the data gathered from these assessments to understand long term disease progression and benefit of riboflavin supplementation using standardised assessments.

It is helpful to note that this chapter was developed and published after the development and publication of chapter four of this thesis. As such, the article presented in this chapter makes reference to previously published materials that form the content of chapter four, namely the development of a functional outcome measure for RTD.

The research presented in this chapter has been published in the journal of *Developmental Medicine and Child Neurology* as the following:

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Authorship Statement

The co-authors of the paper '*Long term outcomes in children with riboflavin transporter deficiency and surveillance recommendations*' confirm that *Jack Fennessy* has made the following contributions:

- Collection of data
- Analysis and interpretation of data
- Drafting and revising of the manuscript and critical appraisal of content

As the primary supervisor of the candidate and this paper, I can confirm that the above authorship attribution statement is correct.

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ORIGINAL ARTICLE

Long-term outcomes in children with riboflavin transporter deficiency and surveillance recommendations

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Abstract

The aim of this longitudinal case series was to describe long-term functional outcome in a group of individuals with riboflavin transporter deficiency (RTD) treated with high-dose oral riboflavin. Data were collected between 2012 to 2022. Eleven individuals with RTD were assessed at 12-month intervals for monitoring of disease progression. Patients had commenced high-dose oral riboflavin from the time of genetic diagnosis. Individuals for whom riboflavin supplementation was initiated early after disease onset had better outcomes compared to those in whom diagnosis was delayed. Despite ongoing riboflavin supplementation, the Charcot–Marie–Tooth disease Pediatric Scale (CMTPedS) total score and the subitems of balance and the 6-Minute Walk Test distance as well as respiratory function worsened, while grip strength improved. There was evidence of improvement in hearing loss and optic atrophy limited to the first 12 months of treatment. While treatment with riboflavin slowed disease progression, patients were left with residual disability. To track disease progression and response to riboflavin supplementation over time, we recommend using the RTD Pediatric Scale and provide a list of clinical measures for regular surveillance of children with RTD.

Riboflavin transporter deficiency (RTD), previously eponymously named Brown–Vialetto–Van Laere syndrome or Fazio–Londe syndrome, is a progressive inherited neuropathy of childhood onset, characterized clinically by pontobulbar palsy (involvement of cranial nerves VII–XII causing facial weakness, dysarthria, and dysphagia), sensory ataxia, hearing loss due to an auditory neuropathy, limb weakness, optic atrophy, and respiratory failure.[1] RTD is a progressive neuropathy/neuronopathy affecting the lower cranial nerves, anterior horn cells/motor nerves, and sensory ganglia/sensory nerves, though upper motor neuron signs like brisk reflexes, upgoing plantars, and clonus are rarely reported.[2] RTD is caused by biallelic mutations in the *SLC52A2* or *SLC52A3* genes which encode the riboflavin transporters RFVT2 and RFVT3 respectively.[3] Without treatment, individuals with RTD develop severe disability and are at risk of death in childhood or adolescence due to respiratory failure. A review of previously published cases showed that of those who did not receive riboflavin supplementation, 46% died with a mean age of death of 11 years 7 months (SD = 11 years 1 month) and a mean time between first presentation and death of 5 years (SD = 7 years 11 months).[1] The only known effective treatment is high-dose oral riboflavin which is life-saving and improves outcomes in multiple functional domains.[4,5]

RTD is a rare condition with an estimated incidence of 1 in 1 million[6] and most reports of outcomes in RTD are short-term descriptions of small groups with a median follow-up period of 12 months and usually without standardized assessments. Of the individuals treated with high-dose oral riboflavin, 71% to 81% show overall clinical improvement while

Abbreviations: CMT, Charcot–Marie–Tooth disease; CMTPedS, Charcot–Marie–Tooth disease Pediatric Scale; RTD, riboflavin transporter deficiency.

the remaining have a stable clinical course, though some individuals are left with residual disability despite early treatment.[5,7] The most common individual domains of function assessed over time with standardized scales or other quantitative methods are hearing,[3,8–15] respiratory function,[8,10,16–18] ataxia,[11,19,20] visual acuity,[15,20,21] and muscle weakness.[10] To provide accurate prognostic information to newly diagnosed families, and to determine if further disease modifying therapies are required, it is important to accurately quantify the long-term benefit that oral riboflavin supplementation has on disease progression in multiple affected domains. The aim of this study was to provide long-term outcome data in multiple functional domains in a group of individuals with RTD who were supplemented with high-dose oral riboflavin and provide recommendations on the modalities that should be monitored as part of regular clinical surveillance and be used as outcome measures in clinical trials.

What this paper adds

- Individuals with riboflavin transporter deficiency who are treated early after symptom onset have better long-term outcomes.
- Despite long-term treatment, patients are left with residual disability.
- Regular surveillance using the Riboflavin Transporter Deficiency Pediatric Scale and clinical measures is recommended.

METHOD

Patient characteristics

In this case series study, 11 individuals with RTD from six unrelated families attended the Peripheral Neuropathy Management Clinic at the Children's Hospital at Westmead, Sydney, Australia during the period 2012 to 2022 and were assessed in multiple domains of function. Patients were followed up at 12-month intervals until they were transitioned to adult services. The study participants are represented by a number code with the first number representing a separate family (e.g. individuals 1.1 and 1.2 are siblings). All 11 individuals had biallelic mutations in the *SLC52A2* (RTD2).

From the time of diagnosis, affected individuals were commenced on high-dose oral riboflavin supplementation at a starting dose of 500 mg per day, grading up to a maximum of 2 g/day (for those above 40 kg) or 50 mg/kg/day per day as tolerated. Other supplements such as flavin mononucleotide, coenzyme Q, L-Carnitine, and fish oil were taken by some patients at their own discretion.

Charcot–Marie–Tooth disease Pediatric Scale

The Charcot–Marie–Tooth disease Pediatric Scale[22] is a well-validated outcome measure for Charcot–Marie–Tooth disease (CMT) and related neuropathies that measures fine and gross motor function, distal strength, sensation, gait, and balance. It provides a disability score based on age- and sex-adjusted normative reference values, allowing for longitudinal assessment of disability and comparison between individuals with CMT. For each of the 11 individual items, participants are given a z-score based on the normative reference values.[23,24] Total disability scores range between 0 (unaffected) to 44 (severely affected) based on category scores across the 11 included items.[25,26] Our group of individuals with RTD were assessed using the CMTPedS at 12-month intervals.

Audiometry

The study group were tested using pure tone audiometry and speech audiometry (speech perception tests) at the time of their genetic diagnosis before commencement of riboflavin supplementation, and at 12-month intervals during follow-up. Both tests were conducted in a sound-attenuated room with THD39 headphones (Telephonics, Farmingdale, NY, USA) or EAR Tone 3A insert phones (Etymotic Research, Elk Grove Village, IL, USA). Outcomes were listed on a spectrum between 'normal' and 'severe' based on decibel thresholds. For pure tone audiometry, thresholds were obtained using the modified Hughson-Westlake technique.[27] Speech audiometry was conducted using the National Acoustic Laboratories Arthur Boothroyd word lists and recorded by an English speaker.[28] Outcomes were listed as 'normal' or 'abnormal'. The hearing outcomes at 24 months of riboflavin supplementation of some children in this study were published in 2016.[9]

Visual acuity and optic atrophy

Visual acuity and optic atrophy were assessed at 12-month intervals during follow-up at the Peripheral Neuropathy Management Clinic. Optic atrophy was assessed by fundoscopy and recorded as 'present' if disc pallor was present.

Respiratory and bulbar dysfunction

Respiratory dysfunction was measured by spirometry at 12-month intervals. Results were recorded as a percentage of the individual's forced vital capacity against age- and sex-matched typically developing controls.

Scoliosis

Scoliosis was diagnosed by spine X-ray and recorded as 'present' or 'absent' based on the radiology report. Neither the severity of scoliosis nor the progression of established scoliosis are reported in this paper.

Other investigations

Samples was taken at clinic visits to measure haemoglobin, mean corpuscular volume, acylcarnitine profile, and riboflavin level. Normal ranges for haemoglobin and mean corpuscular volume were age- and sex-matched. Riboflavin is a precursor to flavin adenine dinucleotide and flavin mononucleotide, essential cofactors for mitochondrial fatty acid β-oxidation, and acylcarnitine metabolites can be deranged in riboflavin deficiency. RTD is associated with elevation of medium chain acylcarnitines (C6–C12) on acylcarnitine profiles collected before riboflavin initiation. The serum acylcarnitine profile was recorded as 'normal' or 'abnormal' based on our institution's reference values. The patient's acylcarnitine profile collected while on riboflavin supplementation was also used as a biochemical marker of adherence to riboflavin supplementation since it is known that all initially abnormal profiles correct to normal with adequate treatment.[1,4]

Data storage and analysis

Written informed consent was obtained from all participants and/or their parents or guardians. Data were gathered from patient medical records and patient files at the Children's Hospital at Westmead with ethics approval (2019/ETH05351). Jamovi (The jamovi project, Version 2.2.5, Sydney, Australia) was used for basic statistical analysis. Given the retrospective nature of the study, not all data points were captured at every 12-month interval and were therefore left as missing in the results section.

RESULTS

Patient characteristics

Detailed information on patient demographics and their presenting features can be found in Table 1. The median age at presentation was 2 years (IQR = 2 years 6 months), and the median age of genetic diagnosis was 9 years 6 months (IQR = 11 years). The first genetic diagnosis of RTD was made in 2012 when the gene for RTD2 was identified. Individual 2.2 died at 3 years 6 months of age before genetic diagnosis. The most common presenting symptom was ataxia ($n = 9$), followed by hearing loss ($n = 4$), nystagmus ($n = 2$), and upper limb weakness ($n = 1$). The median age of ataxia onset was 3 years (IQR = 3 years), and the median age of hearing loss onset was also 3 years (IQR = 3 years 6 months). Individuals 2.1, 3.1, 3.2, and 3.3 had a long interval (median = 13 years, IQR = 3 years) between first presentation and diagnosis and represent the natural progression of the disease without early initiation of riboflavin supplementation. Individuals 1.1, 1.2, 4.1, 4.2, 5.1, and 6.1 had a relatively short interval (median = 1 year 6 months, IQR = 2 years) between first presentation and diagnosis, and represent most closely the response to early and continued riboflavin supplementation on disease progression.

Riboflavin supplementation

Six individuals presented with an abnormal acylcarnitine profile, while four individuals had a normal profile before initiation of high-dose riboflavin. All abnormal acylcarnitine profiles normalized after initiation of riboflavin supplementation. Concerns over adherence to therapy were raised with individual 1.1 at times during the treatment period indicated by mildly abnormal acylcarnitine profiles between 3 years and 5 years after commencing therapy.

Charcot–Marie–Tooth disease Paediatric Score

A higher total CMTPedS score (Figure 1) indicates greater functional impairment. Individuals 2.1, 3.1, and 3.2 who were treated late had the highest baseline scores. Individuals 1.1, 1.2, and 5.1 showed progression of disease severity despite treatment, while individuals 4.1 and 4.2 remained stable. All early treated individuals remained less affected than late treated individuals. All individuals recorded negative z-scores on the 6-Minute Walk Test (Figure 2) indicating impairment compared to unaffected peers. Most individuals had a decline in their 6-Minute Walk Test results despite treatment. Individuals 3.1, 3.2, and 5.1 walked 0 metres because of being non-ambulant at the time of assessment and so are not included in Figure 2.

Limb muscle weakness

Upper and lower limb muscle weakness was measured longitudinally by handheld dynamometry for hand grip (Figure 3), ankle dorsiflexion, and ankle plantarflexion strength as part of the CMTPedS.[23,24] Most individuals were stable in their hand grip strength after commencement of treatment. Individuals 2.1, 3.1, and 3.2, who were treated a long time after initial presentation, showed the weakest hand grip with individual 2.1 recording 0 Newtons of strength because of severe upper limb weakness.[23,24]

Ataxia

All 11 individuals developed ataxia during their disease course which was assessed with the balance item of the CMTPedS (Figure 4). All individuals had z-scores well below typically developing controls with most individuals not being able to complete any balance item for more than 1 second. Individuals 3.1, 3.2, and 5.1 scored 0 seconds on all items since they were non-ambulant or too unstable.

TABLE 1 Demographics and presentation.

Patient ID	1.1	1.2	2.1	2.2	3.1	3.2	3.3	4.1	4.2	5.1	6.1
Sex	F	F	F	F	M	M	M	M	F	M	M
Ethnicity	ME/L	ME/L	W	W	ME/L	ME/L	ME/L	ME/L	ME/L	W	W
Mutation	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.G306R/ p.L339P	*SLC52A2*; p.G306R/ p.L339P	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.G306R/ p.G306R	*SLC52A2*; p.C126Y/ p.L339P	*SLC52A2*; p.Gln251*/ p.Gly451Ser
Age at presentation (years:months)	8:0	2:0	5:0	2:0	3:0	3:0	2:0	7:0	0:1	1:0	1:0
Age at genetic diagnosis (years)	10	9	15	Deceased at 3 years 6 months of age (before genetic diagnosis)	16	16	21	7	4	2	1
Age at commencement of riboflavin supplementation (years)	10	9	15	N/A	16	16	21	7	4	2	1
Presenting symptoms	Ataxia	Ataxia	Hearing loss	Ataxia, upper limb weakness	Ataxia, hearing loss	Ataxia, hearing loss	Ataxia	Ataxia	Hearing loss	Ataxia, nystagmus	Ataxia, nystagmus
Hb (g/L) at presentation	122	110	-	136	133	128	-	125	105	137	90
MCV (fL) at presentation	71	67	-	78	77	67	-	79	70	80	79
Acylcarnitine profile at presentation	Abnormal	Abnormal	Abnormal	Normal	Normal	Abnormal	-	Abnormal	Abnormal	Normal	Normal
GMFCS level at presentation	I	II	II	II	IV	IV	II	I	I	II	IV
Age at hearing loss onset (years)	10	5	5	N/A	3	3	2	9	1 month	N/A	2
Age at ataxia onset (years)	8	2	7	2	3	3	4	7	3	1	1

Abbreviations: F, female; GMFCS, Gross Motor Function Classification System; HB, haemoglobin; M, male; MCV, mean corpuscular volume; ME/L, Middle-Eastern (Lebanese); W, White.

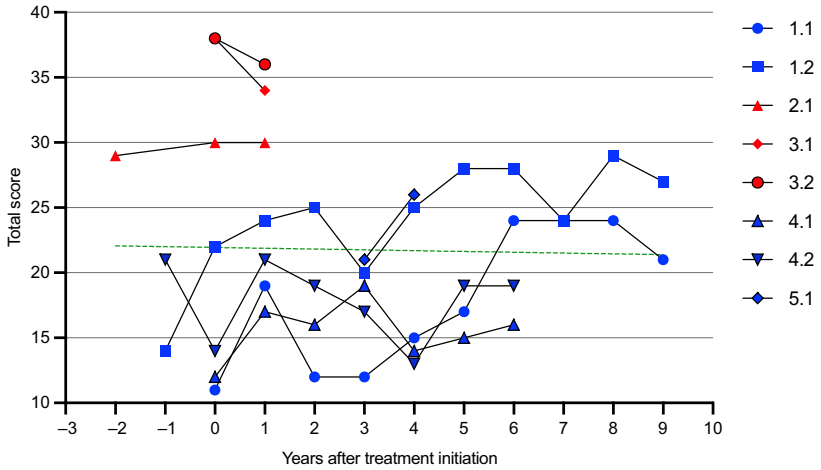

FIGURE 1 Progression of Charcot–Marie–Tooth disease Pediatric Scale total score. A higher total score indicates more functional impairment. 0, on the x axis, indicates time of treatment initiation. Individuals who represent early treatment initiation after onset of symptoms are depicted in blue. Individuals who represent late treatment initiation after onset of symptoms are depicted in red. The line of best fit for all individuals is shown on the graph in green.

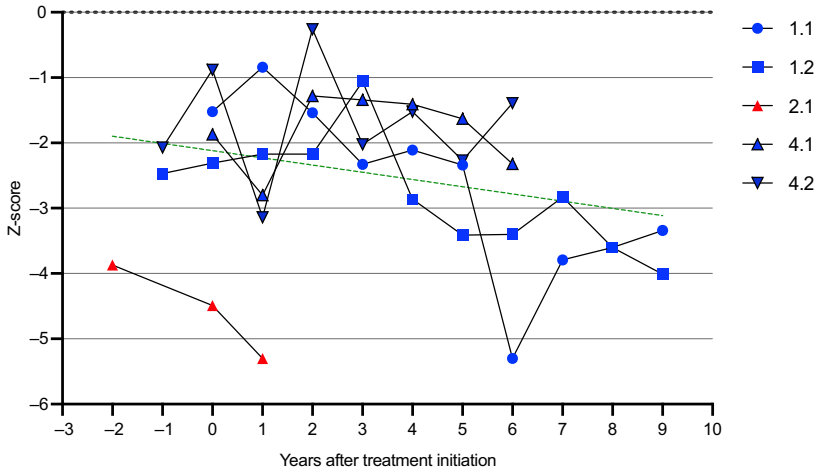

FIGURE 2 Progression of 6-minute walk distance (z-score). A more negative z-score indicates less distance walked compared to age- and sex-matched normative reference data. Individuals who represent early treatment initiation after onset of symptoms are depicted in blue. Individuals who represent late treatment initiation after onset of symptoms are depicted in red. The line of best fit for all individuals is shown on the graph in green.

Audiometry

Individuals treated within 2 years of onset of hearing loss (individuals 1.1 and 4.1) were more likely to have milder hearing loss, with individual 6.1 being the exception (Table 2). Improvements in hearing after initiation of riboflavin supplementation were noted in individuals 1.2, 4.1, and 4.2; however, these improvements were limited to the first 12 months of riboflavin supplementation, with hearing remaining stable in the following years.

Visual acuity and optic atrophy

Of the individuals assessed, individuals 4.1 and 4.2 were the only ones without impairment of visual acuity. Visual acuity worsened in the time from first assessment preceding diagnosis to the time of diagnosis in all individuals (Table 3). Riboflavin supplementation improved the visual acuity of those with visual acuity impairment at diagnosis; however, this improvement was limited to the first 12 months after riboflavin supplementation initiation and remained stable in the following years. Temporal disc pallor by fundoscopy was noted in 8 of 10 individuals. Reversal of temporal disc pallor after riboflavin supplementation was noted in individual 1.1.

Respiratory and bulbar dysfunction

Individuals 1.1 and 1.2 showed decline in their respiratory function, while individuals 4.1 and 4.2 showed relative

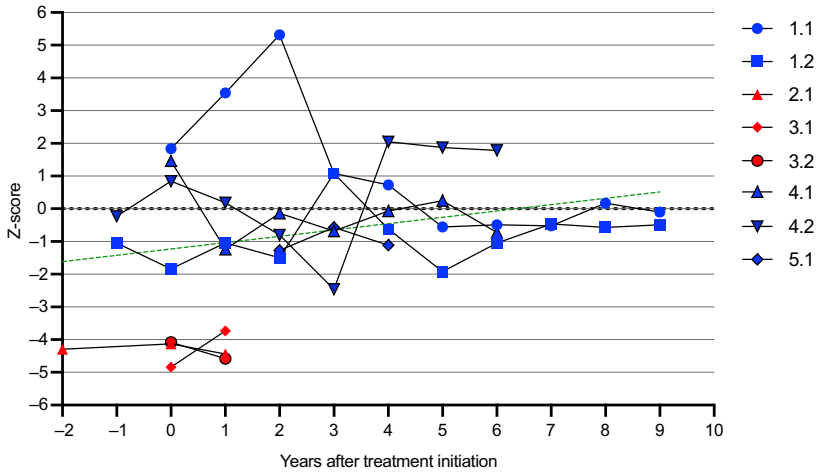

FIGURE 3 Progression of hand grip strength (z-score). A more positive z-score indicates a stronger hand grip strength. Individuals who represent early treatment initiation after onset of symptoms are depicted in blue. Individuals who represent late treatment initiation after onset of symptoms are depicted in red. The line of best fit for all individuals is shown on the graph in green.

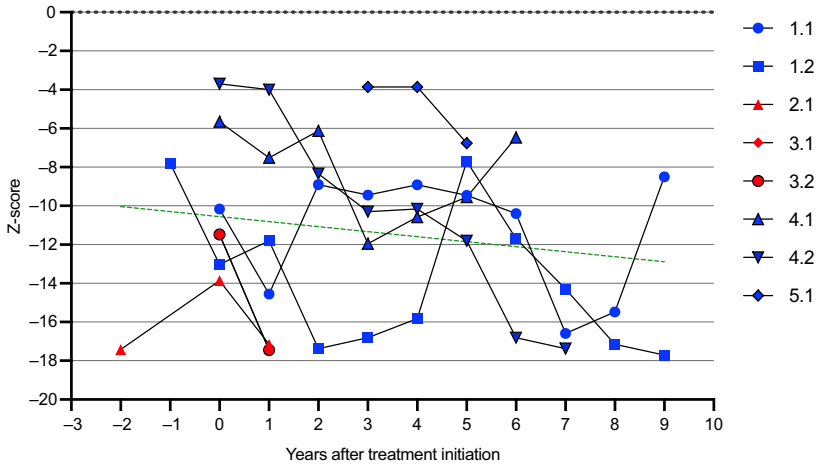

FIGURE 4 Progression of the Charcot–Marie–Tooth disease Pediatric Scale balance item. A more negative z-score indicates increased difficulty balancing. Individuals who represent early treatment initiation after onset of symptoms are depicted in blue. Individuals who represent late treatment initiation after onset of symptoms are depicted in red. The line of best fit for all individuals is shown on the graph in green.

stability in their forced vital capacity (Figure 5). Obstructive sleep apnoea was noted in four individuals (1.1, 1.2, 2.1, 6.1), with three of those individuals requiring continuous positive airway pressure overnight (1.2, 2.1, 6.1). Individuals 3.1 and 3.2 required continuous invasive ventilatory support with pressures of 12/5 and 18/8cmH2O respectively. Individual 2.2 was on ventilation at the time of their death. Individuals 3.1 and 3.2 were also gastrostomy dependent.

Scoliosis

Eight individuals (1.1, 1.2, 2.1, 3.1, 3.2, 4.1, 4.2, 5.1) had radiologically diagnosed scoliosis. The median age of diagnosis of scoliosis was 11 years 6 months (IQR = 4 years). Individual 1.2 required corrective spine surgery for scoliosis. Four individuals (1.2, 2.1, 3.1, 3.2) had scoliosis diagnosed before initiation of riboflavin supplementation. Four individuals had scoliosis diagnosed after initiation of riboflavin supplementation with a median of 4 years difference between supplementation initiation and scoliosis diagnosis (IQR = 1 year 9 months).

Other investigations

Six individuals (1.1, 1.2, 2.1, 3.1, 3.2, 4.2) had microcytic red blood cells at some stage and four individuals (1.1, 1.2, 3.1, 3.2) had microcytic red blood cells in more than 50% of their blood test results. Three individuals (1.1, 1.2, 4.2) had a microcytic anaemia at some stage. Anaemia was corrected after riboflavin supplementation in 1 of the 3 affected individuals. Mean corpuscular volume was corrected after riboflavin supplementation in 2 of the 6 affected individuals. Riboflavin levels rose after treatment with riboflavin but both pre- and post-treatment were within the normal range. Eight individuals had magnetic resonance imaging. Individual 3.2 had a reduced

TABLE 2 Audiometry.

Patient ID	1.1	1.2	2.1	2.2	3.1	3.2	3.3	4.1	4.2	5.1	6.1
Audiometry preceding diagnosis	-	-	R: Mild–Moderate; L: Mild	-	R: Moderate–Severe; L: Moderate–Severe	R: Severe; L: Severe	R: Mild–Moderate; L: Mild–Moderate	-	R: Mild; L: Mild	-	-
Audiometry at diagnosis	R: Mild; L: Mild	R: Moderate–Severe; L: Severe	R: Moderate–Severe; L: Moderate–Severe	-	R: Severe; L: Severe	R: Severe; L: Severe	R: Moderate–Severe; L: Moderate–Severe	R: Severe; L: Severe	R: Moderate–Severe; L: Severe	-	R: Moderate–Severe; L: Moderate–Severe
Audiometry after riboflavin supplementation (years after supplementation initiation)[a]	R: Mild; L: Mild (1)	R: Mild–Moderate; L: Moderate–Severe (1)	R: Moderate–Severe; L:Moderate–Severe (1)	-	-	-	-	R: Mild; L: Mild (2)	R: Moderate–Severe; L: Moderate–Severe (1)	-	-
Speech perception test at diagnosis	Abnormal	Abnormal	Abnormal	-	-	-	Abnormal	-	Abnormal	-	-
Speech perception test after riboflavin supplementation (years after supplementation initiation)[a]	Normal (1)	Abnormal (1)	-	-	-	-	-	Normal (2)	Normal (2)	-	-
Time from hearing loss onset to diagnosis (years)	0	4	10	N/A	13	13	19	2	4	N/A	0

Abbreviations: L, left; N/A, not applicable; R, right.

[a]The result listed is the earliest on record after riboflavin supplementation initiation.

TABLE 3 Optic atrophy.

Patient ID	1.1	1.2	2.1	2.2	3.1	3.2	3.3	4.1	4.2	5.1	6.1
Visual acuity preceding diagnosis	-	-	R: 6/18; L: 6/9	-	R: 6/18; L: 6/18	R: 6/36; L: 6/36	R: 6/15; L: 6/12	-		-	-
Visual acuity at diagnosis	R: 6/24; L: 6/18	R: 6/24; L: 6/18	R: 6/36; L: 6/18	-	R: 6/75; L: 6/60	R: 6/72; L: 6/72	-	R: 6/6; L: 6/6	R: 6/6; L: 6/6	R: 6/60; L: 6/60	-
Visual acuity after riboflavin supplementation (years after supplementation initiation)[a]	R: 6/6; L: 6/6 (1)	R: 6/10; L: 6/9 (1)	R: 6/12; L: 6/12 (1)	-	-	-	-	R: 6/6; L: 6/6 (2)	R: 6/6; L: 6/6 (2)	R: 6/48; L: 6/48 (1)	-
Temporal disk pallor at diagnosis	Present	Present	Present	-	Present	Present	Present	Absent	Absent	Present	Present
Temporal disk pallor after riboflavin supplementation	Absent	-	Present	-	-	-	-	Absent	Absent	Present	Present

Abbreviations: L, left; R, right.

[a]The result listed is the earliest on record after riboflavin supplementation initiation.

cochlear nerve size, and the other seven individuals had no abnormalities detected.

DISCUSSION

This paper has followed the progress in multiple clinical domains of individuals with RTD with mutations in the *SLC52A2* gene. Overall, individuals with RTD had impairments in muscle strength, balance, hearing, visual acuity, and respiratory function compared to age-matched typically developing controls. While high-dose oral riboflavin supplementation resulted in slowing or stabilizing of disease progression, ongoing deterioration in some functional domains were identified. All patients were left with residual disability in multiple domains despite treatment. Individuals for whom riboflavin supplementation was initiated early after first presentation had better outcomes in hearing, optic atrophy, lung function, strength, balance, and functional outcomes compared to those who had delayed treatment initiation.

While the CMTPedS remained stable during the follow-up period, there was variability in the progression of individual items. Irrespective of timing of treatment initiation, there was a progression of ataxia and this was the likely reason for deterioration in the 6-minute walk distance. In contrast, hand grip strength improved for those who were treated early after symptom onset with z-scores mostly ranging between −2 and + 1 with no deterioration with time. Of greatest concern was the gradual decline in the forced vital capacity (12.5% over 9 years), even in those treated early. Three individuals progressed to requiring continuous positive airway pressure despite treatment.

There was evidence of reversal of neuronal injury, with improvements in optic atrophy and audiometry, only in those treated early,[10] suggesting that a prolonged period between disease onset and treatment initiation causes irreversible neuronal loss and reinforcing the need for early diagnosis and treatment of RTD. The reversal of neuronal injury appeared to be limited to the first 12 months after initiation of riboflavin supplementation in most cases, and additional interventions, like cochlear implantation for significantly impaired hearing, can be considered at this time, as further improvement was unlikely. Two individuals required continuous ventilation with both also requiring a gastrostomy, and one individual died from respiratory failure before diagnosis, again reinforcing the importance of maintaining a high degree of suspicion of this disorder in those who present with an auditory neuropathy, ataxia, optic atrophy and nystagmus, or upper limb weakness, despite the rarity of RTD. The acylcarnitine profile is a useful screening test but does not exclude the disorder, and high-dose riboflavin should be considered in those awaiting genetic testing and in whom an alternative cause is not quickly identified.

RTD appears to have an effect on mean corpuscular volume and the development of anaemia as 6 of the 10 tested individuals had microcytic red cells and three individuals had a microcytic anaemia at some stage. This may be due to

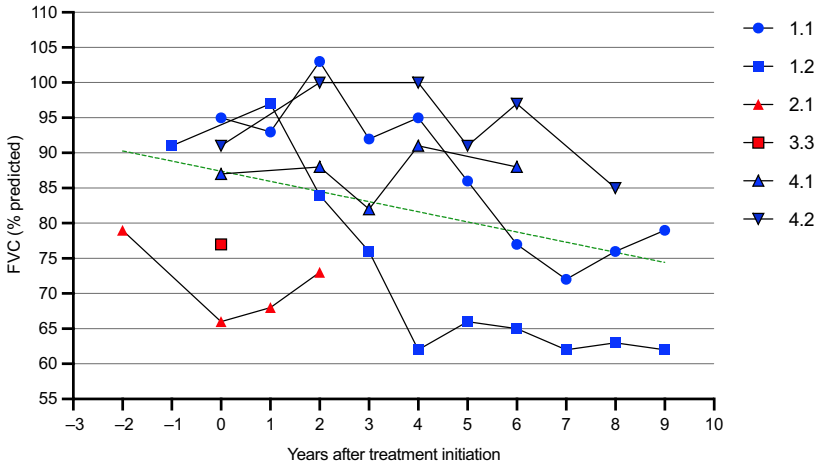

FIGURE 5 Progression forced vital capacity. A higher forced vital capacity (% predicted) indicates better respiratory function. Individuals who represent early treatment initiation after onset of symptoms are depicted in blue. Individuals who represent late treatment initiation after onset of symptoms are depicted in red. The line of best fit for all individuals is shown on the graph in green.

Clinical feature	Surveillance recommendation
Motor and sensory neuropathy (including sensory ataxia)	Annual RTD Pediatric Scale assessment[31]
Auditory neuropathy	Annual: - Pure tone audiometry - Speech discrimination tests[9]
Optic atrophy	Annual assessments of visual acuity Biennial ophthalmology review (more frequent if reduced visual acuity)
Respiratory muscle weakness	Annual spirometry (FVC) Referral to a sleep physician if FVC < 60% predicted or symptoms of respiratory or sleep dysfunction Polysomnograms as per respiratory physician recommendation
Scoliosis	Annual clinical review If scoliosis develops: - Annual X-rays - Referral to a spine surgeon
Biochemical	Annual: - Full blood count - Acylcarnitine profile - Riboflavin/FMN/FAD level (where routinely available at treating institution)

FIGURE 6 Protocol for clinical surveillance in RTD. Abbreviations: FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; FVC, forced vital capacity; RTD, riboflavin transporter deficiency.

riboflavin's interaction with vitamin B_6. Riboflavin converts vitamin B_6 to its metabolically active form piyridoxal-5′-phosphate. Piyridoxal-5′-phosphate functions as a coenzyme of 5-aminolevulinic acid synthase which is involved in the synthesis of heme, thus riboflavin deficiency may produce a microcytic anaemia.[29] While pre- and post-treatment riboflavin levels were within the normal range in our cohort of children with RTD2, we have recommended monitoring of riboflavin/flavin mononucleotide/flavin adenine dinucleotide levels where routinely available at the treating institution as abnormal levels pretreatment with normalization post-treatment has been described with RTD3, caused by mutations in the intestinal transporter.[30]

As RTD is a progressive genetic neuropathy like CMT, the CMTPedS was a reasonable tool for measuring disease progression in these individuals. However, compared to the common forms of CMT, individuals with RTD have more profoundly affected balance. Many components of the balance item showed a ceiling effect in those assessed, producing most z-scores between −6 and − 18. The CMTPedS also does not capture the proximal muscle weakness present in most individuals with RTD. Based on these differences we have recently modified the CMTPedS to create an RTD-specific clinical outcome assessment, the RTD Pediatric Scale, for ongoing monitoring of riboflavin supplementation and use in trials of other therapeutic interventions.[31] This functional outcome measure was pilot tested and validated using five of the individuals with RTD included in this study (1.1, 1.2, 2.1, 3.1, 3.2).

Based on our experience with the individuals included in this study, we recommend regular assessment of children with RTD according to the protocol in Figure 6 as affected children can have disease progression despite high-dose riboflavin. Forced vital capacity has been shown to be both a marker of early respiratory muscle weakness as well as a prognostic marker in other progressive neuromuscular disease.[32] There is a need to identify other disease-modifying agents to improve the long-term outcomes of RTD and reduce residual disability. We also suggest that these outcome measures, especially the RTD Pediatric Scale, to be used in future clinical trials as well as international RTD registries.

The limitations of this study were that the group of individuals assessed was relatively small and it was a retrospective case series study. Additionally, this study only included individuals with mutations in *SLC52A2* (RTD2), though there is significant overlap in the clinical phenotype of RTD2 and RTD3 (caused by mutations in *SLC52A3*). Further longitudinal studies in individuals with RTD3 would be helpful to compare disease progression despite riboflavin supplementation to those with RTD2. An international registry using the surveillance protocol we have recommended (Figure 6) will help us clarify if the progression we have recorded in the study group is applicable to the wider population of individuals with RTD.

In conclusion, while treatment with high-dose oral riboflavin supplementation slowed the progression of functional disability and showed improvements in some domains, patients were left with residual disability in balance, walking endurance, hearing, visual acuity, and respiratory function. There is a need for a standardized outcome measure to track disease progression over time and response to riboflavin supplementation. We recommend using the RTD Pediatric Scale and clinical measures for regular surveillance of children with RTD and for future clinical trials.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest relevant to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Bosch AM, Stroek K, Abeling NG, Waterham HR, Ijlst L, Wanders RJ. The Brown-Vialetto-Van Laere and Fazio Londe syndrome revisited: natural history, genetics, treatment and future perspectives. Orphanet J Rare Dis. 2012;7:83. https://doi.org/10.1186/1750-1172-7-83
2. Sathasivam S. Brown-Vialetto-Van Laere syndrome. Orphanet J Rare Dis. 2008;3:9. https://doi.org/10.1186/1750-1172-3-9
3. Johnson JO, Gibbs JR, Megarbane A, et al. Exome sequencing reveals riboflavin transporter mutations as a cause of motor neuron disease. Brain. 2012;135(Pt 9):2875–82. https://doi.org/10.1093/brain/aws161
4. Foley AR, Menezes MP, Pandraud A, et al. Treatable childhood neuronopathy caused by mutations in riboflavin transporter RFVT2. Brain. 2014;137(Pt 1):44–56. https://doi.org/10.1093/brain/awt315
5. Fennessy JR, Cornett KMD, Burns J, Menezes MP. Benefit of high-dose oral riboflavin therapy in riboflavin transporter deficiency. J Peripher Nerv Syst. 2023;28(3):308–16. https://doi.org/10.1111/jns.12587
6. Cali E, Dominik N, Manole A, Houlden H. Riboflavin Transporter Deficiency. 2015 Jun 11 [Updated 2021 Apr 8]. GeneReviews® Accessed 2023. https://www.ncbi.nlm.nih.gov/books/NBK299312/
7. Jaeger B, Bosch AM. Clinical presentation and outcome of riboflavin transporter deficiency: mini review after five years of experience. J

Inherit Metab Dis. 2016;39(4):559–64. https://doi.org/10.1007/s10545-016-9924-2
8. Ciccolella M, Catteruccia M, Benedetti S, et al. Brown-Vialetto-van Laere and Fazio-Londe overlap syndromes: a clinical, biochemical and genetic study. Neuromuscul Disord. 2012;22(12):1075–82. https://doi.org/10.1016/j.nmd.2012.05.007
9. Menezes MP, O'Brien K, Hill M, et al. Auditory neuropathy in Brown-Vialetto-Van Laere syndrome due to riboflavin transporter RFVT2 deficiency. Dev Med Child Neurol. 2016;58(8):848–54. https://doi.org/10.1111/dmcn.13084
10. Menezes MP, Farrar MA, Webster R, et al. Pathophysiology of motor dysfunction in a childhood motor neuron disease caused by mutations in the riboflavin transporter. Clin Neurophysiol. 2016;127(1):911–8. https://doi.org/10.1016/j.clinph.2015.05.012
11. Khadilkar SV, Faldu HD, Udani V, Patil SB, Malvadkar S. Reversible posterior column dysfunction in Brown-Vialetto-Von Laere syndrome. Muscle Nerve. 2017;56(4):E28–E31. https://doi.org/10.1002/mus.25694
12. Thulasi V, Veerapandiyan A, Pletcher BA, Tong CM, Ming X. A Case of Brown-Vialetto-Van Laere Syndrome Due To a Novel Mutation in SLC52A3 Gene: Clinical Course and Response to Riboflavin. Child Neurol Open. 2017;4:2329048X17725610. https://doi.org/10.1177/2329048X17725610
13. Omar R, Rajput K, Sirimanna T, Rajput S, Pagarkar W. The audio-vestibular profile of Brown-Vialetto-Van Laere syndrome. J Laryngol Otol. 2021;135(11):1000–9. https://doi.org/10.1017/S0022215121002395
14. do Amaral MSA, Massuda ET, Mitikami Fenolio GH, Barbosa Reis ACM, Angelo Hyppolito M. Cochlear Implant in Brown-Vialetto-Van Laere Syndrome Patient. J Int Adv Otol. 2022;18(2):192–5. https://doi.org/10.5152/iao.2022.21159
15. Gedik Soyuyuce O, Ayanoglu Aksoy E, Yapici Z. A case report of sudden-onset auditory neuropathy spectrum disorder associated with Brown-Vialetto-Van Laere syndrome (riboflavin transporter deficiency). Int J Audiol. 2022;61(3):258–64. https://doi.org/10.1080/14992027.2021.1921291
16. Davis A, Josifova D, Lloyd-Owen S, Radunovic A, Swash M. Brown-Vialetto-Van Laere syndrome: a 28-year follow-up. Journal of Neurology, Neurosurgery & Psychiatry. 2016;87(6):681–2. https://doi.org/10.1136/jnnp-2014-310088
17. Bashford JA, Chowdhury FA, Shaw CE. Remarkable motor recovery after riboflavin therapy in adult-onset Brown-Vialetto-Van Laere syndrome. Pract Neurol. 2017;17(1):53–6. https://doi.org/10.1136/practneurol-2016-001488
18. Chaya S, Zampoli M, Gray D, et al. The First Case of Riboflavin Transporter Deficiency in sub-Saharan Africa. Semin Pediatr Neurol. 2018;26:10–4. https://doi.org/10.1016/j.spen.2017.03.002
19. Fan J, Fogel BL. Successful treatment of a genetic childhood ataxia due to riboflavin transporter deficiency. Cerebellum & Ataxias. 2018;5(1)https://doi.org/10.1186/s40673-018-0091-0
20. Gorcenco S, Vaz FM, Tracewska-Siemiatkowska A, et al. Oral therapy for riboflavin transporter deficiency - What is the regimen of choice? Parkinsonism Relat Disord. 2019;61:245–7. https://doi.org/10.1016/j.parkreldis.2018.10.017
21. Bamaga AK, Maamari RN, Culican SM, Shinawi M, Golumbek PT. Child Neurology: Brown-Vialetto-Van Laere syndrome: Dramatic visual recovery after delayed riboflavin therapy Neurology. 2018;91(20):938–41. https://doi.org/10.1212/wnl.0000000000006498
22. Burns J, Ouvrier R, Estilow T, et al. Validation of the Charcot-Marie-Tooth disease pediatric scale as an outcome measure of disability. Ann Neurol. 2012;71(5):642–52. https://doi.org/10.1002/ana.23572
23. McKay MJ, Baldwin JN, Ferreira P, et al. Normative reference values for strength and flexibility of 1,000 children and adults. Neurology. 2017;88(1):36–43. https://doi.org/10.1212/WNL.0000000000003466
24. McKay MJ, Baldwin JN, Ferreira P, et al. Reference values for developing responsive functional outcome measures across the lifespan. Neurology. 2017;88(16):1512–9. https://doi.org/10.1212/WNL.0000000000003847
25. Cornett KMD, Menezes MP, Shy RR, et al. Natural history of Charcot-Marie-Tooth disease during childhood. Ann Neurol. 2017;82(3):353–9. https://doi.org/10.1002/ana.25009
26. Cornett KM, Menezes MP, Bray P, et al. Phenotypic Variability of Childhood Charcot-Marie-Tooth Disease. JAMA Neurol. 2016;73(6):645–51. https://doi.org/10.1001/jamaneurol.2016.0171
27. Carhart R, Jerger JF. Preferred Method For Clinical Determination Of Pure-Tone Thresholds. Journal of Speech and Hearing Disorders. 1959;24(4):330–45. https://doi.org/10.1044/jshd.2404.330
28. Travers A. AB word lists: NAL protocols. Chatswood, NSW, Australia : National Acoustic Laboratories. 1990;
29. McNulty H, Pentieva K, Ward M. Causes and Clinical Sequelae of Riboflavin Deficiency. Annu Rev Nutr. 2023;43:101–22. https://doi.org/10.1146/annurev-nutr-061121-084407
30. Bosch AM, Abeling NG, Ijlst L, et al. Brown-Vialetto-Van Laere and Fazio Londe syndrome is associated with a riboflavin transporter defect mimicking mild MADD: a new inborn error of metabolism with potential treatment. J Inherit Metab Dis. 2011;34(1):159–64. https://doi.org/10.1007/s10545-010-9242-z
31. Fennessy JR, Donlevy GA, McKay MJ, Burns J, Cornett KMD, Menezes MP. Development of a functional outcome measure for riboflavin transporter deficiency. J Peripher Nerv Syst. 2024;29(2):185–92. https://doi.org/10.1111/jns.12619
32. Phillips MF, Quinlivan RC, Edwards RH, Calverley PM. Changes in spirometry over time as a prognostic marker in patients with Duchenne muscular dystrophy. Am J Respir Crit Care Med. 2001;164(12):2191–4. https://doi.org/10.1164/ajrccm.164.12.2103052

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Chapter 4

Development of a Functional Outcome Measure for Riboflavin Transporter Deficiency

Preface

The previous chapter presented functional assessments for a cohort of individuals with riboflavin transporter deficiency (RTD) using the Charcot-Marie-Tooth disease Pediatric Scale (CMTPedS) as there is currently no RTD-specific functional outcome measure.

The research presented in this chapter has been published in the *Journal of the Peripheral Nervous System* as the following:

> Fennessy JR, Donlevy GA, McKay MJ, Burns J, Cornett KMD, Menezes MP. Development of a functional outcome measure for riboflavin transporter deficiency. *J Peripher Nerv Syst*. Jun 2024;29(2):185-192. doi:10.1111/jns.12619

The research presented in this chapter was presented at the following conference as a poster presentation:

> Jack R. Fennessy, Gabrielle Donlevy, Kayla M.D. Cornett, Marnee McKay, Joshua Burns, Manoj P. Menezes. Development of a Disability Scale for Riboflavin Transporter Deficiency. Peripheral Nerve Society Meeting. Copenhagen, Denmark 17 – 20th June 2023.

Appendix S1 ('Riboflavin Transporter Deficiency (RTDPedS) equiptment and training resource kit') can be found in the Appendices section on page 57. eTable 1 ('Medline search strategy') can be found on page 76. eTable 2 ('Items included in selected outcome measures') can be found on page 77. eTable 3 ('Patient demographics') can be found on page 78.

Authorship Statement

The co-authors of the paper '*Development of a functional outcome measure for riboflavin transporter deficiency*' confirm that *Jack Fennessy* has made the following contributions:

- Collection of data
- Analysis and interpretation of data
- Drafting and revising of the manuscript and critical appraisal of content

As the primary supervisor of the candidate and this paper, I can confirm that the above authorship attribution statement is correct.

……

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RESEARCH REPORT

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Development of a functional outcome measure for riboflavin transporter deficiency

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Abstract

Background and Aims: Riboflavin transporter deficiency (RTD) is a progressive inherited neuropathy of childhood onset, characterised clinically by pontobulbar palsy, sensory ataxia, sensorineural deafness, muscle weakness, optic atrophy and respiratory failure. A robust and responsive functional outcome measure is essential for future clinical trials of disease-modifying therapies including genetic therapies. The Charcot–Marie–Tooth disease Pediatric Scale (CMTPedS) is a well-validated outcome measure for CMT and related neuropathies, and might have utility for measuring disease progression in individuals with RTD. However, the CMTPedS requires modifications to account for phenotypic differences between children with CMT and RTD. The aim of this study was to develop a functional outcome measure based on the CMTPedS for specific use in individuals with RTD.

Methods: The CMTPedS data collected over the last 10 years in individuals with RTD attending the Peripheral Neuropathy Management Clinic at the Children's Hospital at Westmead (Sydney, Australia) were reviewed to evaluate each item within the CMTPedS. A literature review of articles published until September 2021 for functional outcome measures generated an item pool for pilot testing. The results of this pilot testing, alongside analysis of existing CMTPedS item scores in the RTD cohort, informed the modification of the CMTPedS.

Results: CMTPedS data were reviewed for eight individuals over the past 10 years. Two items were identified as requiring modification or removal and additional items of proximal strength and function needed to be considered. Six studies were

Abbreviations: BOT-2, Bruininks–Oseretsky Test of Motor Proficiency Second Edition; CMT, Charcot–Marie–Tooth disease; CMTPedS, Charcot–Marie–Tooth disease Pediatric Scale; MAND, McCarron Assessment of Neuromuscular Development'; MFM-32, 32-item Motor Function Measure; PBS, Pediatric Balance Scale; RTD, Riboflavin Transporter Deficiency; RTDPedS, Riboflavin Transporter Deficiency Pediatric Scale.

Kayla M. D. Cornett and Manoj P. Menezes are Senior authors contributed equally.

identified in the literature review, and five items were selected for pilot testing. 'Shoulder internal rotation' and the '30-s sit to stand test' were added as proximal measures of strength and function. The composite balance item comprising nine tasks in the CMTPedS showed a ceiling effect and was replaced with the single 'Feet apart on a line eyes open' balance item. 'Pinprick sensation' was removed due to a floor effect.

Interpretation: This study provides preliminary evidence that the Riboflavin Transporter Deficiency Pediatric Scale (RTDPedS) is a functional outcome measure covering strength, upper and lower limb function, balance and mobility for individuals with RTD to assess disease severity and progression in clinical trials and cohort studies.

KEYWORDS
clinical outcome assessment, clinical trials, endpoint, performance outcome measure, riboflavin transporter deficiency

1 | INTRODUCTION

Riboflavin transporter deficiency (RTD) is a progressive inherited neuropathy of childhood onset, characterised clinically by pontobulbar palsy, sensory ataxia, sensorineural deafness, muscle weakness, optic atrophy and respiratory failure.[1] RTD is caused by biallelic mutations in the *SLC52A2* or *SLC52A3* genes encoding the riboflavin transporters RFVT2 and RFVT3, respectively.[2] Without treatment, affected individuals develop severe disability and experience early death due to respiratory failure. High-dose oral riboflavin supplementation is life-saving and improves outcomes in multiple functional domains.[3,4] Despite early treatment, some individuals are left with residual disability.[4]

Most case reports of RTD in the literature provide detailed phenotypic descriptions at a single point in time but only a few consistently and uniformly track the response to riboflavin supplementation over time. This is partially due to the lack of a functional outcome measure validated for RTD. A robust and responsive clinical outcome measure is also essential for future clinical trials of disease-modifying therapies including genetic therapies.

The Charcot–Marie–Tooth disease Pediatric Scale (CMTPedS)[5] is a well-validated outcome measure for children and adolescents with CMT that measures strength, upper and lower limb function, balance and mobility. The 11-item CMTPedS generates a total disability score ranging from 0 (unaffected) to 44 (severely affected) by scaling the scoring system to age and sex-matched normative reference values.[6,7] Natural history studies indicate that the Rasch-built CMTPedS is sensitive to change and able to capture the spectrum of phenotypic variability of CMT and related neuropathies.[8,9] As RTD is a progressive genetic neuropathy like CMT, the CMTPedS is a reasonable tool for measuring disease progression in this cohort. However, in comparison to the common forms of CMT, children with RTD have a more profoundly ataxic gait, difficulty walking and problems with balance, and the CMTPedS may require modifications to account for these phenotypic differences. The aim of this study was to develop a functional outcome measure based on the CMTPedS for specific use in individuals with RTD.

2 | MATERIALS AND METHODS

2.1 | Review of CMTPedS data

The CMTPedS data collected over the last 10 years in a cohort of eight individuals with RTD attending the Peripheral Neuropathy Management Clinic at the Children's Hospital at Westmead (Sydney, Australia) were reviewed to evaluate the distribution of scores from each functional item within the CMTPedS. Items that showed floor or ceiling effects were identified for removal. Any disability of RTD that was not measured by the current items on the CMTPedS was identified, and additional items that measured that disability was noted for inclusion in the RTD-specific scale.

2.2 | Literature review

A review of the literature published until September 2021 was conducted on the Medline database using terms in eTable 1. Experts in paediatric neuromuscular medicine and outcome measure development were contacted to confirm no relevant studies were missed. The reference lists of the included studies were examined to ensure studies not captured by our search strategy could be considered.

All studies that focused on the development of a functional outcome measure capturing strength, upper and lower limb function, balance and mobility for children with a neuromuscular disease were considered and reviewed. Studies that assessed healthy participants were also included. Studies were excluded if the functional outcome measures were designed for children under 4 years of age. The original study was sourced where possible.

2.3 | Generation of the new item pool

The individual items of the included studies were collated and assessed for their appropriateness and availability of normative reference data for use in our cohort of individuals with RTD.

2.4 | Pilot testing

Individual items identified from the literature search that were deemed appropriate for pilot testing were identified. A cohort of five individuals (from the original cohort of eight) with genetically confirmed RTD were assessed using these new items on two occasions 12 months apart. Written, informed consent was obtained from all participants or their parents/guardians. The remaining three were not available for assessment as they had been transitioned to adult services. These individual items were assessed by two different evaluators (KMDC and JRF) who were formally trained by MJM (expert from the 1000 Norms Project),[6,7] in performing these assessments and blinded from each other's assessments. The potential new items were assessed following the first 10 items of the CMTPedS, prior to the 6-min walk test to prevent fatigue from influencing results. Item scores, inter-rater score comparison, ease of instruction delivery and ease of assessment were evaluated.

3 | RESULTS

3.1 | Review of CMTPedS data

The severity frequency of CMTPedS item scores for individuals with RTD is shown in Figure 1. Each item is scored on a scale from 0 (unaffected) to 4 (severely affected) as previously described.[5] Most items demonstrated an even distribution of scores representing the clinical severity of RTD and were appropriate for inclusion in an RTD-specific scale. Pinprick sensation showed a floor effect (most individuals were unaffected), and the composite balance item comprising nine tasks in the CMTPedS showed a ceiling effect. The review of CMTPedS items identified that the proximal weakness in RTD, which is often upper limb predominant in RTD2, is not currently measured.[4,10] These factors informed the literature search that follows which targeted a revised balance assessment and items assessing proximal muscle function and upper limb strength.

3.2 | Screening of studies

A total of 3848 papers were identified with the search strategy. Titles and abstracts were screened and records that met the inclusion criteria were sought for retrieval (Figure 2). The search strategy was focussed on identifying outcome measures for sensory ataxia and proximal weakness and items and studies that did not include these items or were designed for cerebellar ataxia were excluded. The CMTPedS is validated for children from 3 to 20 years of age, and studies that looked at items for those younger than this age group, or for an adult population were excluded.

3.3 | Description of functional outcome measures

The outcome measures in six studies were included in the final review: the strength, balance and function items from the 1000 Norms Project,[6,7] the Bruininks–Oseretsky Test of Motor Proficiency

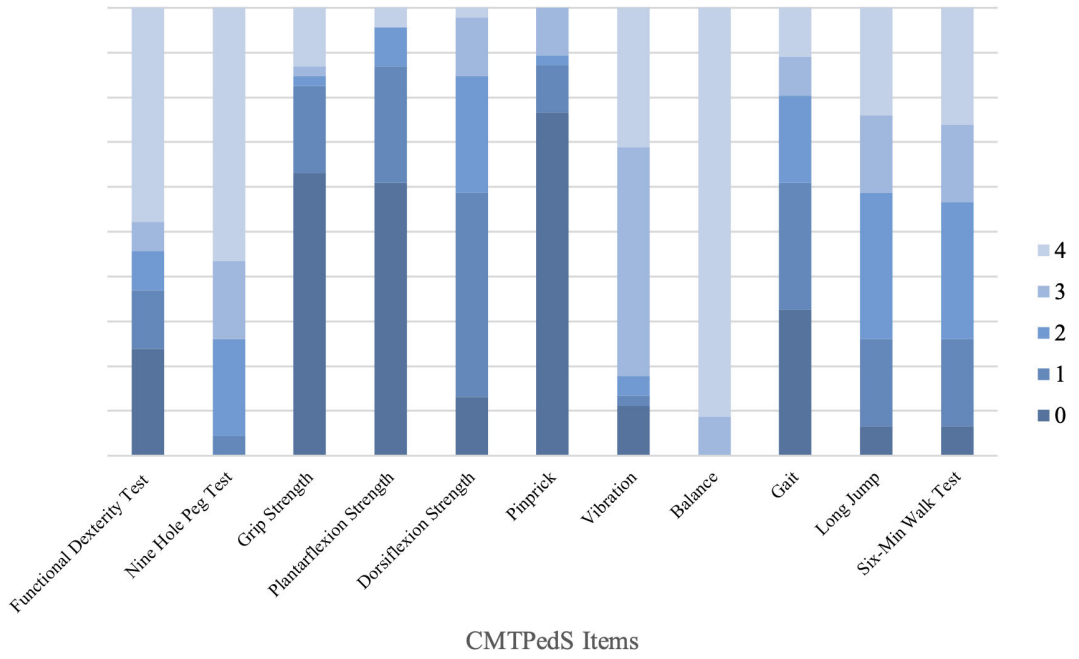

FIGURE 1 Severity frequency of CMTPedS Item Scores. Data include eight individuals at all visits over 10 years. Each item is scored on a scale from 0 (unaffected) to 4 (severely affected).

Second Edition (BOT-2),[11] the Charcot–Marie–Tooth disease Pediatric Scale (CMTPedS),[5] the McCarron Assessment of Neuromuscular Development (MAND),[12] the 32-item Motor Function Measure (MFM-32)[13] and the Pediatric Balance Scale (PBS).[14] These outcome measures were assessed for their target population, measurement domains and the presence/absence of normative values (Table 1).

3.4 | Selection of new items

The factors that influenced the selection of pilot items included the item's appropriateness for individuals with RTD to perform without ceiling or floor effects, its ability to provide a sensitive and quantitative output, the requirement for proximal strength measures, the ceiling effect of the current balance items in the CMTPedS and the presence of normative values. Five items from the 1000 Norms Project (30-s sit to stand, shoulder internal rotation strength, shoulder external rotation strength, elbow flexion strength and feet apart on a line eyes open) met all criteria and were selected to be pilot tested using the previously described protocol.[6,7]

3.5 | Pilot testing

The five individuals with RTD who were available for pilot testing were trialled on two separate occasions 12 months apart with the five new items, alongside their routine CMTPedS assessment (a demographics table can be found in eTable 3). The items were collected in accordance with the protocol described by the 1000 Norms Project.[6,7] The results of the pilot testing are shown in Table 2. Due to RTD being a rare disease (prevalence: approximately 1 in 1 000 000), the resulting small sample size prevented formal validation of these items for use in individuals with RTD.

3.6 | Final RTDPedS

The final version of the RTD Pediatric Scale (RTDPedS) is shown in Figure 3. The RTDPedS is a modified version of the existing CMTPedS. Shoulder internal rotation was added to include an upper limb proximal muscle strength measure and was chosen in preference to elbow flexion and shoulder external rotation based on more proximal location and ease of instruction/muscle isolation. The 30-s sit to stand test was added as a proximal lower limb functional task. The previous balance item, which consisted of nine balance tasks, was removed from the scale due to the ceiling effect (Figure 1), arduousness and length of the assessment. As balance impairment is a key feature of the residual disability experienced by treated patients with RTD it is still critical to measure in future therapies which may address this. The previous balance item was replaced with 'standing with feet apart on a line eyes open' as a single, more attainable measure of balance impairment. Pinprick sensation was removed from the scale because of the floor effect and because there is no current evidence that RTD is a small fibre neuropathy. The symptoms of foot pain, leg cramps, unsteady ankles, hand pain and hand tremor were removed as not common in children with RTD, and requirements for nocturnal non-invasive ventilation and

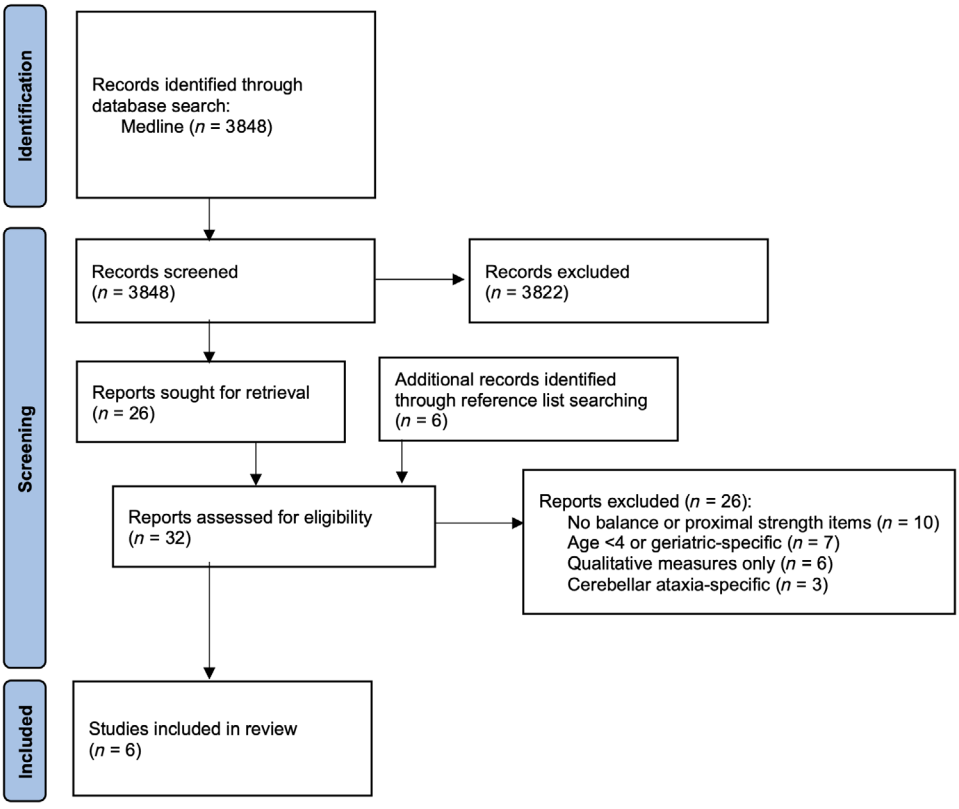

FIGURE 2 PRISMA flow diagram.

TABLE 1 Summary of outcome measures identified in the literature review.

Reference	Outcome measure	Population		Measurement domains	Number of items	Normative values (overall score)	Normative values (individual items)
		Target population	Age range (years)				
McKay et al. (2017)[6]	Items from 1000 Norms Project	Healthy participants	3–101	ROM, strength, FPI, balance, dexterity, endurance, gait	57	No	Yes
Bruininks et al. (2005)[11]	BOT-2	Not specified	4–21	Balance, coordination, dexterity, functional mobility, gait, strength	53	Yes	No
Burns et al. (2012)[5]	CMTPedS	Charcot–Marie–Tooth disease	3–20	Hand dexterity, balance, strength, motor function, sensation	11	Yes	Yes
McCarron et al. (1982)[12]	MAND	None specific	3.5–18	Fine motor, gross, motor, dexterity, balance	10	Yes	No
Berard et al. (2005)[13]	MFM-32	Patients with neuromuscular diseases	6–60	Standing position and transfers, proximal and distal motor function	32	Yes	No
Franjoine et al. (2003)[14]	PBS	Patients with mild to moderate motor impairments	5–15	Balance	14	Yes	No

Note: Items were not included if the score for the item was not quantitative; items that were scored based on whether or not the task could be completed were not included in the table. eTable 2 shows the measurement domains included in each scale. BOT-2, Bruininks–Oseretsky Test of Motor Proficiency Second Edition; CMTPedS, Charcot–Marie–Tooth disease Pediatric Scale; FPI, Foot Posture Index; MAND, McCarron Assessment of Neuromuscular Development; MFM-32, Motor Function Measure (32 item); PBS, Pediatric Balance Scale; ROM, Range of Motion.

TABLE 2 Pilot testing results of new items.

			Elbow flexion	Shoulder internal rotation	Shoulder external rotation	30-s sit to stand	Feet apart on a line eyes open
Patient 1.1	2021	Raw score	144.0	68.7	58.7	15	1.69
		Z-score	−0.641	−1.789	−1.351	−1.779	−10.387
Patient 1.2	2021	Raw score	106.0	59.0	45.3	10	0.00
		Z-score	−1.764	−1.567	−2.004	−2.800	−12.500
Patient 4.1	2021	Raw score	196.7	173.7	106.0	14	2.75
		Z-score	−0.820	−0.075	−0.446	−1.771	−9.062
	2022	Raw score	190.3	173.5	108.8	12	5.90
		Z-score	−0.940	−0.080	−0.326	−2.118	−5.125
Patient 4.2	2021	Raw score	132.0	73.3	74.4	21	1.52
		Z-score	0.074	−0.927	0.959	−0.693	−10.600
	2022	Raw score	110.2	65.8	66.8	17	1.31
		Z-score	−1.127	−1.551	−0.305	−1.430	−10.862
Patient 5.1	2021	Raw score	45.3	48.3	*	*	0.00
		Z-score	−1.968	−0.841	*	*	−12.500
	2022	Raw score	42.7	46.4	*	10	0.00
		Z-score	−2.075	−0.890	*	−2.517	−12.500

Note: Items marked with an asterisk (*) could not be completed at the time of assessment. The labels for individuals 1.1 and 1.2 have been kept consistent with a previous publication which included the same patients.[15] Individuals 1.1 and 1.2 were not available for follow-up in 2022 due to transition to adult services.

gastrostomy were added to a 'support' section of the patient profile. The lunge test and foot posture index were also removed since not clinically relevant tests for children with RTD.

Scoring of the RTDPedS was adapted from the CMTPedS.[5] Raw scores recorded on the score form (Figure 3) are converted *z*-scores using age- and sex-matched normative reference values from the

RTDPedS

Initial evaluation ☐ Re-Evaluation ☐ Date:

Patient Profile				
ID:	DOB:		Age (years):	Gender: Boy ☐ Girl ☐
Height (m):	Weight (kg):	Dominant hand: L ☐ R ☐	Dominant Foot: L ☐ R ☐	Diagnosis:
Symptoms:	Hand weakness ☐	Daily trips and/or falls ☐	Sensory symptoms (e.g. pins and needles, tingling, numbness, prickling) ☐	
Support:	Nocturnal non-invasive ventilation ☐		Gastrostomy ☐	

Upper Limb Function			
1. Functional Dexterity Test (sec)		**2. Nine-hole peg test (sec)**	

Strength	Trial 1	Trial 2	Trial 3	Average
3. Hand grip (*N*)				x2*:
4. Shoulder internal rotation (*N*)				
5. Foot plantarflexion (*N*)				
6. Foot dorsiflexion (*N*)				

*Multiply grip strength by 2 if using citec dynamometer as per manufacturer's instructions.

Sensation	0	1	2	3	4	Score
7. Vibration	Normal	Reduced at first metatarsal bone	Reduced at ankle	Reduced at knee (tibial tuberosity)	Absent at knee and ankle	

Balance	**Assistive device required** (e.g. AFO) Y/N. Describe device and footwear:			
	Raw Score		*Conduct second trial only if examinee does not earn the maximum score (10 sec) on the first trial*	Best score
	Trial 1	**Trial 2**		
8. Standing with feet apart on a line with eyes open (10 sec)				

Lower Limb Function	**Assistive device required** (e.g. AFO) Y/N. Describe device and footwear:		
9. Gait	Foot drop: No ☐ Some ☐ Yes ☐	Difficulty heel walking: No ☐ Some ☐ Yes ☐	Difficulty toe walking: No ☐ Some ☐ Yes ☐
10. Long jump (cm)		**11. 30-second Chair Stand Test (#)**	**12. Six-minute walk test (m)**

Item Scores (0-4): Available for calculation at www.ClinicalOutcomeMeasures.org												Total Score (0-48)
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	

FIGURE 3 Final RTDPedS score sheet.

1000 Norms Project.[6,7] The individual items are then categorized on a 0–4 point scale with a 0 being a *z*-score above normal or between 0 and 1 *z*-scores below normal; 1 is between 1 and 2 *z*-scores below normal; 2 is between 2 and 3 *z*-scores below normal; 3 is between 3 and 4 *z*-scores below normal; 4 is more than 4 *z*-scores below normal for 10 of the 12 items. The vibration and gait items are scored on a 0–4 point scale as per the CMTPedS.[5] The items scores of the 12 items are then summed to give a total score out of 48 with 0 indicating no impairment and a 48 indicating severe impairment. A score of 0–16 is considered mild, 17–32 moderate and 33–48 severe. The median RTDPedS score from the five individuals who were pilot tested was 21/48 (range: 15–28).

4 | DISCUSSION

We have developed a functional outcome measure for children with RTD. The RTDPedS provides a standardised outcome measure that assesses strength, upper and lower limb function, balance and mobility. The RTDPedS grades level of disability in affected individuals to healthy age and sex-matched controls. It also provides a standardised approach to tracking disease progression and treatment response over time. We intend for this scale to be used to monitor the impact of riboflavin supplementation and for future clinical trials assessing novel treatments, including genetic therapies for RTD. The scale takes approximately 30 min to complete and can be scored on the freely available website www.clinicaloutcomemeasures.org. The RTDPedS Equipment and Training Resource Manual provides a detailed methodology for each item of the scale (eAppendix 1). Formal training and reliability assessments should be completed to ensure accurate measurement with this scale.

The RTDPedS can be used to monitor functional disease progression over time, however, there are other aspects of RTD that are not captured by the RTDPedS. Future clinical trials should also consider the use of spirometry for respiratory dysfunction, visual acuity testing for optic atrophy, audiology and speech perception tests for

sensorineural hearing loss, scoliosis assessment and blood tests including acetylcarnitine profile, haemoglobin and mean corpuscular volume to supplement the RTDPedS. Quality of life questionnaires could also be included similar to the paediatric CMT quality of life (CMT-QOL) outcome measure.[16]

There are limitations of this study that should be considered. Because of the small number of identified cases of RTD worldwide and the limited size of the cohort at our centre, we did not have the statistical power to perform well-established validation tests, for example, item, factor and Rasch analysis. Considering this, further studies using the RTDPedS will be required to confirm the reliability of our results, likely requiring an international effort. However, all items used in the RTDPedS have undergone validation studies for either CMT or healthy controls[5–8,17] and reflect the clinical impairments observed in RTD. Our study only included children with RTD2, however, it is expected that the results are relevant to children with RTD3 because of the very similar phenotype. The differences are mainly the frequency and age of onset of some of the features, the upper limb predominance of proximal weakness in RTD2 and the greater frequency of lower cranial nerve signs in RTD3 (facial and bulbar neuropathy).[4,18,19]

In conclusion, this study provides preliminary evidence that the Riboflavin Transporter Deficiency Pediatric Scale (RTDPedS) is a functional outcome measure covering strength, upper and lower limb function, balance and mobility for individuals with RTD to assess disease severity and progression in clinical trials and cohort studies.

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CONFLICT OF INTEREST STATEMENT

The listed authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DISCLOSURES

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REFERENCES

1. Bosch AM, Stroek K, Abeling NG, Waterham HR, Ijlst L, Wanders RJ. The Brown-Vialetto-Van Laere and Fazio Londe syndrome revisited: natural history, genetics, treatment and future perspectives. *Orphanet J Rare Dis*. 2012;7:83. doi:10.1186/1750-1172-7-83
2. Johnson JO, Gibbs JR, Megarbane A, et al. Exome sequencing reveals riboflavin transporter mutations as a cause of motor neuron disease. *Brain*. 2012;135(Pt 9):2875-2882. doi:10.1093/brain/aws161
3. Foley AR, Menezes MP, Pandraud A, et al. Treatable childhood neuronopathy caused by mutations in riboflavin transporter RFVT2. *Brain*. 2014;137(Pt 1):44-56. doi:10.1093/brain/awt315
4. Fennessy JR, Cornett KMD, Burns J, Menezes MP. Benefit of high-dose oral riboflavin therapy in riboflavin transporter deficiency. *J Peripher Nerv Syst*. 2023;28(3):308-316. doi:10.1111/jns.12587
5. Burns J, Ouvrier R, Estilow T, et al. Validation of the Charcot-Marie-tooth disease pediatric scale as an outcome measure of disability. *Ann Neurol*. 2012;71(5):642-652. doi:10.1002/ana.23572
6. McKay MJ, Baldwin JN, Ferreira P, et al. Normative reference values for strength and flexibility of 1000 children and adults. *Neurology*. 2017;88(1):36-43. doi:10.1212/WNL.0000000000003466
7. McKay MJ, Baldwin JN, Ferreira P, et al. Reference values for developing responsive functional outcome measures across the lifespan. *Neurology*. 2017;88(16):1512-1519. doi:10.1212/WNL.0000000000003847
8. Cornett KMD, Menezes MP, Shy RR, et al. Natural history of Charcot-Marie-tooth disease during childhood. *Ann Neurol*. 2017;82(3):353-359. doi:10.1002/ana.25009
9. Cornett KM, Menezes MP, Bray P, et al. Phenotypic variability of childhood Charcot-Marie-tooth disease. *JAMA Neurol*. 2016;73(6):645-651. doi:10.1001/jamaneurol.2016.0171
10. Cornett KMD, Menezes MP, Bray P, Halaki M, Burns J. Established and novel measures of upper limb impairment in children with Charcot-Marie-tooth disease type 1A and riboflavin transporter deficiency type 2. *J Peripher Nerv Syst*. 2018;23(1):29-35. doi:10.1111/jns.12245
11. Bruininks RH, Bruininks BD. *Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2)*. NCS Pearson; 2005.
12. McCarron LT. *MAND: McCarron Assessment of Neuromuscular Development, Fine and Gross Motor Abilities*. McCarron-Dial Systems; 1982.
13. Berard C, Payan C, Hodgkinson I, Fermanian J, Group MFMCS. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord*. 2005;15(7):463-470. doi:10.1016/j.nmd.2005.03.004
14. Franjoine MR, Gunther JS, Taylor MJ. Pediatric balance scale: a modified version of the berg balance scale for the school-age child with

mild to moderate motor impairment. *Pediatr Phys Ther*. 2003;15(2):114-128. doi:10.1097/01.PEP.0000068117.48023.18

15. Menezes MP, Farrar MA, Webster R, et al. Pathophysiology of motor dysfunction in a childhood motor neuron disease caused by mutations in the riboflavin transporter. *Clin Neurophysiol*. 2016;127(1):911-918. doi:10.1016/j.clinph.2015.05.012
16. Ramchandren S, Wu TT, Finkel RS, et al. Development and validation of the pediatric Charcot-Marie-tooth disease quality of life outcome measure. *Ann Neurol*. 2021;89(2):369-379. doi:10.1002/ana.25966
17. Brown T. Structural validity of the Bruininks-Oseretsky test of motor proficiency—second edition (BOT-2) subscales and composite scales. *Journal of Occupational Therapy, Schools, & Early Intervention*. 2019;12(3):323-353. doi:10.1080/19411243.2019.1590755
18. Jaeger B, Bosch AM. Clinical presentation and outcome of riboflavin transporter deficiency: mini review after five years of experience. *J Inherit Metab Dis*. 2016;39(4):559-564. doi:10.1007/s10545-016-9924-2
19. Woodcock IR, Menezes MP, Coleman L, et al. Genetic, radiologic, and clinical variability in Brown-Vialetto-van Laere syndrome. *Semin Pediatr Neurol*. 2018;26:2-9. doi:10.1016/j.spen.2017.03.001

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Chapter 5

Conclusion

5.1 Overview of the Main Findings of the Thesis

This thesis provides a comprehensive analysis of the functional and clinical response of individuals with Riboflavin Transporter Deficiency (RTD) to high-dose oral riboflavin supplementation. Early initiation of riboflavin supplementation is life saving and improves outcomes; however, affected individuls are left with residual disability. This thesis has also developed and trialled a functional outcome measure specific for use in children with RTD, and made recommendations for ongoing monitoring of disease progression. This chapter gives an overview of the main findings of this thesis and the wider implications of these findings.

5.1.1 Response to Riboflavin Supplementation

By reviewing both published cases in the literature and the data gathered from our own cohort of individuals with RTD, oral riboflavin supplementation was confirmed to be life saving. In our review, most individuals showed an overall improvement after the initiation of riboflavin supplementation, and there were no deaths in individuals who were initiated on riboflavin supplementation in comparison to reported death in 46% of those reported prior to identification of causative genes . Short-term improvement was seen in gross motor function, audiology, visual acuity, respiratory function, limb muscle strength and biochemical markers. Ongoing riboflavin therapy slows the progression of disease in the long term. Individuals who are commenced on riboflavin supplementation early after clinical disease onset had better outcomes compared to those who had a delay in diagnosis and therefore treatment initiation. Despite treatment, affected individuals are left with residual disability, and showed deterioration in some domains of function compared to age-and sex-matched controls. This emphasises the importance of early diagnosis and treatment initiation to prevent irreversible neuronal damage and functional impairment.

5.1.2 Development of the RTDPedS

Chapter Four of the thesis developed the Riboflavin Transporter Deficiency Pediatric Scale (RTDPedS), a functional outcome measure recommended for use in children with RTD to assess functional impairment over time compared to age- and sex-matched controls. The scale was developed by modifying the existing Charcot-Marie-Tooth disease Pediatric Scale

(CMTPedS).[1] Historical data gathered from a cohort of children with RTD who were assessed using the CMTPedS. 'Pinprick sensation' was removed from the scale due to its floor effect, and the balance item was replaced due to its ceiling effect. 'Shoulder internal rotation' was added to include an upper limb proximal muscle strength measure, and the '30-second sit to stand test' was added as a proximal lower limb functional task. The RTDPedS was pilot tested on two occasions in a group of five individuals with RTD. The RTDPedS provides a standardised approach to tracking treatment response and disease progression over time, targeted to the manifestations in functional impairments experienced by children with RTD.

5.1.3 Recommendations for Ongoing Monitoring

Chapter Three of this thesis presented the data gathered from a cohort of eleven individuals with RTD who were assessed during the period 1991 to 2022. Audiometry, visual acuity, CMTPedS scores, respiratory function, imaging and biochemical data are included in the analysis. This chapter also made recommendations for ongoing monitoring of disease progression and response to therapy. These recommendations include annual functional assessment using the RTDPedS, pure tone audiometry and speech discrimination tests, visual acuity assessment, spirometry, and serology.

5.2 Implications of the Thesis

The work contained within this thesis has implications for the assessment of children with RTD and ongoing research in the field.

The papers presented in Chapter Two and Three supports work done previously by other groups that confirms the benefit of high-dose oral riboflavin supplementation.[2-4] This thesis also provides evidence of reversal of some of the neuronal injury dysfunction when individuals are diagnosed and treated early.[4] The data presented in this thesis supports conclusions of other works emphasising the importance of early diagnosis and treatment in RTD to prevent irreversible neuronal damage.

The long-term outcome data presented in Chapter Three and the RTDPedS presented in Chapter Four provide valuable assessment tools and prognostic information. Newly

diagnosed individuals and their families will be able to receive prognostic information about the likely benefit from high-dose riboflavin depending on the specific modality of function, and severity of disease at treatment initiation. This thesis provides a standardised assessment protocol that is recommended for use at each follow-up clinic visits. This will standardise the monitoring of disease progression and early detection of treatable impairments associated with the disease such as scoliosis and obstructive sleep apnoea.

The RTDPedS developed in Chapter Four of this thesis serves as a functional outcome measure, an essential component of future clinical trials assessing the effectiveness of new disease-modifying therapies. Given the increased feasibility and current implementation of gene therapy in animal models in related neuropathies such as Charcot-Marie-Tooth disease[5], gene therapies provide great potential for improved outcomes in RTD. Advancing RTD research to the stage of clinical trial readiness will require further validation studies for the RTDPedS.

5.3 Limitations of the Thesis

There are limitations of this research that should be considered. RTD is a rare condition with an estimated incidence of one in one million and approximately one hundred genetically confirmed cases reported in the literature.[6,7] Therefore, the amount of data available for analysis and patient recruitment for cohort studies is limited. A larger patient group in Chapter Three of this thesis would have provided more diverse results and allowed for statistical analyses to be performed. In the same way, the pilot testing of the RTDPedS would have benefited from a cohort larger than the five individuals who were available from our centre. Formal validation of the RTDPedS could not be performed in this research considering we did not have the statistical power to perform well-established validation tests e.g. item, factor and Rasch analysis. Further validation of the RTDPedS will require studies in larger cohorts of affected individuals, likely requiring an international effort. Additionally, much of the research presented in this thesis is retrospective in nature. As a result, some variables could not be controlled for or monitored such as adherence to therapy. Additionally, missing data was inevitable in Chapter Three's case series, particularly prior to 2012 when affected individuals did not have genetic diagnoses. Finally, the participants included in Chapter Three and Four of this thesis had RTD2; our cohort did not include individuals with

RTD3. Further research with long-term follow up data and validation studies in individuals with RTD3 are required.

5.4 Future Directions

While studies looking into clinical outcomes of riboflavin therapy and the development of a functional outcome measure are important for clinical trial readiness, understanding the pathomechanisms underlying RTD are essential for identifying potential therapeutic targets. At the biochemical level, research focuses on the altered metabolic function within motor and sensory neurons caused by riboflavin deficiency in riboflavin. The altered energy metabolism causes an interplay of derangements in cytoskeletal formation, lipid metabolism, antioxidant defences, neuronal differentiation and organelle integrity.[8] In vivo models for RTD have been developed using the fruit fly (*D. melanogaster*)[9], a murine model (*M. musculus*)[10], and a roundworm model (*C. elegans*) as a low-cost and sustainable alternative[11]. Induced pluripotent stem cells (iPSCs) and iPSC-derived motor neurons from individuals with RTD have been very successful in demonstrating derangements in riboflavin transport, cytoskeletal pertubation and altered redox balance.[12-15] Human iPSC technologies offer great potential as in vitro models to study biochemical stress and discover therapeutic targets. An iPSC RTD model has been used to show reduced cellular oxidative stress and improved neurite length after exposure to the antioxidant EPI-743.[16] Intrinsic apoptotic pathways have been studied and identified as possible targets for effective therapeutic approaches.[17] While riboflavin supplementation has been shown to improve neurofilament expression pattern and reduce oxidative stress[12,14], disease-modifying agents such as antioxidants, apoptotic pathway mediators and other biochemical targets will be explored in further studies as they offer potential to improve outcomes and reduce residual disability burden. In addition, the combination of CRISPR-Cas9 gene editing with iPSC technology offers an exciting new era for the treatment of neurological conditions.[18]

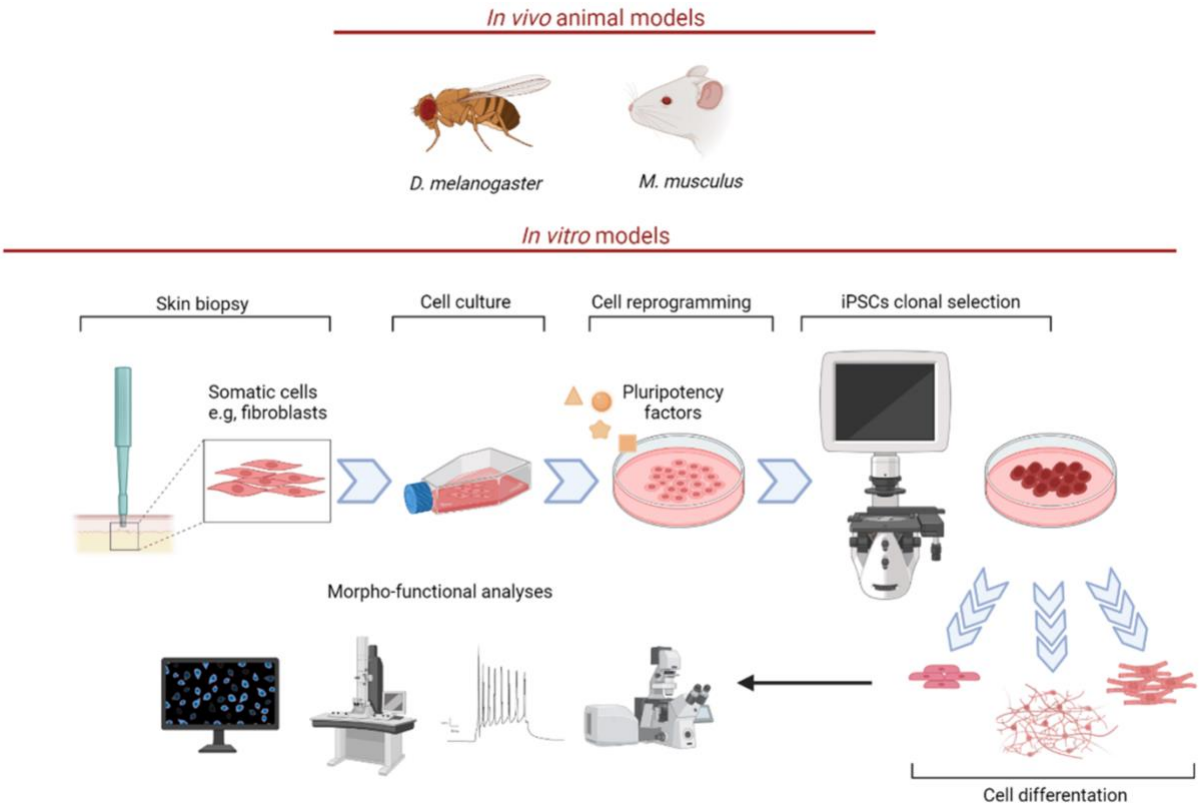


Figure 1. (Obtained from Colasuonno et al., 2022)[8]

RTD in vivo and in vitro models. Proper RTD animal models include M. musculus and D. melanogaster. Note that C. elegans was also used to mimic human RF metabolism deficiency. In vitro models include patient-specific fibroblasts and iPSCs, which can be differentiated into MNs. Indeed, skin biopsy-derived fibroblasts can be reprogrammed into iPSCs, thanks to the introduction of specific pluripotency factors. Human iPSCs may be differentiated into cell types. In the figure, as an example intestinal epithelium, muscle, and neurons, as representative derivatives from endoderm, mesoderm and ectoderm germ layers, are illustrated. Neurons may be studied using diverse morphofunctional approches (e.g., confocal and electron microscopy, electrophysiology).

Future studies using the RTDPedS will help to build the available data of long-term outcomes for individuals with RTD. Incorporating the RTDPedS and our surveillance recommendations into long-term follow-up for all children with RTD will enhance available data on long term outcomes. International collaboration on natural history studies in this population is essential. International collaboration will also provide power for statistical analyses and validation of the RTDPedS.

Finally, considering there have been siblings treated empirically in utero with oral riboflavin supplementation where there is a high index of suspicion for the condition and remained asymptomatic at follow up[19,20], the role of future newborn screening of pathogenic genetic variants can be considered.

5.5 Concluding Statement

This thesis has provided comprehensive data quantifying the effect of riboflavin supplementation in RTD and developed a functional outcome measure for use in children with RTD to ensure standardised assessment and clinical trial readiness.

5.6 References

1. Burns J, Ouvrier R, Estilow T, et al. Validation of the Charcot-Marie-Tooth disease pediatric scale as an outcome measure of disability. *Ann Neurol*. May 2012;71(5):642-52. doi:10.1002/ana.23572
2. Bosch AM, Stroek K, Abeling NG, Waterham HR, Ijlst L, Wanders RJ. The Brown-Vialetto-Van Laere and Fazio Londe syndrome revisited: natural history, genetics, treatment and future perspectives. *Orphanet J Rare Dis*. Oct 29 2012;7:83. doi:10.1186/1750-1172-7-83
3. Foley AR, Menezes MP, Pandraud A, et al. Treatable childhood neuronopathy caused by mutations in riboflavin transporter RFVT2. *Brain*. Jan 2014;137(Pt 1):44-56. doi:10.1093/brain/awt315
4. O'Callaghan B, Bosch AM, Houlden H. An update on the genetics, clinical presentation, and pathomechanisms of human riboflavin transporter deficiency. *J Inherit Metab Dis*. Jul 2019;42(4):598-607. doi:10.1002/jimd.12053
5. Stavrou M, Kagiava A, Sargiannidou I, Georgiou E, Kleopa KA. Charcot-Marie-Tooth neuropathies: Current gene therapy advances and the route toward translation. *J Peripher Nerv Syst*. Jun 2023;28(2):150-168. doi:10.1111/jns.12543
6. Cali E, Dominik N, Manole A, Houlden H. Riboflavin Transporter Deficiency. 2015 Jun 11 [Updated 2021 Apr 8]. GeneReviews® Accessed 10 Jul 2023. https://www.ncbi.nlm.nih.gov/books/NBK299312/
7. Fennessy JR, Cornett KMD, Burns J, Menezes MP. Benefit of high-dose oral riboflavin therapy in riboflavin transporter deficiency. *J Peripher Nerv Syst*. Sep 2023;28(3):308-316. doi:10.1111/jns.12587
8. Colasuonno F, Marioli C, Tartaglia M, Bertini E, Compagnucci C, Moreno S. New Insights into the Neurodegeneration Mechanisms Underlying Riboflavin Transporter Deficiency (RTD): Involvement of Energy Dysmetabolism and Cytoskeletal Derangement. *Biomedicines*. Jun 6 2022;10(6)doi:10.3390/biomedicines10061329
9. Manole A, Jaunmuktane Z, Hargreaves I, et al. Clinical, pathological and functional characterization of riboflavin-responsive neuropathy. *Brain*. Nov 1 2017;140(11):2820-2837. doi:10.1093/brain/awx231
10. Yoshimatsu H, Yonezawa A, Yao Y, et al. Functional involvement of RFVT3/SLC52A3 in intestinal riboflavin absorption. *Am J Physiol Gastrointest Liver Physiol*. Jan 2014;306(2):G102-10. doi:10.1152/ajpgi.00349.2013
11. Leone P, Tolomeo M, Piancone E, et al. Mimicking human riboflavin responsive neuromuscular disorders by silencing flad-1 gene in C. elegans: Alteration of vitamin transport and cholinergic transmission. *IUBMB Life*. Jul 2022;74(7):672-683. doi:10.1002/iub.2553
12. Colasuonno F, Niceforo A, Marioli C, et al. Mitochondrial and Peroxisomal Alterations Contribute to Energy Dysmetabolism in Riboflavin Transporter Deficiency. *Oxid Med Cell Longev*. 2020;2020:6821247. doi:10.1155/2020/6821247

13. Niceforo A, Marioli C, Colasuonno F, et al. Altered cytoskeletal arrangement in induced pluripotent stem cells (iPSCs) and motor neurons from patients with riboflavin transporter deficiency. *Dis Model Mech*. Jan 19 2021;doi:10.1242/dmm.046391
14. Colasuonno F, Bertini E, Tartaglia M, Compagnucci C, Moreno S. Mitochondrial Abnormalities in Induced Pluripotent Stem Cells-Derived Motor Neurons from Patients with Riboflavin Transporter Deficiency. *Antioxidants (Basel)*. Dec 9 2020;9(12)doi:10.3390/antiox9121252
15. Rizzo F, Ramirez A, Compagnucci C, et al. Genome-wide RNA-seq of iPSC-derived motor neurons indicates selective cytoskeletal perturbation in Brown-Vialetto disease that is partially rescued by riboflavin. *Sci Rep*. Apr 6 2017;7:46271. doi:10.1038/srep46271
16. Marioli C, Magliocca V, Petrini S, et al. Antioxidant Amelioration of Riboflavin Transporter Deficiency in Motoneurons Derived from Patient-Specific Induced Pluripotent Stem Cells. *Int J Mol Sci*. Oct 7 2020;21(19)doi:10.3390/ijms21197402
17. Marioli C, Muzzi M, Colasuonno F, et al. Caspase-dependent apoptosis in Riboflavin Transporter Deficiency iPSCs and derived motor neurons. *Cell Death Discov*. Jan 26 2024;10(1):54. doi:10.1038/s41420-024-01812-y
18. Paolini Sguazzi G, Muto V, Tartaglia M, Bertini E, Compagnucci C. Induced Pluripotent Stem Cells (iPSCs) and Gene Therapy: A New Era for the Treatment of Neurological Diseases. *Int J Mol Sci*. Dec 20 2021;22(24)doi:10.3390/ijms222413674
19. Elks N, Wilmshurst JM, Raga SV. Normal Outcome With Prenatal Intervention for Riboflavin Transporter Defect. *Pediatr Neurol*. Jul 2023;144:16-18. doi:10.1016/j.pediatrneurol.2023.04.004
20. Horoz OO, Mungan NO, Yildizdas D, et al. Brown-Vialetto-Van Laere syndrome: two siblings with a new mutation and dramatic therapeutic effect of high-dose riboflavin. *J Pediatr Endocrinol Metab*. Feb 2016;29(2):227-31. doi:10.1515/jpem-2015-0198

Appendices

Supplementary Table S1 ('Patient and clinical outcomes of included articles') from Chapter Two is presented on page 56. **Supplementary Data S1** can be found online in the 'Supporting Information' section at the end of the article on the publisher's website.

Appendix S1 ('Riboflavin Transporter Deficiency (RTDPedS) equiptment and training resource kit') from Chapter Four can be found on page 57. **eTable 1** ('Medline search strategy') can be found on page 76. **eTable 2** ('Items included in selected outcome measures') can be found on page 77. **eTable 3** ('Patient demographics') can be found on page 78.

Supplementary Table 1: Patient and clinical outcomes of included articles

Article	RTD2	RTD3	No. of patients	Audiometry	Ataxia	Weakness	Cranial Nerve Palsy	Bulbar Palsy	Visual Acuity	Respiratory Function	GMFCS
Bosch, 2011		✓	2			✓	✓			✓	✓
Ciccoclella, 2012		✓	3	✓		✓				✓	✓
Johnson, 2012	✓		1	✓		✓			✓		✓
Anand, 2012		✓	1			✓				✓	✓
Haack, 2012	✓		1		✓						
Foley, 2014	✓		14	✓	✓	✓		✓		✓	✓
Spagnoli, 2014		✓	1			✓	✓	✓		✓	✓
Cosgrove, 2015		✓	1				✓	✓		✓	
Davis, 2016		✓	1	✓		✓				✓	✓
Horoz, 2016		✓	2				✓			✓	✓
Menezes, 2016	✓		2	✓		✓				✓	
Cirali, 2017	✓		1			✓				✓	
Petrovski, 2015	✓		1		✓	✓		✓			
Guissart, 2016	✓		2								
Udhayabanu, 2016	✓	✓	1			✓					
Allison, 2017	✓	✓	3	✓	✓	✓				✓	
Bashford, 2017		✓	1				✓	✓		✓	✓
Khadikar, 2017		✓	1	✓	✓	✓		✓			✓
Thulasi, 2017		✓	1	✓	✓	✓	✓	✓		✓	
Camargos, 2018		✓	1				✓	✓		✓	✓
Chaya, 2018		✓	1		✓		✓	✓		✓	
Gowda, 2018		✓	2				✓	✓		✓	
Nimmo, 2018		✓	1							✓	✓
Set, 2018	✓		2								
Woodcock, 2018	✓	✓	2	✓		✓			✓		✓
Abbas, 2018		✓	1				✓			✓	✓
Bamaga, 2018	✓		2	✓	✓	✓		✓	✓		
Fan, 2018	✓		1		✓						
Forman, 2018	✓		1					✓		✓	✓
Garg, 2018		✓	1	✓			✓	✓			
Anderson, 2019	✓	✓	4	✓	✓	✓		✓		✓	
Gorcenco, 2019	✓		1		✓				✓		✓
Mutlu, 2019		✓	1	✓				✓			
Shi, 2019	✓		1	✓		✓		✓		✓	✓
Carreau, 2020	✓	✓	7	✓	✓	✓		✓	✓	✓	✓
Kranthi, 2020	✓		1								
Pillai, 2020	✓		1		✓			✓	✓		✓
Carey, 2021		✓	3	✓	✓		✓	✓			
Frederick, 2021		✓	1			✓		✓		✓	
Gayathri, 2021		✓	4			✓					
Khani, 2021		✓	7								
Liu, 2021	✓		1			✓					✓
Omar, 2021	✓	✓	3	✓	✓	✓			✓		
Yilmaz, 2021	✓		1					✓		✓	✓
do Amaral, 2022		✓	1	✓							
Gedik, 2022		✓	1	✓	✓	✓		✓	✓	✓	✓
Naami, 2022	✓		1								✓

rtdpediatricscale

Riboflavin Transporter Deficiency Pediatric Scale (RTDPedS) equipment and training resource kit

rtdpediatricscale

Contents

RTDPedS

Initial evaluation ☐ Re-Evaluation ☐ Date:

Patient Profile				
ID:	DOB:		Age (years):	Gender: Boy ☐ Girl ☐
Height (m):	Weight (kg):	Dominant hand: L ☐ R ☐	Dominant Foot: L ☐ R ☐	Diagnosis:
Symptoms:	Hand weakness ☐	Daily trips and/or falls ☐	Sensory symptoms (e.g. pins and needles, tingling, numbness, prickling) ☐	
Support:	Nocturnal non-invasive ventilation ☐		Gastrostomy ☐	

Upper Limb Function			
1. Functional Dexterity Test (sec)		**2. Nine-hole peg test (sec)**	

Strength	Trial 1	Trial 2	Trial 3	Average
3. Hand grip (*N*)				x2*:
4. Shoulder internal rotation (*N*)				
5. Foot plantarflexion (*N*)				
6. Foot dorsiflexion (*N*)				

*Multiply grip strength by 2 if using citec dynamometer as per manufacturer's instructions.

Sensation	0	1	2	3	4	Score
7. Vibration	Normal	Reduced at first metatarsal bone	Reduced at ankle	Reduced at knee (tibial tuberosity)	Absent at knee and ankle	

Balance	**Assistive device required** (e.g. AFO) Y/N. Describe device and footwear:			
	Raw Score		*Conduct second trial only if examinee does not earn the maximum score (10 sec) on the first trial*	Best score
	Trial 1	**Trial 2**		
8. Standing with feet apart on a line with eyes open (10 sec)				

Lower Limb Function	**Assistive device required** (e.g. AFO) Y/N. Describe device and footwear:		
9. Gait	Foot drop: No ☐ Some ☐ Yes ☐	Difficulty heel walking: No ☐ Some ☐ Yes ☐	Difficulty toe walking: No ☐ Some ☐ Yes ☐
10. Long jump (cm)		**11. 30-second Chair Stand Test (#)**	**12. Six-minute walk test (m)**

Item Scores (0-4): Available for calculation at www.ClinicalOutcomeMeasures.org												Total Score (0-48)
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	

rtdpediatricscale

List of Equipment

Item

1. **Functional Dexterity Test:** Functional Dexterity Test and digital stopwatch
2. **Nine Hole Peg Test:** Jamar® 9-Hole Peg Test Kit and Digital stopwatch
3. **Grip strength:** Citec hand-hand dynamometer with grip strength applicator, spare batteries (6V) C.I.T. Technics, Haren, The Netherlands: www.citec.nu
4. **Shoulder Internal Rotation Strength:** As above, Citec hand-hand dynamometer, spare batteries (6V)
5. **Plantarflexion strength:** As above, Citec hand-hand dynamometer, spare batteries (6V)
6. **Dorsiflexion strength:** As above, Citec hand-hand dynamometer, spare batteries (6V)
7. **Vibration:** Rydel Seiffer tuning fork, C 64 Hz / c 128 Hz detachable clamps, Arno Barthelmes & Co. GmbH - Tuttlingen – Germany: www.barthelmes.info
8. **Balance:** Digital stopwatch and tape to mark a line (or designate a line on the flooring)
9. **Gait** (not applicable)
10. **Long Jump**: Tape measure (150 cm)
11. **30-Second Chair Stand Test:** Height adjustable bench, http://kayeproducts.com/kaye-adjustable-benches/ and Digital stopwatch
12. **Six-minute walk test:** Two small markers, lap counter, stopwatch, tape measure (30 m)

General Instructions

- Individuals should be instructed to wear comfortable clothing.
- The RTDPedS can be collected in approximately 30 minutes, with training and scoring against normative reference values[1,2] available online via www.ClinicalOutcomeMeasures.org.

Strength Assessments

- Grip strength, shoulder internal rotation, ankle dorsiflexor and ankle plantar flexor strength of the dominant side will be assessed using the Citec hand-held dynamometer. The device should be calibrated before use following the procedures found at the end of the manual.
- The maximum force that can be applied to the Citec is 500 N.
- Perform up to 5 trials; record 3 valid trials which should reflect maximum strength.
- A 10 second rest is given between trials.

Lower extremity function, balance and mobility items of the RTDPedS

- 6-minute walk test, long jump, gait, and balance tasks should be performed barefoot, unless prohibited by your institution, in which case they should wear a standard shoe and document. If assistive devices are absolutely required for any of the items (e.g. ankle-foot orthoses or a walker) the item can still be performed for clinical observation/purposes, however the lowest possible raw value (0 for myometry, balance, long jump, 30-second sit to stand, 6 minute walk test, or yes for all of the gait items) should be given, corresponding with an item score of 4.

- Timed items should be recorded to the nearest tenth of a second (1 decimal point).

Item Instructions

1. Functional Dexterity Test

Background/Purpose: The Functional Dexterity Test (FDT) is a measure of hand dexterity that provides information regarding the use of the fingers and hand for daily tasks requiring 3-jaw chuck prehensions e.g. buttoning, tying shoe laces, screwing a nut and bolt. The FDT has demonstrated good validity and reliability in healthy controls[3,4] and adults with CMT.[5] It has been correlated to light touch-pressure sensation as tested via the Semmes Weinstein monofilament.[6]

Test Position: The child is seated comfortably on a chair with their feet supported on the ground, or by a stool if necessary with their elbows in 90° of flexion, in front of the pegboard. The FDT pegboard is placed on a table, at navel height, in front of him/her with the proximal edge placed 10 cm from the edge of the table. The test may be performed with either the black or white side of the pegs facing upwards.

Testing Procedures: The clinical evaluator explains to each child that the purpose of the test is to turn over the pegs as fast as possible with their dominant hand. The child is instructed to start at the top at the opposite side of the board to the hand and continue in a zigzag manner (left to right and right to left, or vice versa). The clinical evaluator demonstrates by turning over 4 pegs. The child is given a practice trial of turning over 4 pegs. The dominant hand is assessed. It is important to demonstrate that the wrist should not supinate past the neutral position when manipulating the peg.

The evaluator says,

> "This is an assessment of your hand and finger function. I want you to turn over all the pages as quickly as possible one at a time using one hand. I don't want you to turn your hand towards the ceiling, touch the board for help in turning the peg, touch the peg with your other (free) hand, allow the peg to touch your chest, or drop a peg. Here, I want you to watch me and then you can practice."

After the practice trial the clinical evaluator says:

> "I want you to turn over the pegs as fast as you can. "Are you ready?" "Ready, set, go"

Scoring: The evaluator starts the stopwatch at the word 'go' and stops the stopwatch when the child releases the last peg. The time is recorded in seconds to the nearest tenth of a second.

For those unable to perform the FDT due to RTD severity or are very slow, give a score of **150 seconds** which is greater than the maximum childhood RTD value.

Note: No time penalties are used in this protocol. If the participant supinates or touches the board the timer is paused, peg returned to the unturned position and the assessment resumed. If a peg is dropped the timer is paused, the participant retrieves the peg and places it in the unturned position, and then continues to turn the pegs, starting with the returned peg. The timer is re-started from the point it was stopped (not reset and restarted). This repeated effort would capture a time penalty within itself.[7]

2. Nine Hole Peg Test

Background/Purpose: The nine-hole peg test is used to examine fine motor ability, dexterity and hand/eye coordination as a measure of hand function. The nine-hole peg test has been shown to be highly repeatable and valid in healthy children and adults with CMT.[8-11]

Test Position: The participant is seated comfortably at a desk with their feet supported on the floor or stool. The Nine-Hole Peg Test is placed 10 cm from the edge of the table at navel height, centered in front of the participant with the container for the pegs on the same side as the hand being assessed. The dominant hand is assessed, while the non-dominant hand stabilizes the peg board using fingers placed on the small extension of the board.

Testing Procedures: The evaluator demonstrates with 3 pegs. The child is given a practice trial with 3 pegs using their dominant hand.

The evaluator says,

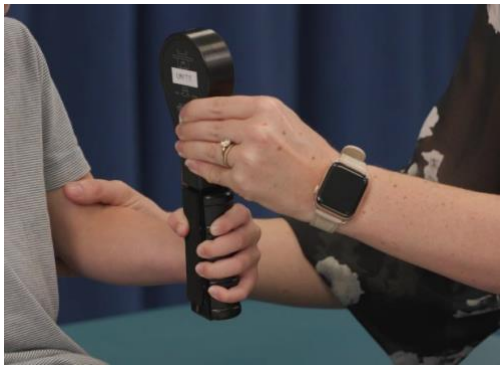
> "This is a timed test to see how fast you can put the pegs in and then take them back out. Pick up each peg one at a time. I want you to do the best that you can. "Are you ready?" "Ready, set, go"

Scoring: The timer is started when the evaluator says 'go' and ends when the last peg is returned to the container. If a peg is dropped off of the board, stop the timer and replace the peg to its initial position. The participant may "fumble" the peg on the board but can keep going if they are able to without too much delay (<1 sec). The time it takes, in seconds, to insert all nine pegs and remove them again is recorded.

For those unable to perform the nine-hole peg test due to RTD severity or are very slow, give a score of **150 seconds** which is greater than the maximum childhood RTD value.

3. Grip strength

Background/Purpose: Grip strength is quantified using the Citec hand-held dynamometer. Please note, the grip applicator must be screwed on all of the way, this may result in the visual display facing in different directions. In addition, only use the grip applicator that came with the dynamometer, these are not interchangeable between devices, as they impact the reliability of measurement. Quantitative muscle testing of hand strength using standardized procedures has been shown to be highly reliable and valid in children and adults with a variety of neuromuscular conditions.[8,10, 12-14]



Test Position: The child is asked to sit in a chair with their feet supported. The testing arm is positioned with the shoulder adducted and in neutral rotation, elbow flexed to 90 degrees, forearm in neutral with the wrist between 0 and 30 degrees of extension and 0 to 15 degrees of ulnar deviation.

Testing Procedure: Three *valid* trials of 3 to 5 seconds of the dominant hand is recorded and averaged. The child is asked to grasp the hand-held dynamometer with the fingers wrapped around the handle. The child is instructed to squeeze the handle as hard as they can.

Please note, the grip applicator must be screwed on all of the way, this may result in the visual display facing in different directions. If the visual display is facing the child, the evaluator can block the view with their hand to eliminate any distraction/motivation factor.

The clinical evaluator says:

> "I am now going to measure your grip strength. I want you to squeeze the device as hard as you can for several seconds. Ready, set, go."

Scoring: Three (3) valid measures of the dominant hand are recorded to the nearest Newton. In accordance with the Citec hand-held dynamometer manufacturer's instructions, the displayed grip values must be **multiplied by 2**, as the grip applicator measures strength in a 1:2 ratio.

For those unable to perform grip strength due to RTD severity or are very weak, score **0 Newtons**.

4. Shoulder Internal Rotation strength

Background/Purpose: Shoulder Internal Rotation sis quantified using the Citec hand-held dynamometer. Using standardized procedures the reliability and validity of using hand-held dynamometry to measure shoulder internal rotation strength has been shown to be acceptable in children and adults.[1]

Hand-held dynamometer set-up:
The hand-held dynamometer can be used with the small square or concave attachment. Choose according to size/shape of the forearm and comfort for the participant. The hand-held dynamometer should be zeroed before placing it against the participant's forearm.

Test Position: The participant is seated upright in a chair, feet supported, shoulder in neutral, elbow in 90° of flexion, and forearm in neutral. The examiner places the HHD against the flexor surface of the forearm, just proximal to the wrist crease.

Testing Procedures: Each child is assessed using the "make" test, whereby the assessor holds the hand-held dynamometer stationary while the child exerts a maximal force against it. The maximum effort should be recorded at the available mid-range position of the foot. A resting period of 10 seconds in between each contraction is given. Standardized verbal encouragement is used.

The examiner says:

> "I am now going to take another measure of your shoulder strength. I want you to push against the device as if trying to rotate your arm inwards as hard as you can for several seconds. Ready, set, go."

Scoring: Three *valid* trials of 3 to 5 seconds are recorded and averaged.

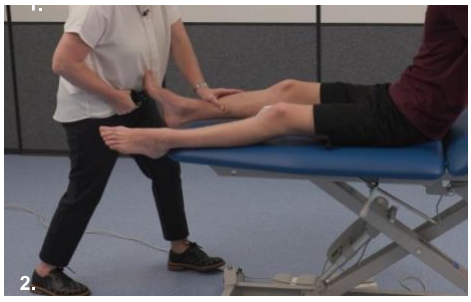
For those unable to perform shoulder internal rotation strength due to RTD severity or are very weak, score **0 Newtons**.

5. Plantarflexion strength
6. Dorsiflexion strength

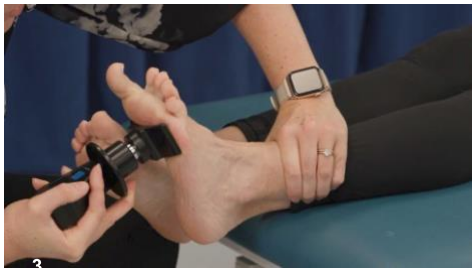
Background/Purpose: Plantarflexion and dorsiflexion strength is quantified using the Citec hand-held dynamometer. Using standardized procedures the reliability and validity of using hand-held dynamometry to measure foot strength has been shown to be acceptable in children[15] and adults.[16]

Hand-held dynamometer set-up:
The hand-held dynamometer can be used with the small square or concave attachment. Choose according to size/shape of the foot and comfort for the participant. The hand-held dynamometer should be zeroed before placing it against the participant's foot.

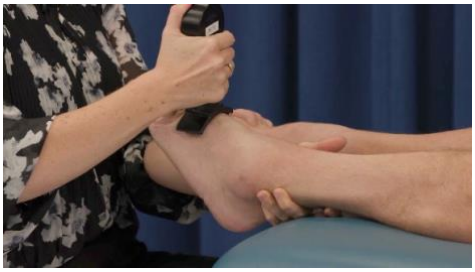
Test Position: The child is seated with hips flexed and knees comfortably extended (long sitting) with their heel off the edge of the plinth. Evaluate the available ROM of the ankle joint. The examiner stabilizes the participant's shank (lower leg) against the plinth with one hand. Be certain not to squeeze the muscle that you are testing when stabilizing the lower leg. With the other hand the examiner positions the foot in mid-range.
The dynamometer is positioned according to the muscle group being tested.



- **Plantarflexion:** The hand-held dynamometer is positioned against the plantar surface of the foot just proximal to the metatarsal heads (Figure 1 & 2).



- **Dorsiflexion:** The hand-held dynamometer is positioned against the dorsal surface of the foot just proximal to the metatarsal heads (Figure 3).



Testing Procedures: Each child is assessed using the "make" test, whereby the assessor holds the hand-held dynamometer stationary while the child exerts a maximal force against it. The maximum effort should be recorded at the available mid-range position of the foot. A resting period of 10 seconds in between each contraction is given. Standardized verbal encouragement is used.

Scoring: Three *valid* trials of 3 to 5 seconds for each muscle group of the dominant foot is recorded and averaged.

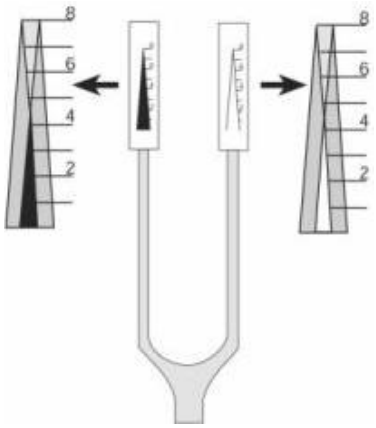
For those unable to perform plantarflexion or dorsiflexion strength due toRTD severity or are very weak, score **0 Newtons**.

7. Vibration

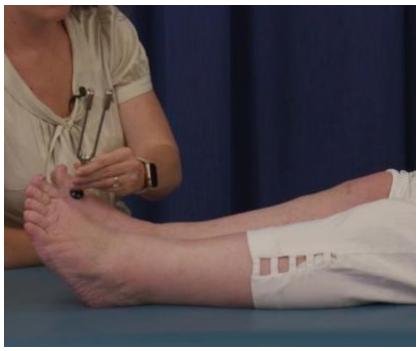
Test Position: Child should be in long sitting. Dominant lower limb only is tested. All children should have their eyes closed during the test, in order to help them keep focus throughout the exam. The clinical evaluator should not hold on to the child's leg or foot.

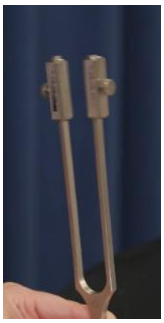
Testing Procedures: Determine if the child is able to feel the tuning fork vibrating on a bony region with "expected" normal sensation (collar bone/forehead). Set the tuning fork into motion by compressing the prongs (tines) in a finger snapping motion. As the prongs start to oscillate, the illusion of two triangles is visible on each damper. The child is asked to indicate the moment when they can no longer perceive the decreasing vibratory stimulus. Read off the black triangle. If the child does not feel any vibration, score 4 and go to the next test. If the child does feel vibration, the test will be performed in the lower limbs (distal-to-proximal direction) starting at the first metatarsal bone. If a score of 5 or higher is obtained, stop and score the item "0" Normal. Any score less than 5 is considered "reduced" and the exam should proceed proximally until a score of 5 or more is reported and the item is graded according to the 4 "reduced" levels described below:

0 Normal ($\geq$5)[17]
1 Reduced at first metatarsal bone
2 Reduced at ankle (medial malleolus)
3 Reduced at knee (tibial tuberosity)
4 Absent at knee and ankle



Note: Only The Reidell Seiffer Tuning Fork in the equipment list should be used as not all tuning forks are calibrated the same way.





8. Balance: Standing with feet apart on a line-eyes open

Set-up:

- Place a target on the wall at the appropriate level for the participant's height, with the bottom of the target at the participant's eye level.
- Using a piece of tape, make a straight line on the floor
- Conduct a second trial only if the participant does not earn the maximum score on their first trial.
- The entire foot should be placed along the line, unless foot deformity precludes this testing position e.g. if foot is fixed in abduction they may use this position for testing.
- The participant must attain the starting position on their own. They may not use a wall or lean on the clinical evaluator for stability.

I----------17 feet (5.2meters) ----------I

Procedure:

- Child stands with feet together, dominant foot on and parallel to the line
- Child places hands on hips
- Child takes one natural step forward, placing non-dominant foot on and parallel to line, and looks at the target

Scoring:

- Record number of seconds, to nearest tenth of a second, that the child maintains proper form, up to 10 seconds
- Stop trial after 10 seconds or if the child steps off line or fails to keep hands on hips

Administration:

- Teach the task to the child. Then, say, "Stand on the line with your feet apart until I tell you to stop. Ready? Begin."
- Begin timing *when the child attains proper form under control*. After 10 seconds or when the child breaks proper form, say, "Stop."
- If the child does not earn the maximum score of 10 seconds, conduct the second trial. If necessary, re-teach the task after you say," Let's try it again."

For those unable to perform balance item (including getting into the test position), a score of **0 seconds** is given.

8. Gait

Background/Purpose: Difficulty toe walking is a gross indicator of plantarflexion weakness, difficulty heel walking is a gross indicator of dorsiflexion weakness (and Achilles tendon shortening), and the presence of foot drop is a sign of dorsiflexion weakness during gait.[18]

Testing procedure:

i. **Foot Drop:** The child is asked to walk 10 steps and the clinical evaluator assesses the presence of foot drop: Note, foot drop is defined as plantarflexion during the mid-swing phase of gait, often with a forefoot strike/slap on loading.

If the child demonstrates NO forefoot strikes/slaps on all steps a score of no is recorded.
If the child demonstrates ANY (1-9) forefoot strikes/slaps during gait or lands flat-footed, a score of some is recorded.
If the child demonstrates forefoot strikes/slaps on ALL (10) steps, a score of yes is recorded.

Detecting foot drop can be subtle, so ask the participant to walk additional steps or confirm during the Six-Minute Walk Test if needed

ii. **Difficulty heel walking:** The child is then asked to heel walk 10 steps: Note, the toes, forefoot, medial, and lateral border all need to clear the floor to count as a successful heel walk.

If the child demonstrates NO difficulty heel walking, a score of no is recorded.
If the child demonstrates difficulty heel walking on ANY step (1-9), a score of some is recorded.
If the child demonstrates difficulty heel walking on ALL (10) steps, a score of yes is recorded.

iii. **Difficulty toe walking:** The child is then asked to tip-toe walk for 10 steps: Note, weight must be over toes/forefoot without any arch/heel contact on ground to count as successful tip-toe walk

If the child demonstrates NO difficulty tip-toe walking, a score of no is recorded.
If the child demonstrates difficulty tip-toe walking on ANY step (1-9), a score of some is recorded.
If the child demonstrates difficulty tip-toe walking on ALL (10) steps, a score of yes is recorded.

9. Long Jump

Background/Purpose: Long Jump is a reliable measure of power and coordination in children.[19]

Test Position: Long jump should be performed barefoot and without an assistive device. If assistive devices are required (e.g. AFO) the test can be performed, however a raw score of 0 should be given. Teach the task to the child and allow practice. All tests should be performed one time only, unless invalid.

Testing Procedures: The child starts standing with the feet even, shoulder width apart behind a taped line on a carpeted floor or firm cushioned mat. The child is instructed to jump as far forward as they can, taking off and landing with both feet. The distance from the start line to the point where the **heel of the foot nearest** to the start line touched the floor is measured using a tape measure.[20] One practice trial is given.

For those unable to perform long jump due to RTD severity, score **0 centimeters**.

10. 30-Second Chair Stand Test

Background/Purpose: The 30-Second Chair Stand Test is used by researchers and clinicians as an assessment of functional lower limb strength.14 Using a standardized protocol, which includes one practice stand by the participant, the 30-Second Chair Stand Test has demonstrated good inter-tester and intra-tester reliability in adult populations.

Assessment Position: A height adjustable flat chair/bench is placed against a wall. The participant sits comfortably, with their sacrum in the middle of the chair. The height of the seat is adjusted so that the participant sits with their hips and knees flexed at 90° and their feet hip width apart. The participant is asked to cross their arms across their chest (hands at shoulders), and not to rest against the back of the chair or wall during the assessment. They are not allowed to use their arms to generate momentum or to push up on the armrests, chair seat or any part of their body and must extend fully when standing. The clinical evaluator stands to the side of the participant holding a stopwatch.

Assessment Procedure: The evaluator demonstrates the task and then the participant is asked to perform one practice stand. The participant is then asked to perform as many repetitions as possible in 30 seconds.

The clinical evaluator says:
"This assessment records how many times you can sit and stand as quickly as possible from the chair in 30 seconds. You must fully sit down with your bottom touching the seat and stand up for the repetition to be counted. When I say go you will start the assessment, you can stop at any time as you need to. Ready, set, go."

If they do not come to a fully extended (erect) position, remind them that they need to come to a fully extended position for the repetition to count.

Scoring: Repetitions where the arms are used for support are not counted. The number of full sit to stands performed in the 30 seconds is recorded. If the timer reaches 30 seconds when the participant is in full stand, it is counted as this test is measuring the number of sit to stand transitions. For those unable to perform this item due to disease severity, **score 0 repetitions.**

11. Six-minute walk test

Background/Purpose: The 6-minute walk test (6MWT) is a clinical measure of submaximal functional endurance capacity[21, 22] The 6MWT is administered according to the American Thoracic Society guidelines. Lack of motivation and understanding can affect the 6MWT performance in children so clear instructions and consistent encouragement at minute intervals are required to ensure test accuracy, feasibility, and safety.

Set-up: The 6MWT is performed indoors, along a long, flat, straight, firm surface. The walking course is 25 meters long and the turnaround points are marked with cones (with the edge on the inside of the course. The direction the participant turns has no effect on the outcome. The 6MWT is performed by one child at a time to preclude any influence of competition between children.

Testing Procedures: Tests should be performed barefoot, unless prohibited by your institution, in which case they should wear a standard shoe and document. If assistive devices are required (e.g. AFO) the test can be performed, however a raw score of 0 should be given. Before the start of the 6MWT, the child sits and rests for 3 minutes. Children are instructed to walk as many laps as possible in 6 minutes without running. Briefly demonstrate the process of walking around the cones. A practice trial is not given for this lengthy test because of concerns about the impact of associated fatigue, and in light of evidence that a familiarization trial does not enhance reliability.[23]

The clinical evaluator says:

> "This test measures how far you can walk in six minutes. When I say go I want you to walk as fast as you comfortably can, without running, for six minutes. I will count your laps and tell you how much time is remaining at each minute. You can stop to take rests if you need to and then keep going, or you can cease the assessment if you can't go any further."

The evaluator asks the child to stand and place his or her toes at the starting line. When the child is ready, the clinical evaluator says, ''Ready, set, go,'' and starts the stopwatch.

At each minute-split, the timer announces the number of minutes completed and the number remaining with standardized encouragement (Ex: you are doing well, keep up the good work).

Each time the child rounds a cone, record the lap on the tally (lap) counter. Parent should not accompany child, but an evaluator can follow behind. The cumulative distance to the nearest lap marker is recorded and any remaining distance is measured to the nearest meter.

Caution: Bandage any loose callosities, blisters, wounds to avoid further trauma. If a fall occurs ensure the child is unharmed, and assist them back to a standing position and resume.

For those unable to perform the 6MWT due to RTD severity or are very weak, score **0 meters**.

Patient Profile Instructions

Complete *ID, DOB, Age, Gender, Height, Weight, Dominant Hand/Foot, Diagnosis*

Symptoms
Each child/parent is asked about the presence or absence of each symptom and marked as present or absent.

Support
Each child/parent is asked about the presence or absence of each symptom and marked as present or absent.

Calibration Tasks

Citec hand-held dynamometer

At least every 3-months, check force readings with a known weight which has been verified by a biomedical department or calibrated scales.

Using the technique shown whereby a 5 kilogram known weight (49 Newtons) is applied to the Citec hand-held dynamometer.

For alternate weight, use Force Convertor website: www.unitconversion.org/unit_converter/force.html

References

1. McKay MJ, Baldwin JN, Ferreira P, et al. Normative reference values for strength and flexibility of 1,000 children and adults. Neurology 2017; **88**(1): 36-43.
2. McKay MJ, Baldwin JN, Ferreira P, et al. Reference values for developing responsive functional outcome measures across the lifespan. Neurology 2017; **88**(16): 1512-9.
3. Aaron DH, Jansen CW. Development of the Functional Dexterity Test (FDT): construction, validity, reliability, and normative data. *Journal of Hand Therapy* 2003; **16**(1): 12-21.
4. Schoneveld K, Wittink H, Takken T. Clinimetric evaluation of measurement tools used in hand therapy to assess activity and participation. *Journal of Hand Therapy* 2009; **22**(3): 221-35.
5. Videler AJ, Beelen A, van Schaik IN, de Visser M, Nollet F. Manual Dexterity in Hereditary Motor and Sensory Neuropathy Type 1A: Severity of Limitations and Feasibility and Reliability of Two Assessment Instruments. *Journal of Rehabilitation Medicine* 2008; **40**(2): 132-6.
6. Melchior H, Vatine JJ, Weiss PL. Is there a relationship between light touch-pressure sensation and functional hand ability? *Disability & Rehabilitation* 2007; **29**(7): 567-75.
7. Lee-Valkov PM, Aaron DH, Eladoumikdachi F, Thornby J, Netscher DT. Measuring normal hand dexterity values in normal 3-, 4-, and 5-year-old children and their relationship with grip and pinch strength. *Journal of Hand Therapy* 2003; **16**(1): 22-8.
8. Burns J, Bray P, Cross L, North KN, Ryan MM, Ouvrier RA. Hand involvement in children with Charcot-Marie-Tooth disease type 1A. *Neuromuscular Disorders* 2008; **18**(12): 970-3.
9. Poole JL, Burtner PA, Torres TA, et al. Measuring dexterity in children using the Nine-hole Peg Test. *J Hand Ther* 2005; **18**(3): 348-51.
10. Solari A, Laura M, Salsano E, Radice D, Pareyson D. Reliability of clinical outcome measures in Charcot-Marie-Tooth disease. *Neuromuscul Disord* 2008; **18**(1): 19-26.
11. Svensson E, Hager-Ross C. Hand function in Charcot Marie Tooth: test retest reliability of some measurements. *Clin Rehabil* 2006; **20**(10): 896-908.
12. Escolar DM, Henricson EK, Mayhew J, et al. Clinical evaluator reliability for quantitative and manual muscle testing measures of strength in children. *Muscle Nerve* 2001; **24**(6): 787-93.
13. Mayhew JE, Florence JM, Mayhew TP, et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. *Muscle Nerve* 2007; **35**(1): 36-42.
14. Merlini L, Mazzone ES, Solari A, Morandi L. Reliability of hand-held dynamometry in spinal muscular atrophy. *Muscle Nerve* 2002; **26**(1): 64-70.
15. Rose KJ, Burns J, Ryan MM, Ouvrier RA, North KN. Reliability of quantifying foot and ankle muscle strength in very young children. *Muscle & Nerve* 2008; **37**(5): 626-31.
16. Burns J, Redmond A, Ouvrier R, Crosbie J. Quantification of muscle strength and imbalance in neurogenic pes cavus, compared to health controls, using hand-held dynamometry. *Foot Ankle Int* 2005; **26**(7): 540-4.
17. Hilz MJ, Axelrod FB, Hermann K, Haertl U, Duetsch M, Neundorfer B. Normative values of vibratory perception in 530 children, juveniles and adults aged 3-79 years. *Journal of the Neurological Sciences* 1998; **159**(2): 219-25.
18. Burns J, Ryan MM, Ouvrier RA. Evolution of foot and ankle manifestations in children with CMT1A. *Muscle & Nerve* 2009; **39**(2): 158-66.
19. Castro-Pinero J, Gonzalez-Montesinos JL, Mora J, et al. Percentile values for muscular strength field tests in children aged 6 to 17 years: influence of weight status. *J Strength Cond Res* 2009; (8): 2295-310.

20. Rose KJ, Burns J, North KN. Relationship between foot strength and motor function in preschool-age children. *Neuromuscular Disorders* 2009; **19**(2): 104-7.
21. Lammers AE, Hislop AA, Flynn Y, Haworth SG. The six-minute walk test: Normal values for children of 4 -11 years of age. *Arch Dis Child* 2008; **93**(6): 464 - 8.
22. Geiger R, Strasak A, Treml B, et al. Six-Minute Walk Test in Children and Adolescents. *Journal of Pediatrics* 2007; **150**(4): 395-9.e2.
23. Thompson P, Beath T, Bell J, et al. Test-retest reliability of the 10-metre fast walk test and 6-minute walk test in ambulatory school-aged children with cerebral palsy. *Dev Med Child Neurol* 2008; **50**(5): 370-6.

eTable 1. Medline search strategy

A. Measurement terms combined with 'OR'	B. Population terms combined with 'OR'	C. Function terms combined with 'OR'
Measure*[Title]	Child*[Title/Abstract]	Motor function*[Title/Abstract]
Tool*[Title]	Pediatric*[Title/Abstract]	Gross motor[Title/Abstract]
Scale*[Title]	Paediatric*[Title/Abstract]	Balance[Title/Abstract]
Score*[Title]	Adolescent*[Title/Abstract]	Strength[Title/Abstract]
Assess*[Title]		Disability[Title/Abstract]
Results of searches A, B and C combined with "AND" operator		

eTable 2. Items included in selected outcome measures

	1000 Norms Project	BOT-2	CMTPedS	MAND	MFM-32	PBS
Balance						
Single leg stance eyes closed	X	X	X	X		
Single leg stance eyes open		X	X	X	X	X
Feet apart on a line eyes closed	X	X	X			X
Feet apart on a line eyes open	X	X	X			X
Standing feet together						X
Single leg on a balance beam eyes closed		X	X			
Single leg on a balance beam eyes open		X	X			
Standing heel-to-toe on balance beam		X	X			
Walking heel-to-toe on a line		X	X	X		
Walking forward on a line		X	X		X	
Hopping on one leg					X	
Proximal muscle strength						
Shoulder external rotation strength	X					
Shoulder internal rotation strength	X					
Hip internal rotation strength	X					
Hip external rotation strength	X					
Elbow flexion strength	X					
Elbow extension strength	X					
Hip abduction strength	X					
Knee flexion strength	X					
Knee extension strength	X					
30-second sit to stand test	X					
V-up		X				
Wall sit		X				
Knee or full push-ups		X				
Sit-ups		X				
Standing long jump	X	X	X	X		

eTable 3. Patient demographics

Patient ID	1.1	1.2	4.1	4.2	5.1
Gender	Female	Female	Male	Female	Male
Ethnicity	Middle-Eastern (Lebanese)	Middle-Eastern (Lebanese)	Middle-Eastern (Lebanese)	Middle-Eastern (Lebanese)	Caucasian
Mutation	p.G306R/p.G306R	p.G306R/p.G306R	p.G306R/p.G306R	p.G306R/p.G306R	p.C126Y/p.L339P
Age at presentation	8	2	7	0	1
Age at genetic diagnosis	10	9	7	4	2
Age at commencement of riboflavin supplementation	10	9	7	4	2
Age at 2021 Assessment	20	18	15	12	7
Presenting symptoms	Ataxia	Ataxia	Ataxia	Hearing loss	Ataxia, Nystagmus