University of Cincinnati

Date: 12/29/2017

I, Fatima E Amir, hereby submit this original work as part of the requirements for the degree of Master of Science in Genetic Counseling.

It is entitled:

The Clinical Journey of Patients with Riboflavin Transporter Deficiency Type 2

Student's name: **Fatima E Amir**

This work and its defense approved by:

Committee chair: Carrie Atzinger, M.S., C.G.C.

Committee member: John Greinwald, Jr.

Committee member: Lisa Hunter

Committee member: Maggie Kettler, Au.D.

Committee member: Elizabeth Ulm, M.S.

29153

The Clinical Journey of Patients with Riboflavin Transporter Deficiency Type 2

A thesis submitted to the
Graduate School
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of

Master of Science

in the Department of Pediatrics
of the College of Medicine
March 2018

By

Fatima Amir
B.S. Carnegie Mellon University
May 2016

Committee Chair:
Carrie Atzinger, MS, LGC

Committee Members:
Margaret Kettler, AuD, FAAA
John Greinwald, MD, FAAP
Lisa Hunter, PhD, FAAA
Elizabeth Ulm, MS, LGC

ABSTRACT

Purpose: To identify what symptoms, genetic testing, and healthcare interactions patients with RTD Type 2 have before receiving the diagnosis. **Methods:** Parents of children with RTD Type 2 (n=10) from the Cure RTD Foundation patient registry were invited to participate in the study. Interviews were conducted to collect information on the patient's clinical journey and diagnosis. Descriptive statistics were used to report various aspects of the clinical journey. **Results:** The average diagnostic delay for our patients was 27.6 months. Neurologists were the most common provider seen by patients (90%). The most common symptoms before diagnosis were gait ataxia, nystagmus, and upper body muscle weakness. Gait ataxia was the most common symptom within the first year of the clinical journey. Mitochondrial disease was the most common suspected diagnosis (30%). **Conclusion:** Despite clinical variability, there are common features in the clinical journey of patients with RTD Type 2. The addition of the *SLC52A2* gene to gene panels and the newborn screen should be considered. With readily available treatment, we hope this study leads to earlier diagnoses, earlier treatment, and better outcomes for patients with RTD Type 2.

Key Words: Riboflavin Transporter Deficiency, *SLC52A2*, Brown-Vialetto-Van Laere syndrome, Fazio Londe, clinical journey, genetic testing, diagnostic odyssey

(This page is intentionally left blank.)

ACKNOWLEDGEMENTS

The authors would like to thank the families who participated in this research for sharing their journeys with us. We also thank Dr. Keith Massey and the Cure RTD Foundation for assisting with recruitment.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES	vi
INTRODUCTION	1
MATERIALS AND METHODS	4
Participants	4
Interview Guide	4
Medical Record Review	5
Data Collection	5
Data Analysis	5
RESULTS	5
Participants	5
Clinical Journey of RTD Type 2	6
Constellation of Symptoms before Diagnosis in RTD Type 2	6
Other Genetic Testing, Incorrect, and Suspected Diagnoses in Patients with RTD Type 2	7
DISCUSSION	8
Conclusion	11
References	13
Tables and Figures	15
Appendix A: Interview Guide	21
Appendix B: Data Collection Tool	26

LIST OF TABLES AND FIGURES

Table 1: Clinical journey of patients with RTD Type 2

Figure 1: Frequencies of common symptoms during the clinical journey of patients with RTD Type 2

Table 2: Catalog of symptoms within first year of clinical journey

Figure 2: Symptom map showing the range of ages at presentation of the various symptoms for RTD Type 2 with the median age of presentation included

Table 3: Previous genetic testing ordered

Table 4: Incorrect and suspected diagnoses

INTRODUCTION

Riboflavin Transporter Deficiency (RTD) is a progressive neurologic disorder with an estimated 70 diagnoses worldwide per year (Manole and Houlden 2015). In the literature, it is also referred as Brown-Vialetto-Van Laere Syndrome (BVVL) and Fazio-Londe syndrome. It is most commonly characterized by sensorineural hearing loss and bulbar palsy (Sathasivam 2008). Other common symptoms are facial weakness, respiratory compromise and muscle weakness (Bosch et al. 2012). The age of onset is variable but in a recent review of 70 patients who had a molecular diagnosis of RTD, the mean age of presentation was 4.1 years, ranging from 0.25 years to 27 years (Jaeger and Bosch 2016). The main symptoms upon presentation include hearing loss, weakness of the limbs and neck, bulbar weakness leading to feeding difficulties, balance and gait problems and respiratory complaints such as dyspnea or stridor. Visual and speech problems are less frequently reported as the presenting symptom. The progressive nature of the condition results in a worsening of and an increase in symptoms over time. Additional symptoms reported include cranial nerve deficits and sensory symptoms such as an ataxic gait (Srour et al. 2014, Jaeger and Bosch 2016).

Pathogenic variants in the *SLC52A2* and *SLC52A3* genes cause RTD Type 2 and 3 respectively (Green et al. 2010, Johnson et al. 2012). All types of RTD are inherited in an autosomal recessive pattern (Malheiros et al. 2007, Mégarbané et al. 2000). However, a recent study identified six individuals with heterozygous *SLC52A3* mutations that did not differ considerably phenotypically or by age of presentation from those with homozygous mutations (Manole et al. 2017). The phenotypic presentation of pathogenic variants in each of these genes has been shown to be different (Foley et al., 2013). Some studies have shown that patients with variants in *SLC52A2* seem to have a higher prevalence of sensory ataxia and optic atrophy than patients with variants in *SLC52A3*. However, due to the small number of patients in these studies and the lack of symptoms reported, further studies are needed to explore the variations in phenotype by genotype (Foley et al. 2013, Jaeger and Bosch 2016). For the purposes of this study, only patients with RTD Type 2 who have pathogenic variants in the *SLC52A2* gene are included.

The *SLC52A2* gene encodes a 445 amino acid riboflavin transporter RFVT2 (Yonezawa and Inui 2013), and is expressed ubiquitously but in especially higher amounts in the brain and salivary glands (Yao et al. 2010). The riboflavin transporter, as the name suggests, is involved in the transport of riboflavin (commonly known as Vitamin B2). Since the human body cannot synthesize riboflavin, it must be supplied by daily food intake. RFVT2 is believed to play a significant role in the transport of riboflavin from the blood to the brain tissue, allowing neuronal maintenance and homeostasis of riboflavin (Yonezawa and Inui 2013, Timmerman and De Jonghe 2014). Riboflavin is a precursor required for the biosynthesis of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Both FAD and FMN play a significant role in oxidative stress by forming parts of redox enzymes, which are involved in signal transduction, apoptosis and DNA repair. Hence, biallelic or compound heterozygous mutations in *SLC52A2* lead to disease by interfering with the riboflavin transport function of RFVT2 (Ciccolella et al. 2013, Foley et al. 2013, Timmerman and De Jonghe 2014, Nabokina, Subramanian and Said 2012).

After the discovery of these genes, case reports showed that treatment with high dose riboflavin supplementation in patients with RTD greatly reduced their clinical symptoms and improved their overall health (Anand et al. 2012, Foley et al. 2013). A better prognosis is reported for patients who start treatment earlier in the course of the illness (Foley et al., 2013). Starting the treatment as early as possible is important because some progressive neurological symptoms of the disease, such as diaphragmatic paralysis, could be irreversible (Bashford, Chowdhury and Shaw 2016). A review of patients with a molecular diagnosis of RTD who are not treated with riboflavin supplementation reported gradual deterioration and eventually death in some cases due to respiratory insufficiency (Jaeger and Bosch 2016, Green et al. 2010, Johnson et al. 2012, Ciccolella et al. 2012, Ciccolella et al. 2013, Dezfouli et al. 2012, Malafronte et al. 2013, Bandettini Di Poggio et al. 2014). On the contrary, patients with a molecular diagnosis of RTD who have been treated with riboflavin have shown improvement or stabilization either immediately, up to a month, or sometimes multiple months after treatment (Bosch et al. 2011, Anand et al. 2012, Haack et al. 2012, Foley et al. 2013, Spagnoli et al. 2014, Cosgrove, Datta and Busby 2015,

Menezes et al. 2016, Horoz et al. 2016). The mechanism of high dose riboflavin supplementation in improving clinical outcome is not well understood, however it has been suggested that transport of riboflavin may occur through passive diffusion or overdrive of the other riboflavin transporter that is still functional (Haack et al. 2012, Shashi et al. 2015).

Since RTD has a variety of clinical symptoms and because it is rare, patients often receive incorrect diagnoses before receiving the correct diagnosis (Bosch et al. 2011). One example in the literature describes a patient with RTD who was misdiagnosed with autoimmune demyelinating polyneuropathy upon initial presentation, but was correctly diagnosed with RTD after 10 years (Kliegman et al. 2017). The biochemical and clinical abnormalities of RTD can make it difficult to distinguish from late-onset Multiple Acyl-coA Dehydrogenase Deficiency (MADD), also leading to possible misdiagnoses (Grünert 2014). Differential diagnoses of RTD include Madras motor neuron disease, Boltshauser syndrome, Nathalie syndrome, Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy (Sathasivam 2008, Peeters, Chamova and Jordanova 2014). Patients with RTD may also experience a long clinical journey without any diagnoses. Clinical testing of the *SLC52A2* and *SLC52A3* genes has become available only in the past few years. Prior to that, patients may have received a diagnosis through exome sequencing on a clinical or research basis. Currently, a two gene Riboflavin Transporter Deficiency panel is clinically available that consists of the *SLC52A2* and *SLC52A3* genes. These genes are also present on some panels for neuropathy, muscular dystrophy, Amyotrophic Lateral Sclerosis, and ataxia.

An earlier diagnosis for RTD Type 2 leads to earlier treatment. Knowing that timely treatment with riboflavin supplementation leads to better outcomes, it is important that patients get a correct diagnosis as early as possible. Identifying what symptoms present first in patients with RTD Type 2 and which healthcare providers patients have seen before diagnosis, will allow for targeted education to allow providers to better recognize this disorder. Identifying the specialists seen and evaluations performed before receiving the correct diagnosis of RTD Type 2 will identify if there are common gene panels that should include the *SLC52A2* and *SLC52A3* genes. If these genes were tested when symptoms initially

presented, the time of diagnosis for patients could significantly decrease. Therefore, the goal of this study was to identify what symptoms, evaluations, genetic testing, and healthcare interactions patients with RTD Type 2 have before receiving the diagnosis.

MATERIALS AND METHODS

This study was approved by the Cincinnati Children's Hospital Institutional Review Board in July of 2017 (IRB#2017-2631).

Participants

Parents or legal guardians who were fluent in English and had at least one child under the age of 18 who had a diagnosis of RTD, BVVL syndrome, or Fazio Londe syndrome were recruited for this study. Due to the variability in phenotype participants were limited to those whose child had confirmed pathogenic variants in the *SLC52A2* gene. Participants were recruited through providers at Cincinnati Children's Hospital Medical Center (CCHMC). In addition, an ad for the study was posted on the National Society of Genetic Counselors and the American Academy of Audiology discussion boards to allow healthcare providers to distribute the ad to their patients. The ad was also sent to the founder of the CureRTD foundation, who distributed it among the families who fit participation criteria and posted it on the CureRTD foundation Facebook page. The ad included contact information for interested participants to contact the study team directly. Interested participants were electronically sent information on the study including eligibility criteria.

Interview Guide

Eligible participants were scheduled for approximately one hour long interviews held over the phone. An interview guide was created to gather information on the child's demographics, diagnosis of RTD and the clinical journey of the patient from the time of the first symptom till the time they received a genetic diagnosis. A data collection tool was used to record information during the interview.

Medical Record Review

Patients who had previously sent their medical records to the founder of the CureRTD Foundation, or who were exclusively seen at CCHMC were eligible for a medical record review. The medical record review for eligible patients consisted of a review of the patient's genetic testing results and audiology records. The purpose of the medical record review was to verify information collected in the interview for this subset of patients.

Data Collection

A data collection tool was created alongside the interview guide to assist with recording the data from the interview. Any information verified by the medical record review was also noted on the data collection tool. Interviews were held from September 2017 till January 2018 by the lead author over the phone. All data collected from the interviews and medical records were entered into a secure REDCap database.

Data Analysis

Various elements of the patient's clinical journey were added to the database. These elements included symptoms, ages of symptom onset, type of healthcare provider seen for each symptom, encounter details, referrals made, genetic testing ordered, type of healthcare provider that ordered the genetic testing and incorrect or suspected diagnoses made. Descriptive statistics were used to report frequencies and proportions of the symptoms, ages of symptoms onset, healthcare providers encountered, and previous suspected or incorrect diagnoses made.

RESULTS

Participants

Data was collected from 9 interviews with parents of patients with RTD Type 2, for a total of 10 patients, including one sibling pair. Medical records were obtained for one patient, and it did not change

any of the information gathered from the interview. Of the 10 patients included in the study, 6 patients were female (60%) and 4 patients were male (40%). The ages of the patients at the time of the interview ranged from 3 years old to 11 years old, with the median age being 6 years old. All patients were of Caucasian descent, while one patient was also of Asian descent. Six patients were from North America (60%), and 4 patients were from Europe/Australia (40%).

Clinical Journey of RTD Type 2

Table 1 gives an overview of the clinical journeys of each of the patients in this study. Common first symptoms observed in the patients were dysphagia (n=3), gait ataxia (n=3), not tracking with eyes (n=2), and vision loss (n=2). The onset of these symptoms ranged from 3 months to 72 months. Neurologists were the most common healthcare providers to order genetic testing that led to the diagnosis of RTD Type 2, making up 60% of ordering providers. The remaining ordering providers consisted of Geneticists (30%) and a Metabolic Specialist (10%). The diagnosis was made using whole exome sequencing in 50% of the patients. Other types of testing ordered included whole genome sequencing, panel testing, and site specific testing for a known familial mutation. The length of the clinical journey, or the diagnostic delay, represents the time between when the first symptom presented till the time the diagnosis was made. Excluding the patient who was a sibling of another patient, the average diagnostic delay for our patients was 27.6 months (n=9). All patients except the patient who was a sibling of another patient were seen by a neurologist at some point during their clinical journey, making neurologists the most common provider seen by patients with RTD Type 2 during their clinical journey (90%). Pediatricians were the second most common provider seen by patients with RTD Type 2 (70%).

Constellation of Symptoms before Diagnosis in RTD Type 2

Figure 1 shows the frequency of symptoms in RTD Type 2 before diagnosis reported in more than one patient in this study, along with the frequency of symptoms reported within the first year of the clinical journey. The most common symptoms before diagnosis in this study were gait ataxia, nystagmus,

and upper body muscle weakness. Each of these symptoms presented in 70% of patients. Dysphagia, optic nerve atrophy, and sleep apnea each presented in 50% of patients. Vision loss, blue spells, and gait ataxia presented as a symptom within the first year of the clinical journey in all of the patients who presented this symptom before their diagnosis.

Table 2 displays the catalog of symptoms presented in patients within the first year of their clinical journeys. Gait ataxia presented as a symptom within the first year of the clinical journey in 70% of patients, while nystagmus presented within the first year of the clinical journey in 50% of patients. Blue spells and upper body muscle weakness each presented as symptoms within the first year of the clinical journey in 40% of patients, followed by dysphagia and vision loss each presenting within the first year of the clinical journey in 30% of patients.

Figure 2 shows a symptom map for the variability in ages of presentation for the various symptoms, along with the median age of presentation of each of these symptoms. Symptoms that only presented in a single patient were not included in this map. Not tracking with eyes presented as early as 3 months of age to as late as 95 months of age, with the median age of presentation being 20 months. Similarly dysphagia and nystagmus each presented as early as 3 months to as late as 90 months of age, with the median age of presentation being 4 months and 15 months respectively. Hearing loss had a relatively smaller variation in age of presentation, reported as early as 22 months of age to as late as 44 months of age, with the median age of presentation being 28.5 months.

Other Genetic Testing, Incorrect, and Suspected Diagnoses in Patients with RTD Type 2

Genetic tests ordered prior to the testing that resulted in the diagnosis of RTD Type 2 are listed in Table 3. Previous genetic testing was reported for 6 patients (60%). Across the 6 patients, there were a total of 8 genetic tests ordered prior to the genetic testing that made the diagnosis of RTD Type 2. The chromosomal microarray, Friedreich Ataxia panel, and testing for mitochondrial diseases were ordered for the same patient.

A comprehensive list of incorrect and suspected diagnoses made prior to the final diagnosis of RTD Type 2 are shown in Table 4. Most commonly, mitochondrial disease was suspected in 30% of patients, followed by the suspicion of a brain tumor and Opsoclonus Myoclonus Syndrome each in 20% of patients.

DISCUSSION

To our knowledge, our sample size of 10 patients with a molecular diagnosis of RTD Type 2, is the second largest reported sample size for individuals with RTD Type 2 in a single study after Foley et al. 2013. The description of the symptoms presented within the first year and before diagnosis in children with RTD Type 2 will allow healthcare providers to consider it as a differential diagnosis when they may see children with similar phenotypes. Currently, studies in the literature regarding riboflavin transporter deficiency report collectively on RTD Type 2 and Type 3. Besides case reports, there is only one study that has aimed to look at RTD Type 2 separately (Foley et al. 2013). Having a more specific understanding of RTD Type 2 can highlight the differences in clinical presentation between Type 2 and Type 3, perhaps leading to a more specific diagnostic criteria for each condition. Our study is the first that has aimed to describe the clinical journey of patients with RTD Type 2. The goal of this would be earlier diagnoses, leading to earlier treatment, and hence, better prognoses for patients with RTD Type 2.

The results show that despite the variability in clinical journey of patients with RTD Type 2, half or more of the patients displayed muscle weakness, nystagmus, gait ataxia, sleep apnea, optic nerve atrophy, and dysphagia before diagnosis. The nystagmus in particular was not reported as a common symptom in previous literature reviews on RTD, while in our study, it was one of three most common symptoms reported in 70% of our cohort (Jaeger and Bosch 2016, Sathasivam 2008). Common first symptoms in our cohort included dysphagia, gait ataxia, not tracking with eyes, and vision loss. Dysphagia and gait ataxia have both been described as first symptoms in a previous literature review on RTD, however, not tracking with eyes, and vision loss are not described as common first symptoms. On the contrary, hearing loss is described as a first symptom in a previous review, while none of the children

in our cohort presented with hearing loss as their first symptom (Jaeger and Bosch 2016). Some of the most common symptoms that presented within the first year of the clinical journey in the patients with RTD Type 2 were gait ataxia, nystagmus, blue spells, and upper body muscle weakness. None of the previous literature reviews of RTD have reported common symptoms presenting within the first year of the clinical journey. A challenge to making the diagnosis of RTD Type 2 is that individually, these symptoms may generally present in a range of other conditions. However, if any number of these symptoms are seen together, it is important that providers have RTD Type 2 on their differential particularly given the potential for treatment.

Hearing loss was reported in 40% of the subjects' clinical journey prior to diagnosis. A previous study that aimed to understand the characteristics of hearing loss in patients with RTD Type 2 identified that the hearing loss in all the patients in their study was due to auditory neuropathy (Menezes, 2016). Auditory neuropathy is a rare condition, reported in only about 0.42% of the general population, and about 8% of children per year who are newly diagnosed with hearing loss (Gökdoğan et al. 2016). Therefore, RTD Type 2 should be considered in individuals who are diagnosed with hearing loss due to auditory neuropathy. Adding the *SLC52A2* gene to testing panels for children with auditory neuropathy may improve clinical sensitivity for these panels and shorten the time to diagnosis. In addition, most states use otoacoustic emissions for testing as part of their universal hearing screening program. This test will not identify auditory neuropathy, and therefore children with RTD may have auditory neuropathy early but may not be identified until later.

With gait ataxia and muscle weakness both being frequently presenting symptoms in patients with RTD Type 2, it is no surprise that 90% of the subjects were seen by a Neurologist during their clinical journey. Neurologists were also the most common provider to order the genetic testing that established a diagnosis of RTD Type 2 in our patients. There are currently no other studies in the literature that describe healthcare interactions for patients with RTD Type 2, or even for patients with RTD in general. Based on our findings, neurologists are the most frequently involved medical specialists

in the care of children with RTD Type 2 early in their presentation. Therefore, we hope that the description of symptoms in this patient population that is presented in this paper may be helpful to neurologists who may see children with a constellation of some of the symptoms described.

In addition, mitochondrial disease was a suspected diagnosis in 3 patients. Therefore, the *SLC52A2* gene could be added to diffuse neurologic panels, panels associated with the common symptoms in RTD Type 2, and to nuclear gene panels for mitochondrial disease. Even though the addition of this gene on the various panels might have low yield, the impact of diagnosis is high when effective treatment with riboflavin supplementation is readily available.

Given the nonspecific nature of the common first symptoms in RTD Type 2, it is worth considering the addition of the *SLC52A2* gene to the newborn screen. This could result in diagnoses made early in infancy, allowing riboflavin treatment to be started early, resulting in the best possible clinical outcome for patients with RTD Type 2. There are six key questions that are used to evaluate whether or not a condition should be added to the recommended uniform newborn screen. First, is there evidence to suggest that screening at birth for the condition leads to improved outcomes for the individual and possibly their family? Second, what is the incidence, prevalence, natural history and spectrum of disease of the condition? Is there an impact of recognizing and treating the condition earlier versus later? Third, is there a screening test or screening test algorithm available for the condition that has sufficient analytical validity? Fourth, has the clinical validity of the screening test or algorithm in combination with the diagnostic test or algorithm been determined? If so, is the validity adequate? Fifth, what is the clinical utility of the screening test or screening algorithm? Sixth, is the screening diagnosis and treatment for this disorder more cost effective than the usual clinical case detection and treatment (Calonge et al. 2010)?

This paper supports the case for RTD Type 2 to meet the evaluation criteria for a condition to be added to the newborn screen. Our data shows that children with RTD Type 2 may present with symptoms as early as 1 month. While it is difficult to find a precise value for the prevalence of RTD Type 2, the impact of diagnosing this condition in infancy is high due to the availability of riboflavin supplementation

as an effective treatment option (Foley et al. 2013). Since RTD Type 2 is a progressive condition with some of the neurologic symptoms being irreversible, earlier treatment with riboflavin may provide these children with the best possible outcome. Since our data shows that patients with RTD Type 2 may go up to 55 months without a diagnosis, identifying individuals with RTD Type 2 on the newborn screen may also prove to be a more cost effective approach than the long diagnostic odysseys that patients may otherwise face with numerous tests, procedures and appointments spent trying to get to a diagnosis. The non-specific nature of the individual symptoms and clinical variability paired with the readily available treatment makes RTD Type 2 a strong candidate for the newborn screen.

This study has some limitations. First, the data was primarily collected using interviews with parents of patients who had a diagnosis of RTD Type 2. Interviewing can inevitably lead to recall bias that may affect the accuracy of the data. For future research, performing a complete chart review to collect data regarding the clinical journey of patients may result in more objective data collection. Second, the patients in our study received their healthcare across three continents. The differences in healthcare systems, such as costs and coverage of services, as well as availability of testing technology, could influence the clinical journeys of the patients, therefore leading to differences in healthcare interactions, and testing ordered. Lastly, our patients were primarily of Caucasian descent, with only one patient who also was of Asian descent. Therefore, the data presented may not be generalizable across patients of all ethnic backgrounds. As awareness around RTD increases, future research may utilize larger sample sizes to explore the genotype-phenotype relationships. Perhaps this may explain the clinical variability within RTD Type 2. Furthermore, research that can compare the phenotype of RTD Type 2 to RTD Type 3 may aid in understanding the natural history of both types separately.

Conclusion

Our study shows that despite the clinical variability among patients with RTD Type 2, there are common features observed across the clinical journey before diagnosis of 10 patients from 9 families. The common features, delay in diagnosis, and long list of incorrect or suspected diagnoses in our reported

patients, the case can be made for the *SLC52A2* gene to be added to various gene panels, and possibly newborn screening. This is especially important because riboflavin supplementation has been shown to serve as effective and readily available treatment for individuals with RTD Type 2. Therefore, our hope is that our study leads to earlier diagnoses, resulting in the administration of earlier treatment, and therefore, better outcomes for individuals with RTD Type 2.

References

- Anand, G., N. Hasan, S. Jayapal, Z. Huma, T. Ali, J. Hull, E. Blair, T. McShane & S. Jayawant (2012) Early use of high-dose riboflavin in a case of Brown–Violetto–Van Laere syndrome. *Developmental Medicine & Child Neurology*, 54, 187-189.
- Bandettini Di Poggio, M., M. Monti Bragadin, L. Reni, L. Doria-Lamba, C. Cereda, M. Pardini, L. Roccatagliata, A. Rossi & A. Schenone (2014) Brown-Violetto-Van Laere syndrome: clinical and neuroradiological findings of a genetically proven patient. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 15, 141-144.
- Bashford, J. A., F. A. Chowdhury & C. E. Shaw (2016) Remarkable motor recovery after riboflavin therapy in adult-onset Brown—Violetto—Van Laere syndrome. *Practical neurology*, practneurol-2016.
- Bosch, A. M., N. G. G. M. Abeling, L. Ijlst, H. Knoester, W. L. van der Pol, A. E. M. Stroomer, R. J. Wanders, G. Visser, F. A. Wijburg & M. Duran (2011) Brown-Violetto-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. *Journal of inherited metabolic disease*, 34, 159-164.
- Bosch, A. M., K. Stroek, N. G. Abeling, H. R. Waterham, L. Ijlst & R. J. A. Wanders (2012) The Brown-Violetto-Van Laere and Fazio Londe syndrome revisited: natural history, genetics, treatment and future perspectives. *Orphanet journal of rare diseases*, 7, 1.
- Calonge, N., N. S. Green, P. Rinaldo, M. Lloyd-Puryear, D. Dougherty, C. Boyle, M. Watson, T. Trotter, S. F. Terry & R. R. Howell (2010) Committee report: method for evaluating conditions nominated for population-based screening of newborns and children. *Genetics in Medicine*, 12, 153.
- Ciccolella, M., M. Catteruccia, S. Benedetti, I. Moroni, G. Uziel, C. Pantaleoni, L. Chiapparini, A. Bizzi, A. D'Amico & F. Fattori (2012) Brown–Violetto–van Laere and Fazio–Londe overlap syndromes: A clinical, biochemical and genetic study. *Neuromuscular Disorders*, 22, 1075-1082.
- Ciccolella, M., S. Corti, M. Catteruccia, S. Petrini, G. Tozzi, T. Rizza, R. Carrozzo, M. Nizzardo, A. Bordini & D. Ronchi (2013) Riboflavin transporter 3 involvement in infantile Brown-Violetto-Van Laere disease: two novel mutations. *Journal of medical genetics*, 50, 104-107.
- Cosgrove, J., S. Datta & M. Busby (2015) Adult onset Brown-Violetto-Van Laere syndrome with opsoclonus and a novel heterozygous mutation: a case report. *Clinical neurology and neurosurgery*, 128, 1.
- Dezfouli, M. A., S. Yadegari, S. Nafissi & E. Elahi (2012) Four novel C20orf54 mutations identified in Brown–Violetto–Van Laere syndrome patients. *Journal of human genetics*, 57, 613-617.
- Foley, A. R., M. P. Menezes, A. Pandraud, M. A. Gonzalez, A. Al-Odaib, A. J. Abrams, K. Sugano, A. Yonezawa, A. Y. Manzur & J. Burns (2013) Treatable childhood neuronopathy caused by mutations in riboflavin transporter RFVT2. *Brain*, awt315.
- Green, P., M. Wiseman, Y. J. Crow, H. Houlden, S. Riphagen, J.-P. Lin, F. L. Raymond, A.-M. Childs, E. Sheridan & S. Edwards (2010) Brown-Violetto-Van Laere syndrome, a ponto-bulbar palsy with deafness, is caused by mutations in c20orf54. *The American Journal of Human Genetics*, 86, 485-489.
- Grünert, S. C. (2014) Clinical and genetical heterogeneity of late-onset multiple acyl-coenzyme A dehydrogenase deficiency. *Orphanet journal of rare diseases*, 9, 117.
- Gökdoğan, Ç., S. Altinyay, B. Gündüz, Y. K. Kemalolu, Y. Bayazit & K. Uygur (2016) Management of children with auditory neuropathy spectrum disorder (ANSO). *Brazilian journal of otorhinolaryngology*, 82, 493-499.
- Haack, T. B., C. Makowski, Y. Yao, E. Graf, M. Hempel, T. Wieland, U. Tauer, U. Ahting, J. A. Mayr & P. Freisinger (2012) Impaired riboflavin transport due to missense mutations in SLC52A2 causes Brown-Violetto-Van Laere syndrome. *Journal of inherited metabolic disease*, 35, 943-948.

- Horoz, O. O., N. O. Mungan, D. Yildizdas, Ö. Hergüner, S. Ceylaner, D. Kör, H. Waterham & T. Coskun (2016) Brown-Vialetto-Van Laere syndrome: two siblings with a new mutation and dramatic therapeutic effect of high-dose riboflavin. *Journal of Pediatric Endocrinology and Metabolism*, 29, 227-231.
- Jaeger, B. & A. M. Bosch (2016) Clinical presentation and outcome of riboflavin transporter deficiency: mini review after five years of experience. *Journal of inherited metabolic disease*, 39, 559-564.
- Johnson, J. O., J. R. Gibbs, A. Megarbane, J. A. Urtizberea, D. G. Hernandez, A. R. Foley, S. Arepalli, A. Pandraud, J. Simón-Sánchez & P. Clayton (2012) Exome sequencing reveals riboflavin transporter mutations as a cause of motor neuron disease. *Brain*, aws161.
- Kliegman, R. M., B. J. Bordini, D. Basel & J. J. Nocton (2017) How Doctors Think: Common Diagnostic Errors in Clinical Judgment—Lessons from an Undiagnosed and Rare Disease Program. *Pediatric Clinics of North America*, 64, 1-15.
- Malafronte, P., H. B. Clark, I. Castaneda-Sanchez, J. Raisanen & K. J. Hatanpaa (2013) Brown-Vialetto-Van Laere syndrome: clinical and neuropathologic findings with immunohistochemistry for C20orf54 in three affected patients. *Pediatric and Developmental Pathology*, 16, 364-371.
- Malheiros, J. A., S. T. Camargos, J. T. d. Oliveira & F. E. C. Cardoso (2007) A Brazilian family with Brown-Vialetto-van Laere syndrome with autosomal recessive inheritance. *Arquivos de neuro-psiquiatria*, 65, 32-35.
- Manole, A. & H. Houlden (2015) Riboflavin Transporter Deficiency Neuronopathy.
- Manole, A., Z. Jaunmuktane, I. Hargreaves, M. H. R. Ludtmann, V. Salpietro, O. D. Bello, S. Pope, A. Pandraud, A. Horga & R. S. Scalco (2017) Clinical, pathological and functional characterization of riboflavin-responsive neuropathy. *Brain*, 140, 2820-2837.
- Menezes, M. P., M. A. Farrar, R. Webster, J. Antony, K. O'Brien, R. Ouvrier, M. C. Kiernan, J. Burns & S. Vucic (2016) Pathophysiology of motor dysfunction in a childhood motor neuron disease caused by mutations in the riboflavin transporter. *Clinical Neurophysiology*, 127, 911-918.
- Mégarbané, A., I. Desguerres, E. Rizkallah, V. Delague, R. Nabbout, A. Barois & A. Urtizberea (2000) Brown-Vialetto-Van Laere syndrome in a large inbred Lebanese family: Confirmation of autosomal recessive inheritance? *American journal of medical genetics*, 92, 117-121.
- Nabokina, S. M., V. S. Subramanian & H. M. Said (2012) Effect of clinical mutations on functionality of the human riboflavin transporter-2 (hRFT-2). *Molecular genetics and metabolism*, 105, 652-657.
- Peeters, K., T. Chamova & A. Jordanova (2014) Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. *Brain*, 137, 2879-2896.
- Sathasivam, S. (2008) Brown-Vialetto-Van Laere syndrome. *Orphanet journal of rare diseases*, 3, 9.
- Shashi, V., S. Petrovski, K. Schoch, R. Crimian, L. E. Case, R. Khalid, M. A. El-Dairi, Y.-H. Jiang, M. A. Mikati & D. B. Goldstein (2015) Sustained therapeutic response to riboflavin in a child with a progressive neurological condition, diagnosed by whole-exome sequencing. *Molecular Case Studies*, 1, a000265.
- Spagnoli, C., M. C. Pitt, S. Rahman & C. de Sousa (2014) Brown-Vialetto-van Laere syndrome: a riboflavin responsive neuronopathy of infancy with singular features. *European Journal of Paediatric Neurology*, 18, 231-234.
- Srour, M., M. L. Putorti, J. Schwartzenruber, V. Bolduc, M. I. Shevell, C. Poulin, E. O'Ferrall, D. Buhas, J. Majewski & B. Brais (2014) Mutations in riboflavin transporter present with severe sensory loss and deafness in childhood. *Muscle & nerve*, 50, 775-779.
- Timmerman, V. & P. De Jonghe (2014) Promising riboflavin treatment for motor neuron disorder. *Brain*, 137, 2-3.
- Yao, Y., A. Yonezawa, H. Yoshimatsu, S. Masuda, T. Katsura & K.-i. Inui (2010) Identification and comparative functional characterization of a new human riboflavin transporter hRFT3 expressed in the brain. *The Journal of nutrition*, 140, 1220-1226.
- Yonezawa, A. & K.-i. Inui (2013) Novel riboflavin transporter family RFVT/SLC52: identification, nomenclature, functional characterization and genetic diseases of RFVT/SLC52. *Molecular aspects of medicine*, 34, 693-701.

Table 1: Clinical Journey of Patients with RTD Type 2

Patient ID#	First symptom(s)	Age and Date of onset	Age and Date of Molecular diagnosis	Provider who made the Molecular Diagnosis	Type of Genetic Testing	Diagnostic Delay
1	Dysphagia, Lethargic & Not Tracking with Eyes	3 months (10/2011)	27 months (10/2013)	Metabolic Specialist	Whole Exome Sequencing	24 months
2	Dysphagia	4 months (11/2012)	20 months (04/2014)	Geneticist	Whole Exome Sequencing	16 months
3	Gait Ataxia	25 months (09/2011)	55 months (03/2014)	Neurologist	Whole Exome Sequencing	30 months
4	Gait Ataxia	15 months (10/2015)	26 months (09/2016)	Neurologist	Whole Exome Sequencing	11 months
5	Vision Loss and Hearing Loss	34 months (06/2009)	79 months (03/2013)	Neurologist	Whole Genome Sequencing	45 months
6	Vision Loss	72 months (12/2013)	100 months (04/2016)	Geneticist	Whole Exome Sequencing	28 months
7	Not Tracking with Eyes	20 months (04/2013)	58 months (06/2016)	Geneticist	Site Specific testing (known familial mutation)	38 months
8	Respiratory Problems	1 month (04/2014)	24 months (03/2016)	Neurologist	Unknown Panel	23 months
9	Dysphagia	4 months (03/2015)	20 months (07/2016)	Neurologist	Unknown Panel	16 months
10	Gait Ataxia	11 months (06/2011)	66 months (01/2016)	Neurologist	Unknown	55 months

Figure 1: Frequencies of common symptoms during the clinical journey of patients with RTD Type 2

Table 2: Catalog of Symptoms within first year of Clinical Journey

Symptom	Number of patients
Gait Ataxia	7
Nystagmus	5
Blue Spell	4
Upper Body Muscle Weakness	4
Dysphagia	3
Vision Loss	3
Fever	2
Not Tracking with Eyes	2
Persistent Coughing	2
Respiratory problems	2
Generalized Muscle Weakness	1
Hearing Loss	1
Hyperflexible	1
Lethargic	1
Optic Nerve Atrophy	1
Pontobulbar palsy	1
Proprioception Nerve Damage	1
Seizure	1
Severe Anemia	1
Sleep Apnea	1
Tremors	1

Figure 2: Symptom Map showing the range of ages at presentation of the various symptoms for RTD Type 2 with the median age of presentation included

Table 3: Previous Genetic Testing ordered

Previous Genetic Testing	Patients
Lysosomal Storage panel	1
<i>SLC52A3</i> testing	1
Unknown panel including SMA genes	1
Chromosomal Microarray	1
Friedreich Ataxia panel	1
Mitochondrial disease	1
Infantile Neuroaxonal Dystrophy	1
Unknown familial dystonia panel	1

Table 4: Incorrect and Suspected Diagnoses

Incorrect and/or Suspected Diagnosis	Patients	Healthcare Provider who made or suspected the diagnosis
Mitochondrial disease	3	Neurologist, Metabolic Specialist
Brain Tumor	2	Pediatrician
Opsoclonus Myoclonus Syndrome	2	Neurologist
A type of Familial Dystonia	1	Neurologist
Autoimmune disorder	1	Neurologist
Diamond-Blackfan Anemia	1	Hematologist
Friedreich's Ataxia	1	Geneticist
Guillain-Barre syndrome	1	Neurologist
Hereditary Hemorrhagic Telangiectasia	1	Geneticist
Interstitial Lung disease	1	Pulmonologist
Lyme disease	1	Neurologist
Metachromatic Leukodystrophy	1	Metabolic Specialist
Mucopolysaccharidosis (MPS)	1	Geneticist
Niemann-Pick disease	1	Geneticist
Optic Nerve Hypoplasia	1	Ophthalmologist
Residual brain damage from Parbovirus	1	Ophthalmologist, Hematologist, Pulmonologist
Short Chain Acyl-CoA Dehydrogenase deficiency (SCAD)	1	Metabolic Specialist
Spinal Muscular Atrophy (SMA)	1	Neurologist

Appendix A: Interview Guide

Hello, I'm Fatima Amir from Cincinnati Children's Hospital Medical Center Division of Human Genetics. Could I speak with Mr. /Mrs. _____?

Thank you for your willingness to participate in this interview. This interview is part of an ongoing research study. The purpose of the study is to understand the clinical journey of patients diagnosed with a condition known by several names including Riboflavin Transporter Deficiency, Brown-Vialetto-Van Laere Syndrome or Fazio Londe Syndrome. Which name are you most familiar with? _____ *(Depending on their answer, we will refer to the condition with the term that they are familiar with throughout the rest of the interview. For the purposes of this script, I will simply write it out as RTD)*

When we say we want to understand the clinical journey of patients diagnosed with *RTD*, we mean we want to understand the **order of their symptoms**, what **genetic testing** the patient has had until their final diagnosis of *RTD*, whether or not they have received any **incorrect diagnoses** before their diagnosis of *RTD* and their **healthcare interactions** – that is, what kinds of doctors they were seen by and in what order. We are mainly focusing on the journey up until the time of their genetic diagnosis with *RTD*.

During this interview, I will be recording responses as we go along so you may expect some pauses from my side after you answer a question.

To highlight some of the important elements of the consent, we would like to remind you that you are being asked to participate in this research because your child has a diagnosis of *RTD*. You should not participate in this research study if your child is over 18 years old and has not had a positive result for a mutation in the *SLC52A2* gene. Your interview will take approximately one hour. Data collected from this research study will be maintained in a database that can be used for future studies on *RTD*. You will be asked for your permission to be involved in future research. You have the right to withdraw your information from the database at any time. About 20 people will take part in this study at Cincinnati Children's Hospital Medical Center. There are no known risks associated with the study. There may be unknown or unforeseen risks associated with study participation such as a loss of confidentiality. If you agree to take part in the research study, there is no direct medical benefit to you. The information learned from this study may benefit other patients with *RTD*. The information learned from this study may also help health care providers better understand the condition. It is hoped that this information will decrease the time to diagnosis, resulting in earlier treatment and a better prognosis for patients. Every effort will be made to maintain the confidentiality of your child's medical and research information. There are no costs to you for participation in this research study. There will be no payment or reimbursement to participate in this research study. If you refuse to participate, your rights concerning treatment, payment for services, and enrollment in a health plan or eligibility for benefits will not be affected.

We would like to remind you that your participation in this research study is completely voluntary and you may withdraw your consent at any time.

We would like to ask you some questions about your child's clinical journey. To help us better understand the more technical aspects of the journey, we may also like to review your child's medical records. We will ask your permission to access these records only if you have already sent these records to Dr. Keith Massey in the past, or if you were exclusively seen at Cincinnati Children's Hospital Medical Center. We will not ask you to collect and send us any records.

Would you like to participate in this study?

If no: Thank you for your time.

If yes: Thank you for volunteering to participate in our study. As the email explained, participating involves taking part in a phone interview and a possible review of your child's medical records. During the phone interview, we will ask you questions on your child's clinical journey till they received a diagnosis of *RTD*. For the medical records, if you have already sent your child's medical records to Dr. Keith Massey, then we can ask him to send them to us if you give us the permission to do so. If your child was seen exclusively at Cincinnati Children's, then we will ask you for permission to access your child's medical records.

I will now ask some demographic questions about your child.

What is the name of your child?

What is your relationship to *Patient*?

What is *Patient*'s date of birth?

What is *Patient*'s sex?

What is *Patient*'s ethnicity?

Have you sent *Patient*'s medical records to Dr. Keith Massey?

If yes: Would you give us permission to ask Dr. Keith Massey for any of *Patient*'s genetic testing results and audiology records up to the point of the final genetic testing confirming *Patient*'s diagnosis?

If no: Was *Patient* seen exclusively at Cincinnati Children's Hospital Medical Center?

If yes: We will mail you a release of information form that you can either mail back to us, or you can send us a scanned copy of, if you choose to allow us to access *Patient*'s medical records directly.

If no: We will not be conducting a medical record review for *Patient*.

At this time do you have any questions before we begin the interview?

If yes: Answer question as appropriate

If no: Continue with interview.

Was *Patient's* diagnosis made before confirming it with genetic testing, or was *Patient's* diagnosis made first with genetic testing?

If clinical dx first:

When was *Patient's* diagnosis of *RTD* made?

What kind of doctor made the diagnosis?

When was *Patient's* diagnosis of *RTD* confirmed with genetic testing?

If genetic dx first: When was genetic testing ordered that made a diagnosis of *RTD*?
Continue with interview.

What kind of doctor ordered the genetic testing?

Do you know what kind of testing was done? Examples include Whole Exome Sequencing, a Gene Panel or Single gene testing for the *SLC52A2* gene.

Thank you for that information. Now I will try to understand *Patient's* clinical journey until the diagnosis of *RTD* was made.

Going back in time a little bit, what was the first symptom that *Patient* developed?

What age was *he/she*? Or if you prefer to use dates, what month and year was that?

What doctor did you see at that time?

What was the outcome of that visit? Did they refer you to any other doctors?

What was the next symptom to develop?

(continue to ask and clarify order of symptoms and doctor visits up to the point of diagnosis)

I would like to summarize the information you have given me to make sure I have recorded it accurately. Please let me know if I have missed any information you deem relevant.

(Summarize information in table, and correct as appropriate)

(If genetic testing has not been mentioned) Was there any other genetic testing ordered by any other doctors along the way? What did it test for? Who ordered it? What were the results?

Were there any diagnoses that *Patient* was given that were incorrect before his/her correct diagnosis of *RTD*? Who made these diagnoses?

Now I will ask you some questions specifically related to Audiology.

What age was *Patient* identified with hearing loss (*if not already mentioned*)? Did *Patient* get hearing aids? Did *Patient* use cochlear implants? How well does *Patient* communicate now? How well do you think your child can hear with their devices?

Does *Patient* use speech to communicate? (*If yes, then do not ask remaining questions*)

Does *Patient* use sign language to communicate?

Does *Patient* use tactile signs to communicate?

Does *Patient* use a communication board to communicate?

Does *Patient* use gesturing to communicate?

Thank you. We value your opinion, so now I will ask you some general questions before we end the interview.

What research would you like to see done about *RTD* in the future?

Would you like to be contacted for any future research?

Do you have any other comments or feedback for us?

If records with Keith or CCHMC patient:

As mentioned earlier, we would also like to review some of *Patient's* medical records to get a more comprehensive picture.

If records with Keith: We will be getting in touch with Dr. Keith Massey and ask him to send us a copy of all of *Patient's* clinic notes, genetic testing results and audiology records that he has up to the time of diagnosis. Would you allow us to review these records?

If seen at CCHMC: Since your child was seen at Cincinnati Children's, we will be mailing you a release of information form in the next few days. If you give us permission to review your child's medical records, then you may sign and send the form back to us either by mail or by scanning or faxing it back to us. Then we will be able to access *Patient's* clinic notes, genetic testing results and audiology records up to the time of diagnosis. What mailing address can we use to mail the release of information form to you?

Thank you very much for agreeing to participate in this phone interview from the Division of Human Genetics at Cincinnati Children's Hospital Medical Center. Do you have any questions at this point?

I am the contact person for this study in case you have any questions that come up later. My contact information was included in the email previously sent to you regarding this study. If you

would like I can go over it again. Would you like me to review my contact information with you right now?

If yes:

Fatima Amir

Division of Human Genetics

Cincinnati Children's Hospital Medical Center

3333 Burnet Avenue, MLC 4006

Cincinnati, OH 45229-3039

Phone: (513)302-4363

Email: Fatima.Amir@cchmc.org

If no: *Continue with interview.*

Thank you again for your time. It was my pleasure speaking with you. Good bye!

Appendix B: Data Collection Tool

Interviewee & Patient Information:

Interview ID: _____

Name of Interviewee(s):

Name of Patient:

Patient Sex:

Relationship(s) to Patient:

Patient DOB:

Patient Ethnicity:

Source: ☐ Parent report ☐ MR

Data Collection:

☐ Clinical Diagnosis (made before a genetic diagnosis ☐ Yes/ ☐ No)

☐ __/__(mm/yyyy)

○ Type of specialist who made diagnosis: _____

Source:

☐ Parent report ☐ MR

☐ Genetic Diagnosis (made before a clinical diagnosis ☐ Yes/ ☐ No)

○ __/__(mm/yyyy)

○ Type of specialist who ordered genetic testing: _____

Source:

☐ Parent report ☐ MR

○ Type of genetic test:

☐ Targeted testing for *SLC52A2*

☐ Panel

☐ Whole Exome Sequencing

☐ Other: _____

Source:

☐ Parent report ☐ MR

Age/Date	Symptom	Doctor/HCP	Encounter	Referral	Genetic Testing (with Result)	Source

- Previous Incorrect/Suspected Diagnoses, Age & Who made the diagnosis:

Source:

☐ Parent report ☐ MR

- Audiology Data

- ☐ Age of diagnosis of Hearing Loss: _____
- ☐ Hearing aids: ☐ Yes / ☐ No
- ☐ Cochlear Implants: ☐ Yes / ☐ No
- ☐ How well does the patient communicate: _____
- ☐ Hearing with devices: _____
- ☐ How does your child communicate?
 - ☐ Speech ☐ Sign Language ☐ Tactile signs
 - ☐ Communication board ☐ Gesturing

Source:

☐ Parent report ☐ MR

- Future research:

Contact for future research? ☐

- Comments/Feedback:

- Medical Records

- ☐ Dr. Keith Massey

- ☐ CCHMC only

- ☐ No permission to review Medical Records

- Mailing Address of Interviewee (☐ NA)