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Jessica MacWilliams April 20th, 2019

Hand Fine Motor Control Quantification in Children and Adults with Classic Galactosemia

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Abstract

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Classic galactosemia (CG) is a rare inborn error of galactose metabolism that results from reduced or deficient activity of the enzyme galactose-1-phosphate uridylyltransferase (GALT). Motor control problems are a known outcome of classic galactosemia. This study made use of a publically available software called Neuroglyphics (NG) and a digitizing tablet to quantitatively score Archimedes spirals drawn by a sample 53 CG participants and 80 controls. The purpose of this study was to use NG software to determine the proportion of children and adults with CG affected by motor control problems. Additionally, we asked if participants with CG exhibited a lack of dominant hand advantage, and if there was a difference between males and females in motor outcomes. Results indicated that about 30-35% of adults and children with classic galactosemia were affected by motor control problems. Additionally, about 66% of those affected exhibited no dominant hand advantage, meaning their dominant hand did not perform significantly better than their non-dominant hand. No difference in motor control was found between males and females. Neuroglyphics is a quick and effective tool for measuring motor control problems in patients with classic galactosemia and will be advantageous for measuring motor outcomes in future clinical trials and/or intervention studies.

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CHAPTER 1: INTRODUCTION

CLASSIC GALACTOSEMIA GENETICS AND BIOCHEMISTRY

Classic Galactosemia (CG) is a rare inborn-error of galactose metabolism resulting from deficient activity of the second enzyme of the Leloir pathway, galactose-1-phosphate uridylyltransferase (GALT). As of April 2017, 229 sequence variations of the GALT gene had been identified, with the most commonly reported mutations being Q188R, L195P, S135L, K285N, and T138M[1]. These mutations alter the conformation of the GALT enzyme resulting in an inability to convert galactose-1-phosphate (gal-1P) into uridine diphosphogalactose (UDP-gal).[2] Some mutations appear to be associated with less severe long-term outcomes, which is likely due to differences in residual activity of the GALT variants; for example, homozygocity of the Q188R allele is often associated with poor outcomes and measures of RBC GALT activity are essentially zero.[3] In contrast, presence of even one S135L allele confers up to a few percent activity and milder long-term outcomes.[4] In a study of 33 adults, the proportion of participants with motor or speech problems was not associated with genotype, but the effect of genotype on long-term outcome is unclear.[5]

GALT is responsible for catalyzing the transfer of a uridyl group from UDP-Glc to Gal-1-P through a double displacement mechanism involving a transiently uridylated histidine residue (Figure 1).[6] The inactivity of GALT leads to accumulation of galactose, galactose-1-phosphate, galactitol, and galactonate metabolites. Whether levels of UDP-galactose and UDP-glucose are perturbed remains a point of controversy.[7] It is believed that galactitol accumulation within cells may contribute to cellular and organ failure—a potential explanation for the long-term outcomes of CG—however, definitive evidence has not confirmed this hypothesis.[8]

Even with dietary restriction, endogenous production of galactose contributes to total body galactose synthesis.[9] Quantitative measurements estimate 0.48 to 1.7 mg/kg/h of whole body galactose, and endogenous galactose production is highest in childhood and decreases throughout adulthood.[10]

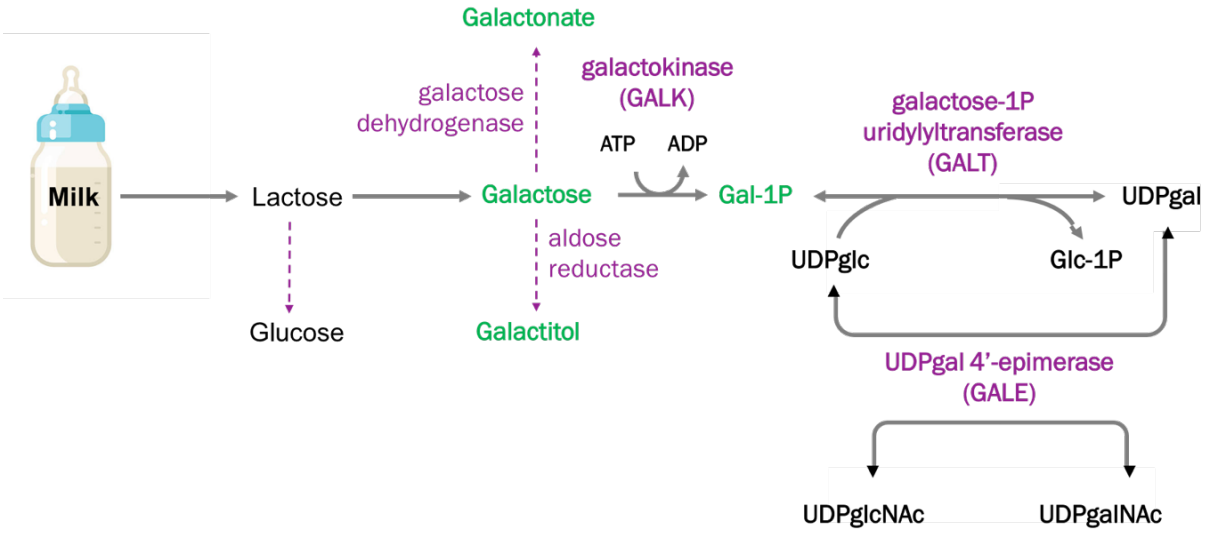

FIGURE 1. LELOIR PATHWAY OF GALACTOSE METABOLISM. ACCUMULATION OF GALACTOSE, GALACTOSE-1-PHOSPHATE, GALACTITOL, AND GALACTONATE METABOLITES (GREEN). ENZYME NAMES ARE IN PURPLE.

PATIENT EXPERIENCE

CG is an enigmatic condition whereby the majority of neonates diagnosed and treated with rigorous galactose restriction within the first few days following birth nonetheless grow to experience complications that can persist into adulthood.[11] Known long-term outcomes of CG with estimate of percent affected include: cognitive disability (50%), movement disorders (estimates range from 18%[12] to 66%[13]), speech problems (60%), delayed growth, and ovarian dysfunction in girls and women (> 80%).[4,11,14] The puzzling nature of CG has resulted in differential approaches for diagnosis and treatment. A small, global assessment of different

treatment protocols found no differences in long-term outcomes among treatment sub-groups; however, this conclusion pointed to the reality that no "best approach" to CG had been identified, and more extensive studies might expose notable insights into the impacts of different approaches.[11]

CG has an incidence of 1 in 40,000-60,000 live-births and can be detected by newborn screening.[14] A common method for CG diagnosis is the measurement of GALT enzyme activity in RBC, sometimes accompanied by measurements galactose, Gal-1-P and/or galactitol in the blood and/or galactitol in the urine. Elevated RBC Gal-1-P may also occur in benign GALT variants and in conditions unrelated to GALT, so this method should not be used alone to diagnose CG.[15] Gal-1-P and galactitol levels are higher in cases before and sometimes even after dietary restriction as compared to control infants, and thus provides a reliable measurement for following CG.[16] However, other conditions that compromise galactose metabolism or liver function can also give elevated galactose metabolites. The most reliable diagnostic tool is activity measurements of GALT in RBC. GALT activity in CG patients is often undetectable or less than 1% of control activity.[7] Initial symptoms commonly include: poor feeding, diarrhea, vomiting, and lethargy. Progressive toxicity leads to sepsis, cataracts, and eventually infant death.[15]

Arguments against the inclusion of screening for CG are based on reasoning that diagnoses can be made clinically following birth, and long-term outcomes may still develop despite early intervention. However, CG is rare, and in the US, many doctors miss this diagnosis without new born screening. Another factor to consider is that parental stress tends to be lower

in families of children diagnosed through newborn screening as compared to those diagnosed by clinical evaluation.[17]

Ambivalence also arises concerning the role of strictness of dietary galactose restriction as a modifier of outcomes. Immediate dietary galactose restriction is the current standard of care. Of note, dietary restriction eliminates the acute clinical presentation of CG. A study evaluating various long-term outcomes of CG reported that the rigor of non-dairy galactose restriction showed no correlation with the severity of these outcomes.[4] Additionally, even though blood galactose levels fall quickly following implementation of a galactose restricted diet, the levels remain mildly elevated in many CG patients, indicating inevitability of elevated RBC galactose even with extreme dietary restriction which may reflect endogenous galactose production.[19]

MOTOR CONTROL OUTCOMES IN CLASSIC GALACTOSEMIA

The specific purpose of this study was to evaluate hand fine motor control in people with CG. Most information known about motor disorders in CG comes from self-report, retrospective surveys, and informal neurological examination. Kuiper et al. (2018) assessed 37 patients diagnosed with CG through use of subjective self-report surveys and interviews as well as an objective video assessment. The objective examination included walking, posturing tasks, kinetic tasks, and functional tasks, such as writing. The videotaped neurological exam was scored by five members of an expert panel. Of the 37 participants included in the study, 48.6% met criteria for either fine motor, gross-motor or both motor disorders, and one third of all patients tested were considered to fall in the range of moderate to severe motor problems according to evaluation by

the expert panel.[20] The three motor disorders most frequently diagnosed by the expert panel included: tremor, ataxia, and dystonia.

Neurologists have identified different types of tremor that fall into the larger category. Essential Tremor (ET) is characterized by a tremor amplitude of less than one centimeter with a regular, high frequency (8-12 Hz). The tremor amplitudes are all in a unidirectional axis, with right-handed spirals commonly following an 8-2 o'clock direction and left-handed spirals following a 10-4 o'clock direction.[21] In contrast, dystonic tremors commonly contain fluctuating tremor amplitudes that fall along a multidirectional axis. The frequency of dystonic tremor is usually less than 7 Hz, and there is usually a more forceful pen pressure.[21] Lastly, functional tremor is characterized by inconsistency. Variability within one drawn spiral or variability between repeated spiral drawings is a key feature of this tremor type.[21]

Ataxia is a movement disorder that results from degeneration of the nervous system. Slurred speech, stumbling, falling, and lack of coordination are common. Therapy and some medications may help, but there are no known cures for ataxia.[22] Dystonia is a movement disorder characterized by slow repetitive and involuntary muscle contractions. These muscle contractions may be painful and can be combined with other neurological features.[23]

From self-report surveys, 43.2% (N=37) of the participants in the Kuiper et al. (2018) study perceived problems with fine motor tasks, and some also reported difficulties with gross motor skills (running or balance). In 36.8% (n=19) of adults, the symptoms were self-perceived as progressive. Over three fourths of patients believed that their motor symptoms originated before 10 years of age. Speech problems were also frequent, appearing in 59.5% (N=37) of

participants. The only significantly associated clinical variable with presence of a motor disorder was delayed motor milestones development. Sex, age, time of diagnosis, strictness of dietary compliance, speech, fertility in women, special education, and employment status were not significantly associated with presence of motor disorders.[20]

The neurological component of speech disorders and their correlation with motor disorders in patients with CG was previously evaluated by Potter et. al (2013) in a study of 32 CG children with neurologic speech disorders and 130 controls. Participants were tested for coordination as well as hand and tongue strength. Results indicated that CG children had weaker hand and tongue strength than controls, and 66% (N=32) of cases were assessed to have significant coordination disorders of balance and/or manual dexterity.[14] The high prevalence of co-occurring speech, coordination, and strength disorders may be evidence of a common underlying etiology. These disorders are hypothesized to be a result of diffuse cerebellar damage rather than being distinct disorders.[14]

Speech disorders in the CG population were defined to be of neurologic origin and were classified into three subtypes in the study by Potter et. al: childhood apraxia of speech (CAS), dysarthria, or a motor speech disorder not otherwise specified. CAS entails a problem with motor planning or programming, whereas dysarthria is a deficit in neuromuscular control.[14] CAS is estimated to have a prevalence of 18 percent in the galactosemia population which is 180 times the estimated risk for CAS in the general population.[24] Tests used in the study by Potter et al. (2013) for motor control included: manual dexterity, ball skills, and balance. From these three tests, all scores were added together to create a total impairment score.

Males and females with CG made more articulation errors than did controls, and children diagnosed with CAS or dysarthria had more speech errors than children classified as having a non-specified motor disorder. Additionally, children with galactosemia had weaker tongue strength than controls, and within the galactosemia group, tongue strength was not significantly different among diagnoses.

No significant difference between the dominant hand strength compared to non-dominant hand strength was found between cases and controls; however, males and females had overall weaker dominant and non-dominant hand strength as compared to controls. The standardized scores of coordination for all age groups of children with galactosemia (4-5 and 6-16 years of age) were markedly below those of the general population. Finally, children with galactosemia and speech disorders were found to be 3.5 times more likely to have a co-occurring coordination disorder as compared to the general population with both speech and language disorders.[14]

Co-occurrence of speech and motor disorders is common in the general population as well as in Galactosemia.[25] Motor disorders are defined as deficits in strength and coordination. Out of 3,000 children referred for assessment of developmental delays, one third of the children with speech disorders also had a coordination disorder. However, two thirds of children with Galactosemia are estimated to experience a co-occurring coordination disorder.[22] In the general population, males are more likely to experience developmental disorders and have twice the prevalence of coordination and speech disorders compared to females.[14] The ratio of males to

females with coordination disorders in the galactosemia population is currently unknown and is a specific interest of this study.

It has been proposed that speech and motor disorders should not be considered two distinct disorders because they appear to result from a common underlying cause that affects several motor domains. The cerebellum is responsible for maintaining balance (midline of the cerebellum), refining motor movements and motor learning (right and left lateral cerebellar hemispheres), and speech production (superior lateral area of the right cerebellar hemisphere). Two studies, with a total of 19 CG participants, showed evidence of cerebellar degeneration in cases. The breadth of area affected suggests diffuse damage across the cerebellum rather than distinct focal areas of damage.[14,26,27] A case study of a 10-year-old girl with CG presenting with decreased level of consciousness was found to have galactitol accumulation and associated edema of the brain.[28] This patient was on a galactose restricted diet, and even though the lesion was reversible and the patient recovered with no focal neurological deficits, this case emphasized the importance of evaluating acute and chronic brain damage caused by galactosemia.[28]

NEUROGLYPHICS SOFTWARE

Neuroglyphics (NG) is a software program publicly available for download at (http://neuroglyphics.org). The software provides an interface to record and analyze hand-drawn digitized Archimedes spirals. Archimedes spirals drawn with pen and paper have been a part of clinical assessment of tremor for over 200 years and give clinicians valuable information about hand fine motor control. Archimedes spirals are used specifically because they offer a

standardized action that is similar to those of daily living, such as writing.[29] Neuroglyphics is advantageous in that it allows for storage of images of collected spirals, as well as evaluation of spirals' position, velocity, and acceleration in both time- and frequency-domains. The value of this software is that it provides better sensitivity than visual ratings in capturing minute inaccuracies in spiral drawings.[29] It also allows assessment by a trained user who is not a movement specialist.

Investigation of validity, reliability, and sensitivity of tremor-intensity in essential tremor (ET) patients indicates that collection and analysis of hand-drawn spirals using NG is a more effective and more sensitive method for evaluating hand find motor control than visual rating.[29] Neuroglyphics has been previously used to evaluate hand fine motor control in patients with essential tremor, Parkinson's, multiple sclerosis, functional (psychogenic) tremor, Niemann-Pick disease type C, neurodegenerative ataxias, Huntington disease, and tick disorder.[30–34] However, it has not previously been used in patients with CG to our knowledge.

Utilization of NG in the context of classic galactosemia is important because motor problems have been frequently reported, but severity and prevalence of hand fine motor problems among CG patients has not been well studied. Use of NG provides a reliable method for standardizing assessment of hand motor disorders in classic galactosemia. Motor problems during task-specific actions such as writing have a great impact on the daily life of patients, and thus it is important to standardize a method of assessing severity so that effectiveness of interventions can be evaluated. The sensitive, quantitative measures may also be useful for clinical assessments.

The feature used in this study to measure hand fine motor control is root mean square (RMS) distance in millimeters from the “ideal spiral.” Time points are collected at a frequency of 100 Hz, and the root mean square distance from the ideal spiral is summed together over a uniform distance of the drawn spiral. The ideal spiral is the exact path of a spiral equidistant from each side of the path (Figure 3). This measure was chosen because it is a continuous outcome that can give insight into small differences in hand control. Control subjects are expected to exhibits smaller deviations from the ideal spiral, and thus smaller RMS values.

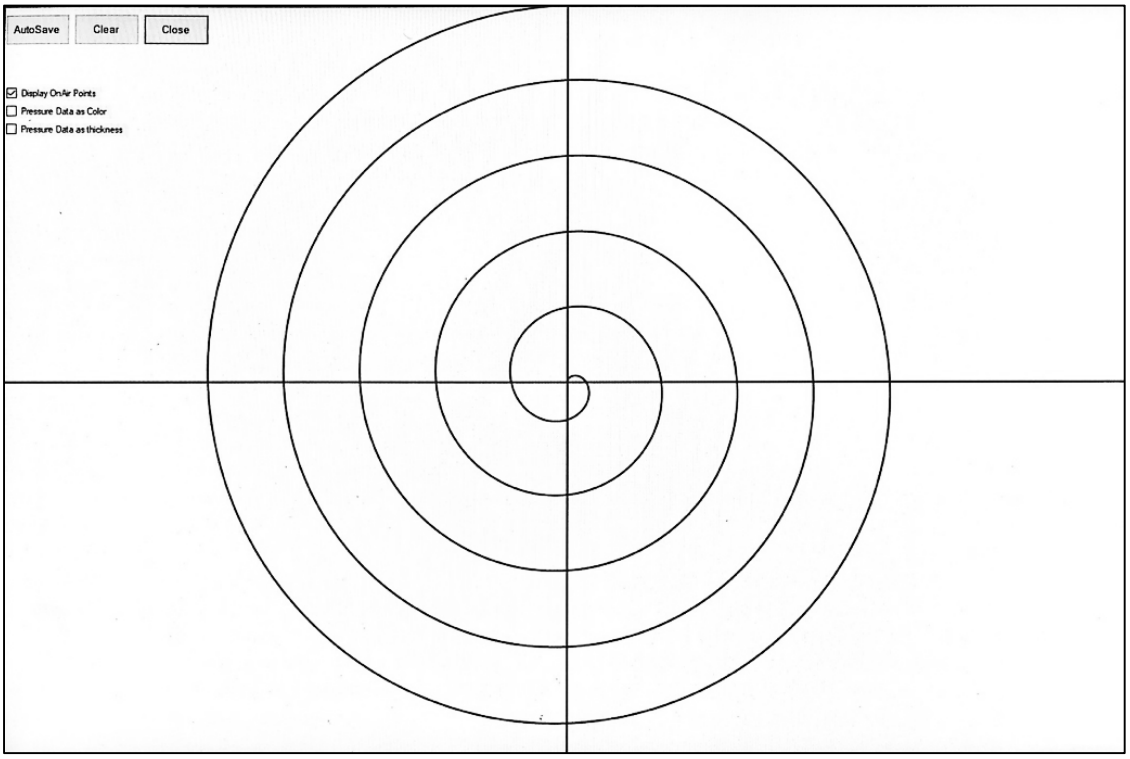

FIGURE 2. BLANK NG DATA COLLECTION SURFACE. PARTICIPANTS WERE INSTRUCTED TO START IN THE MIDDLE OF THE AXIS AND DRAW A SPIRAL ALONG THE MIDDLE OF THE DESIGNATED PATH.

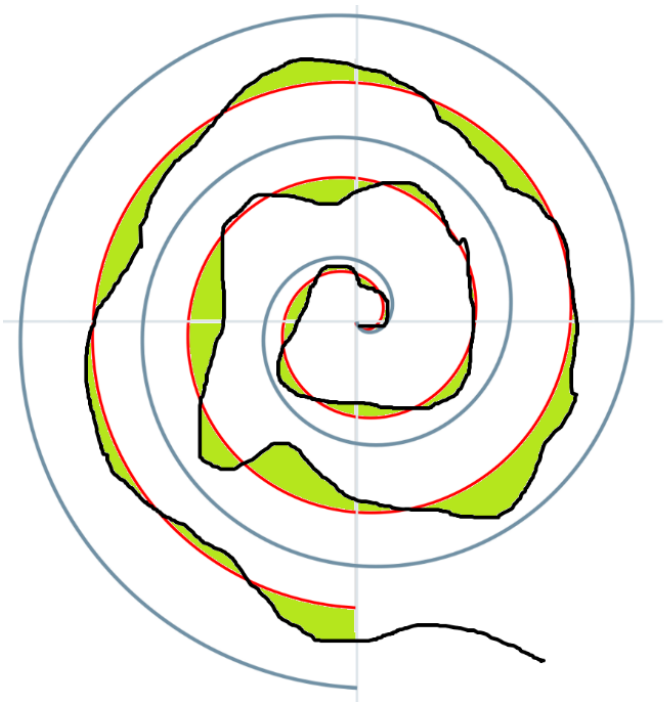

FIGURE 3. EXAMPLE OF DRAWN SPIRAL COMPARED TO WHAT IS CONSIDERED THE "IDEAL SPIRAL." THE BLUE CURVE REPRESENTS THE DESIGNATED SPIRAL PATH. RED INDICATES THE PATH OF THE IDEAL SPIRAL. BLACK IS THE EXAMPLE OF A DRAWN SPIRAL. GREEN FILLS THE SPACE BETWEEN THE DRAWN AND THE IDEAL SPIRAL. NEUROGLYPHICS HAS A SAMPLING RATE OF 100 HZ., MEANING THAT 100 TIMES PER SECOND THE DISTANCE BETWEEN THE DRAWN SPIRAL AND THE IDEAL SPIRAL IS MEASURED IN MILIMETERS, AND THEN THE ROOT MEAN SQUARE (RMS) IS CALCULATED TO GIVE EACH SPIRAL A SCORE.

While NG offers an innovative approach to the assessment of hand find motor control in patients with CG, it is important to note a few limitations. Crudely drawn spirals that do not stay within the space of the designated path are poorly quantified and often present as outliers, which we will have to exclude from some analyses (Figure 4). However, it is important to acknowledge the presence of these spirals because even though they can't be quantified by the software, they represent extreme cases of motor disability. These extreme cases that are unable to be quantified by NG software are included in calculations of motor control prevalence. It must also be noted that data are not recorded by NG when the pen leaves the surface of the tablet. For an individual with a severe tremor, it is possible that the pen tip may leave the surface of the tablet as a result of an uncontrollable tremor, but this is not recorded by the software. Even with

these limitations, NG has been validated as a quick and effective tool for measuring hand motor problems.[29]

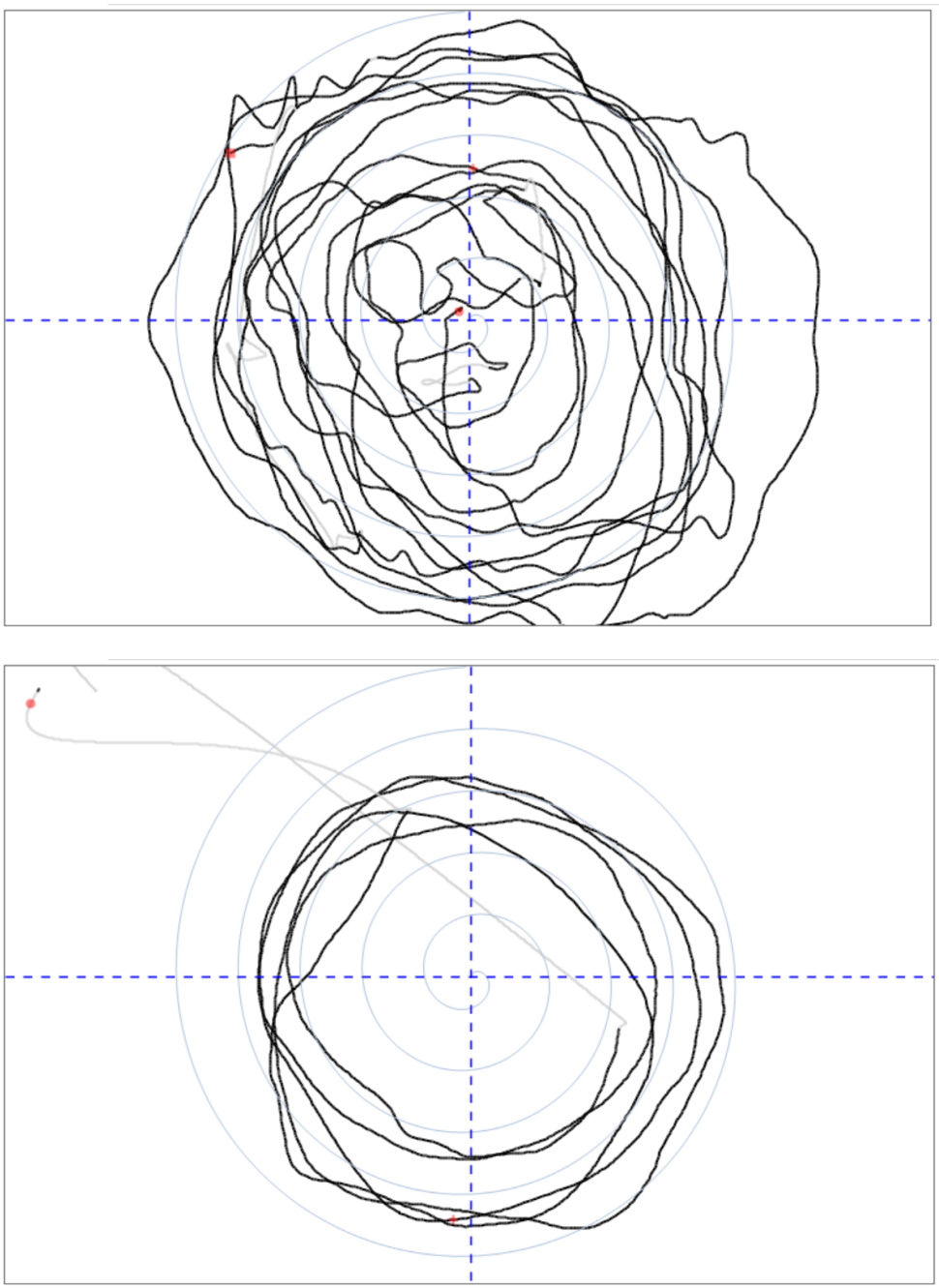

FIGURE 4. TWO CASES EXCLUDED FROM ANALYSIS BECAUSE THEY WERE UNQUANTIFIABLE BY NG SOFTWARE. THE FIRST ATTEMPT WITH THE DOMINANT HAND IS SHOWN FOR EACH EXCLUDED CASE. (A) 35-YEAR-OLD MALE (B) 6-YEAR-OLD MALE

CHAPTER 2: A SUBSET OF CHILDREN AND ADULTS WITH CLASSIC GALACTOSEMIA EXHIBIT NO DOMINANT HAND ADVANTAGE IN FINE MOTOR CONTROL

INTRODUCTION

During data collection, Neuroglyphics records the time taken to draw the spiral (spiral time). Participants were not instructed on how fast or slow to draw their spirals. Spiral time was suspected to be a possible covariate of RMS score, so the first step of this analysis investigated the relationship between RMS score and spiral time. Additionally, we wanted to use the variability in repeated trials of the same hand to assess whether the variability between dominant and non-dominant hands was greater or equivalent to the variability in repeated trials of the same hand. This finally leads us to the question of whether there is a dominant hand advantage in children and adults with classic galactosemia. In this study, participants who lack "dominant hand advantage" are those with poor motor control (scores beyond the 95^{th} percentile determined by the control distribution) who do not have a dominant hand that performs significantly better than their non-dominant hand.

METHODS

STUDY PARTICIPANTSE

Participants were attendants of the 2018 Galactosemia Foundation Conference in Denver, Colorado. Many families attend the conference, and family members of the CG individual frequently participated as controls. Table 1 presents summary statistics of the study sample.

Table 1. Sample Summary Statistics

		Case (n)	Control (n)	Total [N(%)]
sex				
	female	24	46	70 (52.63)
	male	29	34	63 (47.37)
dominant hand				
	right	51	76	129 (95.49)
	left	2	3	5 (3.76)
	ambidextrous	1	0	1 (0.75)
age (years)				
	6 to < 12	14	19	33 (24.81)
	12 to < 20	15	20	35 (26.32)
	20 to < 30	15	16	31 (23.31)
	30 +	9	25	34 (25.56)
total		53	80	133 (100.00)

TEST ADMINISTRATION

All participants were given the same directions before completing their spiral drawings, and the test administrator demonstrated how to complete the test before each participant began their first task. Starting with the tip of the pen in the middle of the tablet, and with elbow lifted from the table, participants were instructed to follow the middle of the path of the spiral. First, two spirals were drawn in the clockwise direction using the right hand and then two spirals were drawn in the counterclockwise direction using the left hand (Figure 5).

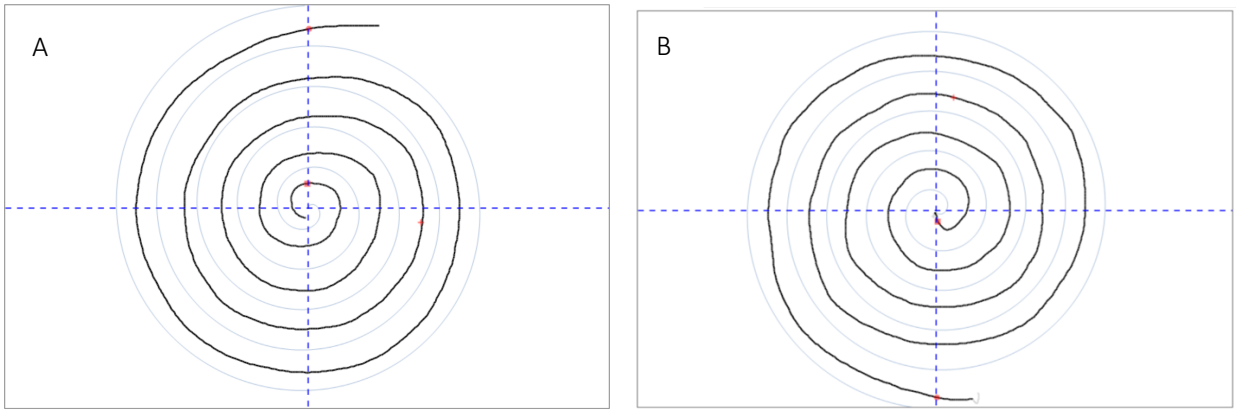

FIGURE 5. (A) CLOCKWISE ARCHIMEDES SPIRAL DRAWN WITH RIGHT HAND. THE SMALL, RED SQUARE REPRESENTS WHERE THE RMS CALCULATION BEGINS AND THE SMALL, RED CIRCLE REPRESENTS THE END OF THE CALCULATION. (B) COUNTERCLOCKWISE ARCHIMEDES SPIRAL DRAWN WITH LEFT HAND.

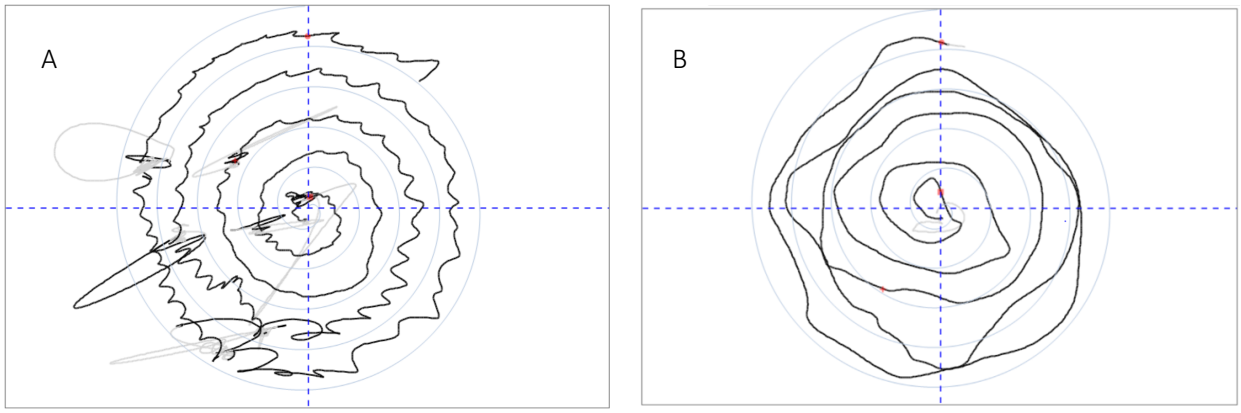

FIGURE 6. (A) CLOCKWISE SPIRAL WITH TREMOR. LIGHT GRAY STROKES REPRESENT MOMENTS WHEN THE PEN TIP LEFT THE TABLET SURFACE BUT WAS CLOSE ENOUGH TO STILL BE RECORDED BY THE TABLET. (B) CLOCKWISE SPIRAL DRAWN WITH THE INABILITY TO FOLLOW THE DESIGNATED SPIRAL PATH

Statistical methods

As mentioned in the introduction, two cases are excluded from all analyses using RMS scores because the spirals were unable to be scored by NG. For all other subjects, the relationship between spiral time and RMS score was assessed using simple linear regression. Slope and R^2 were used to assess the relationship between variables. Correlation between

dominant and non-dominant hands was assessed using Spearman correlation for non-parametric data.

The control mean and standard deviation of the difference between dominant hand trials 1 and 2 were used to determine whether the average dominant hand RMS score was better, worse, or equivalent to the average non-dominant hand RMS scores for each individual participant. Figure 7 outlines the methods and results for determining dominant hand advantage.

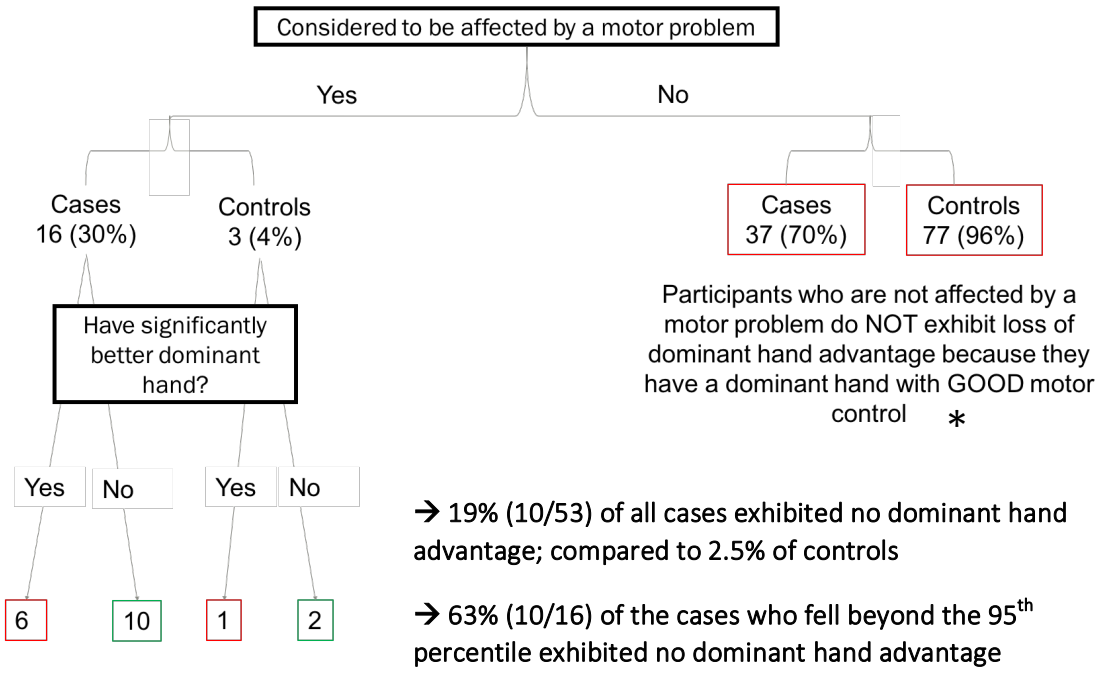

FIGURE 7. METHOD AND RESULTS OF DETERMINING THE PROPORTION OF CASES WHO EXHIBITED NO DOMINANT HAND ADVANTAGE. BOXES IN GREEN INDICATE THE NUMBER OF CASES AND CONTROLS THAT WERE CONSIDERED TO EXHIBIT NO DOMINANT HAND ADVANTAGE; 19% (10/53) OF CASES AND 2.5% (2/80). *GOOD MOTOR CONTROL IS DEFINED AS SPIRALS WITH RMS SCORES THAT FELL WITHIN THE 95TH PERCENTILE OF CONTROLS.

RESULTS

Cases and controls spent the same amount of time drawing dominant and non-dominant hand spirals (Table 2). Additionally, there was no significant difference between the dominant and non-dominant hand spiral times within cases or controls ($p = 0.44$ cases; $p = 0.83$ controls).

Table 2. Average Spiral Times for Cases and Controls in Seconds

	Case (mean ± SD)	Control (mean ± SD)
Dominant hand	23.12 ± 8.97	23.01 ± 6.84
Non-dominant hand	22.00 ± 7.90	23.21 ± 5.91
p-value (2-sample t-test; dominant hand spiral time vs. non-dominant hand spiral time within group)	$p = 0.44$	$p = 0.83$

Simple linear regression of spiral time and the respective RMS score indicated that spiral time is not a good predictor of RMS score. R^2 values indicate that only about 0.1% – 6% of the variation in RMS scores can be explained by the time taken to draw the spiral. Combining all cases and controls together, results indicated no predictive relationship between the two variables: $R^2 = 0.02$, $m = 0.01$ (Table 3). This was also the case when data were split up by cases and controls and by dominant and non-dominant hands. Furthermore, the slope of the best line in each group was approximately zero, supporting this conclusion. For these reasons, RMS scores were not adjusted to reflect spiral time.

A significant, moderate to strong linear relationship was observed between scores for dominant and non-dominant hand spirals for cases and controls ($p < 0.001$; $r = 0.67$; Spearman

correlation). Those with low RMS scores for their dominant hand tended to have lower scores for their non-dominant hand (Figures 7 - 9).

Table 3. Relationship Between Spiral Time and RMS Score

		All	Case	Control
Both hands combined				
	r-squared	0.019	0.056	0.002
	slope	0.011	0.016	-0.006
Dominant hand only				
	r-squared	0.080	0.007	0.175
	slope	0.025	-0.032	0.028
Non-dominant hand only				
	r-squared	< 0.001	0.001	< 0.001
	slope	-0.001	-0.004	0.001

*r-squared represents the amount of variation in RMS score that is explained by spiral time. Slope (mm/s) represents the change in RMS score divided by change in spiral time.

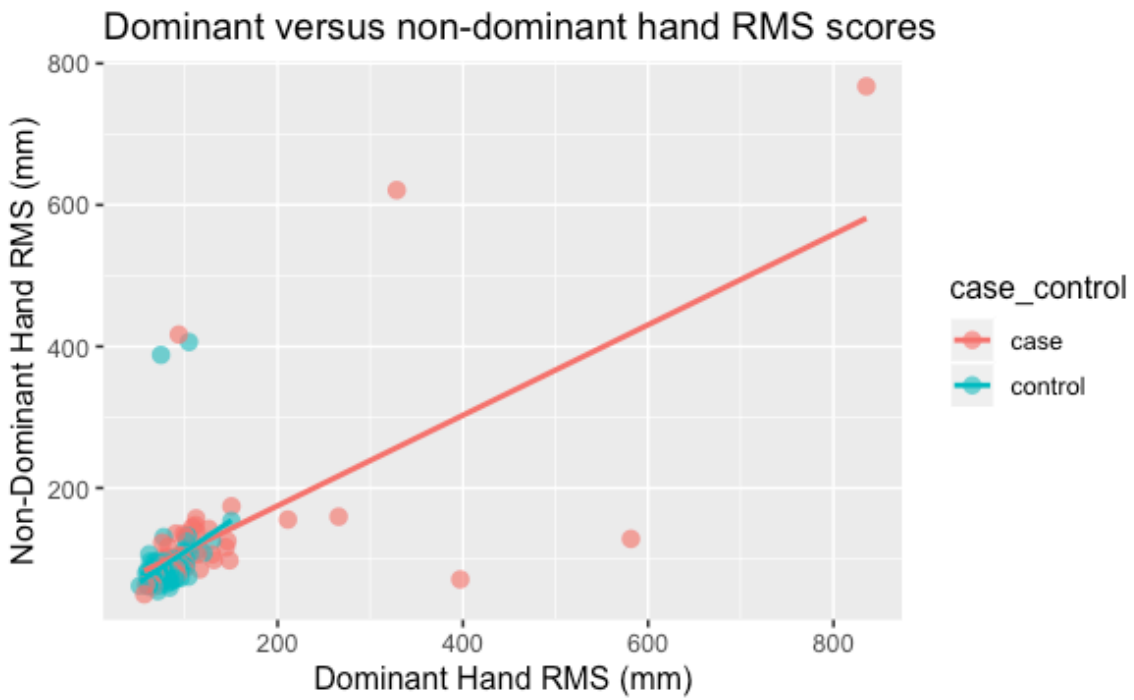

FIGURE 8. DOMINANT VERSUS NON-DOMINANT HAND AVERAGE RMS SCORES FOR CASES AND CONTROLS. A SIGNIFICANT CORRELATION BETWEEN DOMINANT AND NON-DOMINANT HANDS WAS OBSERVED AS WELL AS A MODERATE TO STRONG LINEAR RELATIONSHIP ($P < 0.001$; $R = 0.674$).

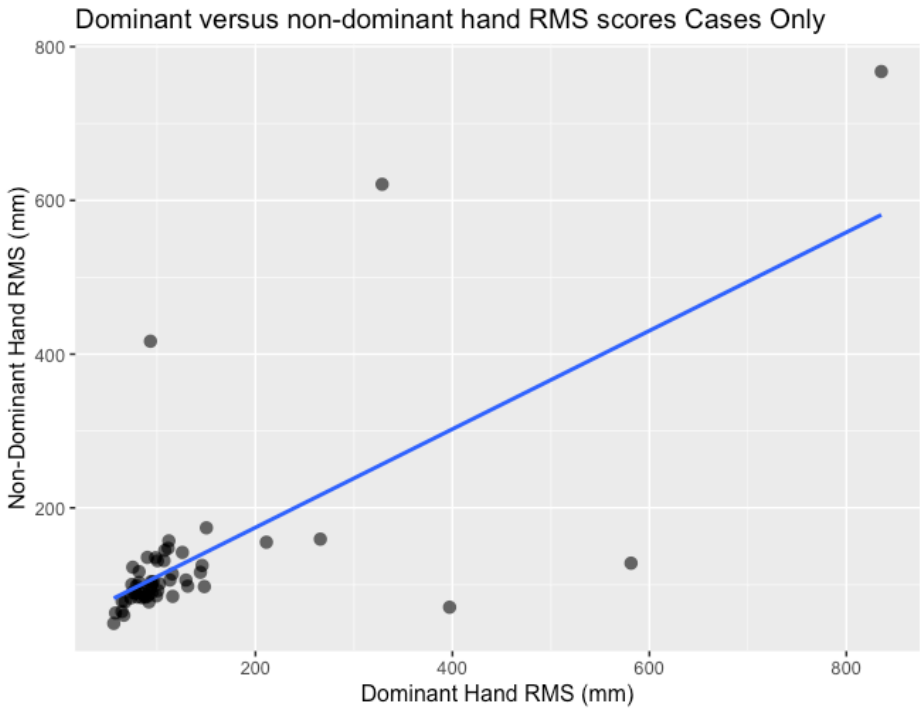

FIGURE 9. LINEAR RELATIONSHIP BETWEEN DOMINANT AND NON-DOMINANT HAND RMS SCORES. R-SQUARED = 0.4684, AND SLOPE=0.6399.

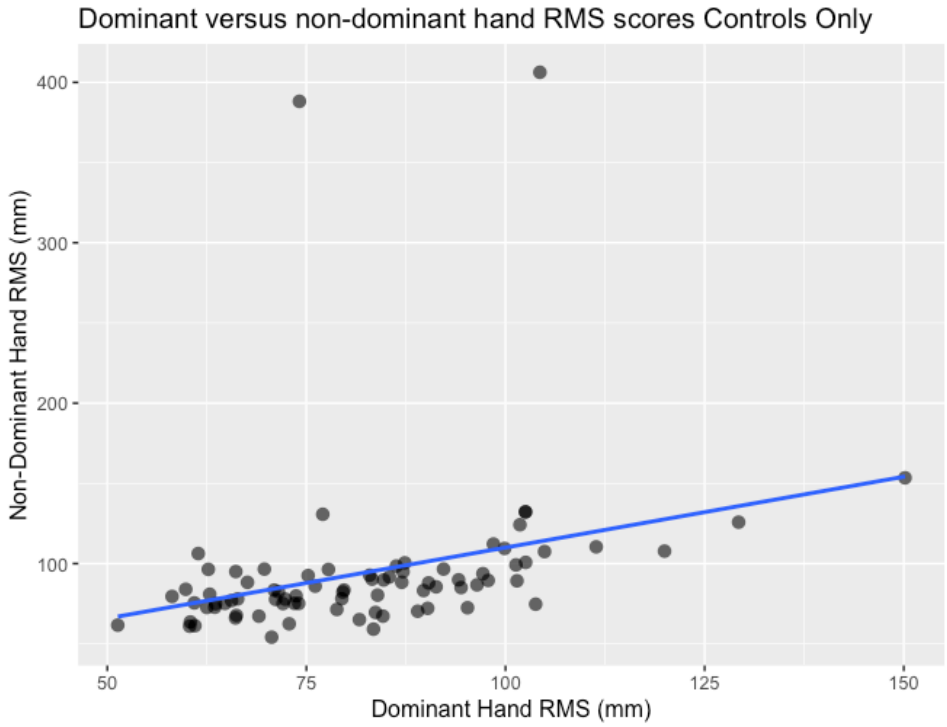

FIGURE 10. LINEAR RELATIONSHIP BETWEEN DOMINANT AND NON-DOMINANT HAND RMS SCORES. NOTE TWO OUTLIERS THAT DO NOT FOLLOW LINEAR RELATIONSHIP. R-SQUARED = 0.0858, AND SLOPE=0.8728.

Each participant drew two spirals with their dominant hand (the two spirals will be called A1 and A2). Each participant also drew two spirals with their non-dominant hand (the two spirals will be called B1 and B2). A1 minus A2 gives the difference in RMS scores between the two spirals drawn with the subject's dominant hand, and B1 minus B2 gives the difference between the two RMS scores of the non-dominant hand. A1 minus A2 for each individual was averaged for all cases and controls. The same was done with B1 minus B2. These results are presented in Table 4 ($\bar{x} \pm SD$). Zero was contained within the 95% confidence interval for cases and controls within each age group. This means that there was no detectable difference in RMS scores between repeated spiral drawings of the same hand.

The mean and standard deviation of A1 minus A2 (-0.97 ± 30.06 for ages 6 to <12; 5.52 ± 15.66 for ages 12 +) was the amount of variation expected between two repeated trials of the dominant hand for a control subject. The 95% confidence interval was used to determine whether the average dominant hand RMS score was significantly different from the average non-dominant hand RMS score. If the difference between dominant and non-dominant hand RMS scores was outside of -0.97 ± 30.06 for ages 6 to <12 or 5.52 ± 15.66 for ages 12 +, we considered there to be a significant difference between dominant and non-dominant hands. Otherwise, the dominant and non-dominant hands were considered equivalent (Table 5).

As stated earlier, an individual with no dominant hand advantage was considered to be a subject who had poor motor control (fell outside of the 95^{th} percentile of controls) and did not have a dominant hand that performed better than their non-dominant hand. A total of 19% of cases were determined to exhibit no dominant hand advantage (Figure 7). Of the 16 cases

identified to have motor control problems (see Chapter 3), 63% exhibited no dominant hand advantage.

Table 4. Average Difference in Repeated Trials of Spiral Drawing with the Dominant and Non-Dominant Hands [$\bar{x}$ ± SD]

		Cases	Controls
Dominant (A1 minus A2)			
	6 to < 12	24.42 ± 200.67	-0.97 ± 30.06 *
	12 +	-22.91 ± 112.15	5.52 ± 15.66 *
Non-dominant (B1 minus B2)			
	6 to < 12	47.25 ± 209.62	-5.79 ± 23.39
	12 +	-0.77 ± 20.85	20.91 ± 105.23

* Control mean and standard deviation used to determine whether the difference between average dominant RMS score and average non-dominant RMS score were significant.

Table 5. Classification of Cases and Controls as Having Equivalent, Better Dominant, or Better Non-Dominant RMS Scores [N(%)]

	Cases	Controls
equivalent RMS scores	24 (45.28)	55 (68.75)
better dominant hand RMS score	21 (39.62)	21 (26.25)
better non-dominant hand RMS score	8 (15.09)	3 (3.75)
total	53 (100.00)	80 (100.00)

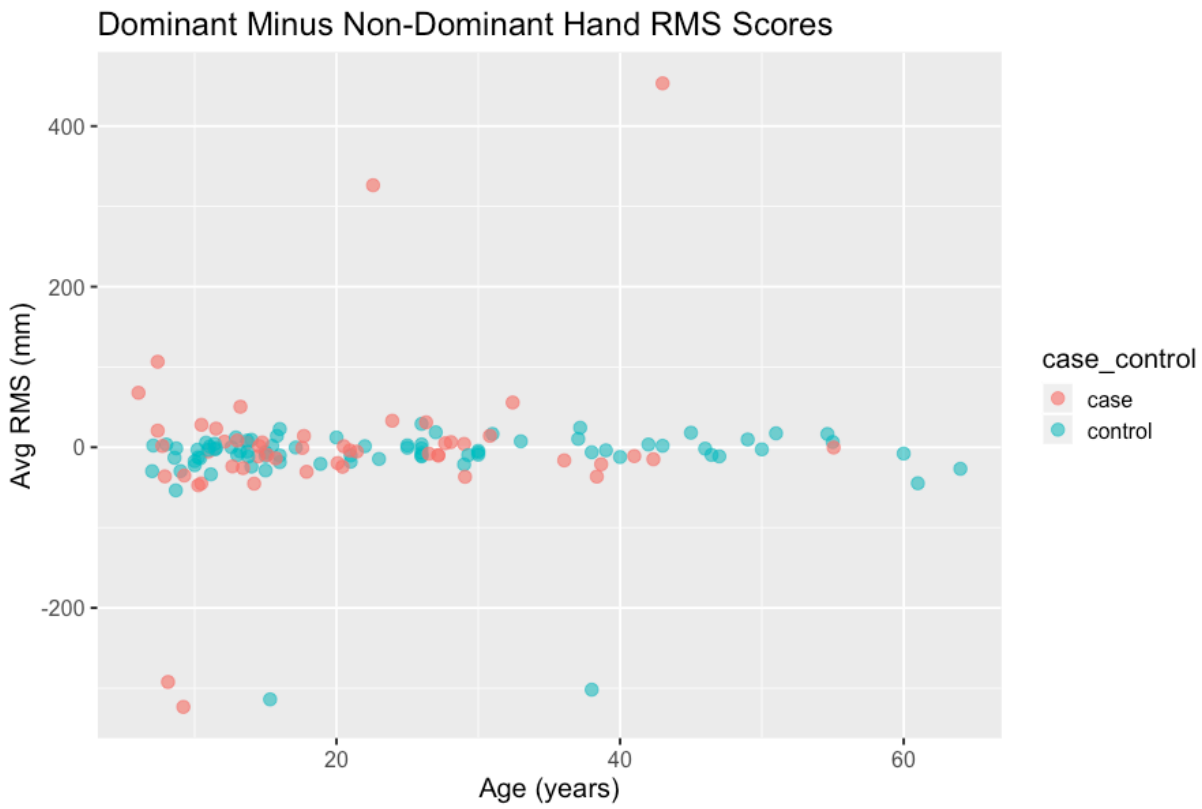

FIGURE 11. DIFFERENCE BETWEEN DOMINANT AND NON-DOMINANT HAND RMS SCORES. MOST POINTS FOR CASES AND CONTROLS FALL NEAR THE X-AXIS.

DISCUSSION

Participants were not instructed how fast or slow to draw their spirals, and spiral time was thought to be a possible covariate. However, results indicate no predictive relationship between spiral time and RMS score.

Dominant and non-dominant hand RMS scores were linearly correlated within cases and controls which suggests that motor control affects both hands similarly. Participants with higher RMS scores for the dominant hand also tended to have higher RMS scores for the non-dominant hand. In addition, participants with lower dominant hand RMS scores tended to have lower RMS scores for their non-dominant hand.

The majority of controls and about half of cases had approximately equivalent RMS scores for both hands. A possible explanation for the high proportion of cases and controls with equivalent hand RMS scores is that some participants focused more while drawing their non-dominant spirals, being aware of the increased difficulty of completing a spiral with the non-dominant hand.

Another notable feature of Table 4 is that about 15% of cases had better non-dominant hand control than dominant hand control. The cases with better non-dominant hand control were highly represented in the group of cases that fell beyond 95th percentile of RMS scores (95th percentile calculated from control distribution; see Chapter 3). The scores that fall beyond the 95th percentile of controls are considered to be affected by a motor control problem. The 8 cases in the "better non-dominant hand RMS group," were all beyond the 95th percentile of controls. The 8 cases in this group included 2 children in the 6 to < 12 age group, and 6 participants in the 12 and above age group.

Figure 11 outlines the methods and results of the calculation of dominant hand advantage. The majority (66%) of the participants affected by motor control problems exhibited no dominant hand advantage, meaning their dominant hand RMS score was not significantly better than their non-dominant hand RMS score. An interpretation of this result is that it is possible that these participants chose a hand to be their "dominant hand" even if they really did not favor that side. Of the 10 participants with no dominant hand advantage, 9 reported that they were right-handed and one reported that they were left-handed. Right handedness is more

common than left, and if it was difficult to use both hands, it makes sense that one might choose the right hand to be the so-called dominant.

One of the three controls whose dominant hand RMS score fell outside the 95th percentile had a significantly better dominant than non-dominant hand RMS score. The remaining two controls exhibited no dominant hand advantage. Where 19% of cases exhibited no dominant hand advantage, only 2.5% of controls fell into this category. We can conclude that cases experience worse hand fine motor control than controls, and a majority of affected cases do not have a dominant hand that is significantly better than their non-dominant hand.

CHAPTER 3: CHILDREN AND ADULTS WITH CLASSIC GALACTOSEMIA HAVE MORE ERROR IN ARCHIMEDES SPIRAL DRAWINGS THAN AGE-MATCHED CONTROLS

INTRODUCTION

An estimate of fine motor problems based on self-report and expert examination is about 40% in patients with classic galactosemia.[20] Since hand fine motor problems have never been assessed for patients with CG using digitized tablets and NG software to our knowledge, a main question of interest in this study was whether Neuroglyphics can detect a difference between cases and controls, and what proportion of cases score outside of the normal range as determined by control 95th percentile limits.

Additionally, we wanted to examine whether symptoms worsen with age, as self-reports indicate perceived worsening over time.[20] No longitudinal data on NG scores are yet available, but cases stratified by age offer a first approach to this question.

METHODS

STATISTICAL METHODS

The purpose of the statistical analysis was to test for possible differences between cases and controls. Non-stratified cases and controls were evaluated for significant difference between dominant hand RMS scores. Exact Wilcoxon was used to assess significance. Children age 6 to 11.99 were separated from subjects ages 12 and above.

Cases and controls 12 years old and above were then separated to form similarly sized groups. Each age stratum was then evaluated separately. Using dominant hand only and all four spirals together, cases and controls were tested using the 2-sample exact Wilcoxon. Functional

significance of abnormality is often designated to be two standard deviations outside the control mean. Since the RMS score distribution is non-normal and asymmetrical, functional significance was determined to be outside the 95% percentile of the control sample. The Kruskal-Wallis Test was used to determine significant differences between all four age groups.

RESULTS

Median RMS scores for cases and controls were significantly different ($p < 0.001$) with control median and quartiles substantially lower than case median and quartiles (Table 6). Further, all stratified age groups showed significant differences between cases and controls (Table 7). The 6 to < 12 age group had significantly higher RMS scores for both cases and controls than did all other age groups ($p = 0.001$ controls; $p = 0.01$ cases) (Table 8). The older three age groups were all not significantly different from one another ($p = 0.1085$; Kruskal-Wallis Test). Means increased with age among the three age-groups above 12 years of age, but this was largely due to outliers, and medians did not increase with age (Figure 13).

The distribution of dominant hand RMS scores is right-skewed, so control percentile scores were used to determine functionally relevant cutoffs instead of mean and standard deviation. Two cases had spirals that were unquantifiable by NG software because the spirals were too crudely drawn for RMS error from ideal spiral to be calculated. Because these individuals represent patients with poor motor control, they need to be counted in the results of the study even though their spirals were not able to be quantified. Because these spirals were beyond the limit of quantification, they were considered as the upper end of severity in terms of motor control problems. To determine the number of cases and controls that fell beyond

functionally defined normality, all participants were split into age groups 6 to < 12 and 12 and above since controls in the 6 to < 12 group were significantly different from controls in the 12 and above group. About 30% (n=55) of cases (including the two unquantifiable cases) as well as 7.50% (n=80) of controls fell beyond the 95th percentile limit when calculated using the dominant hand spiral scores only. Using all four spirals from both hands combined, about 36% (n=55) of cases and under 4% (n=80) of controls fell beyond the 95th percentile limit (Table 7). We thus estimate that about 30 to 36% of cases are affected by hand fine motor control problems. Additionally, 23.64% of cases and 1.25% of controls fell outside the 97th percentile, and 16.36% cases fell outside the 100th percentile for controls.

Table 6. Dominant and Non-Dominant Hand RMS Scores [Mdn (Q1, Q3)]

	Cases (n=53)	Controls (n=80)
Dominant hand	95.39 (81.80, 115.97)	80.68 (69.38, 94.27)
Non-dominant hand	100.77 (85.23, 131.38)	83.98 (74.95, 96.43)
Both hands combined	99.43 (73.96, 93.02)	82.65 (73.96, 93.12)

Table 7. P-values and Test Statistics for Age Stratified RMS Scores Using Exact Wilcoxon

	Group	P-value
Dominant hand only		
	All Cases and Controls (n=53 cases; n=80 controls)	< 0.001
	6 to < 12 (n=14 cases; n=19 controls)	0.007
	12 to < 20 (n=15 cases; n=20 controls)	0.043
	20 to <30 (n=15 cases; n=16 controls)	0.018
	30+ (n=9 cases; n=25 controls)	0.007
Both hands combined		
	All Cases and Controls (n=53 cases; n=80 controls)	< 0.001
	6 to < 12 (n=14 cases; n=19 controls)	0.001
	12 to < 20 (n=15 cases; n=20 controls)	0.074*
	20 to <30 (n=15 cases; n=16 controls)	0.006
	30+ (n=9 cases; n=25 controls)	0.002

*indicates a non-significant result; alpha = 0.05

Table 8. Comparison of RMS Scores for Individuals Younger and Older Than 12 Years

		age group	n	median	IQR
Cases					
	Dominant hand only				
		older than 12	39	91.86	29.98
		younger than 12	14	113.84	52.44
	Both hands combined				
		older than 12	39	92.39	32.33
		younger than 12	14	129.82	113.21
Controls					
	Dominant hand only				
		older than 12	61	78.82	22.76
		younger than 12	19	94.11	27.31
	Both hands combined				
		older than 12	61	79.64	17.88
		younger than 12	19	100.25	29.14

Table 9. Cases and Controls Outside the 95th, 97th, and 100th Percentiles‡

	Quantile	Cases [n(%)]	Controls [n (%)]
Dominant hand only			
95th percentile			
6 to <12 (n = 14)	150.16	4 (28.57)*	0
12 + (n = 39)	102.53	12 (30.77)**	3
Total of all cases or controls		16 (30.19)	3 (3.75)
97th percentile			
6 to <12 (n = 14)	150.16	4 (28.57)*	0
12 + (n = 39)	104.32	12 (30.77)**	1
Total of all cases or controls		16 (30.19)	1 (1.25)
100th percentile			
6 to <12 (n = 14)	150.16	4 (28.57)*	0
12 + (n = 39)	119.96	8 (20.51)**	0
Total of all cases or controls		12 (22.64)	0 (0.00)
Both hands combined			
95th percentile			
6 to <12 (n = 14)	151.81	5 (35.71)*	0
12 + (n = 39)	104.67	14 (35.90)**	3
Total of all cases of controls		19 (35.85)	3 (3.75)
97th percentile			
6 to <12 (n = 14)	151.81	4 (35.71)*	0
12 + (n = 39)	231.14	3 (7.69)**	1
Total of all cases of controls		7 (13.21)	1 (1.25)
100th percentile			
6 to <12 (n = 14)	151.81	4 (35.71)*	0
12 + (n = 39)	255.29	2 (5.13)**	0
Total of all cases and controls		6 (11.32)	0 (0.00)

‡Cells contain count and joint percentages outside specified percentile. Percentiles determined by control distribution. Highlighted rows contain total count of both age groups combined and the percent of all cases or controls.

* extreme case (6 years of age) is added to count

** extreme case (34 years of age) is added to count

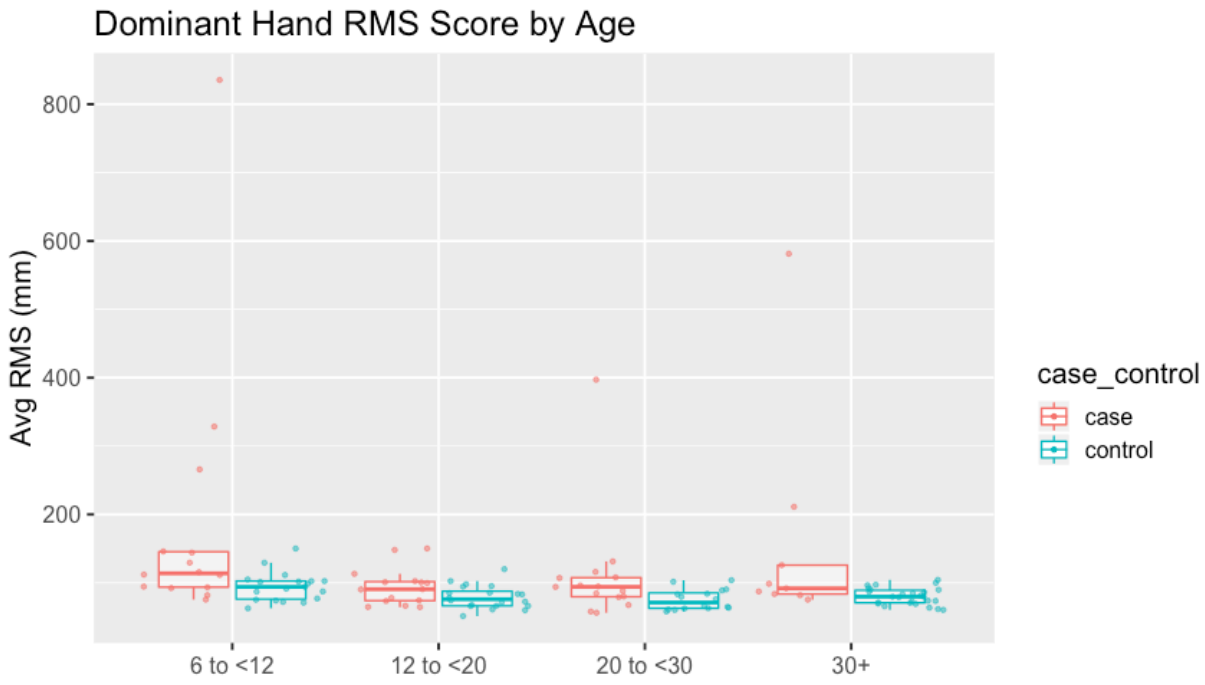

FIGURE 12. SIDE BY SIDE BOXPLOTS OF CASES (RED) AND CONTROLS (BLUE)FOR EACH AGE GROUP USING DOMINANT HAND ONLY. CASES AND CONTROLS WERE SIGNIFICANTLY DIFFERENT WITHIN EACH GROUP, AND GROUP 6 TO < 12 HAD SIGNIFICANTLY HIGHER RMS SCORES THAN THE OLDER AGE GROUPS.

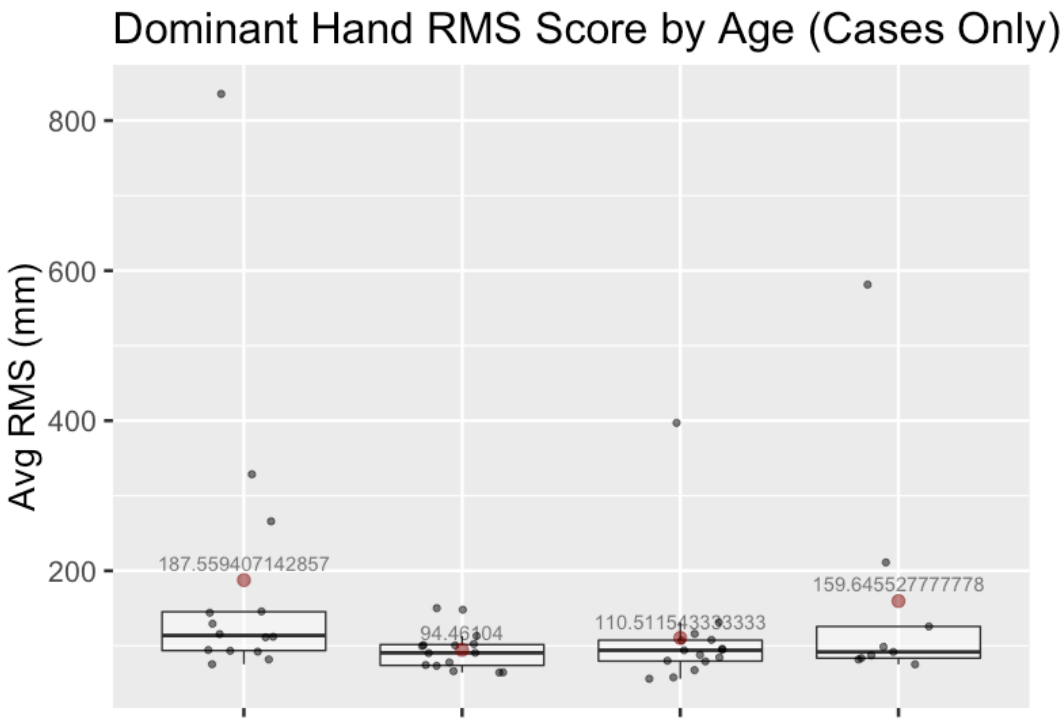

FIGURE 13. OBSERVATION SHOWS THAT MEANS (RED POINT) INCREASE IN THE OLDER THREE GROUPS. HOWEVER, MEDIANS ARE NOT SIGNIFICANTLY DIFFERENT FOR THE OLDER THREE AGE GROUPS.

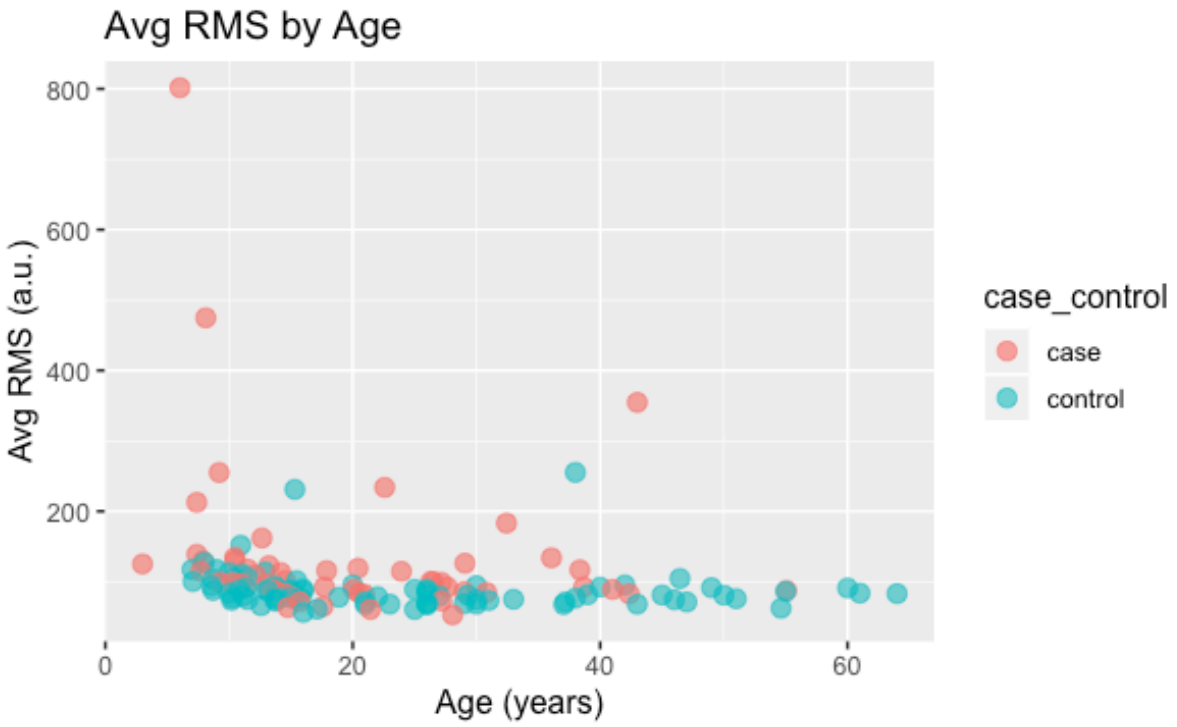

FIGURE 14. SCATTERPLOT OF AVERAGE RMS SCORES FOR CASES (RED) AND CONTROLS (BLUE). AGE CUTOFFS WERE MADE BASED ON THESE DATA WHICH SHOW THAT RMS SCORES DECREASE IN CHILDREN FROM AGE 6 TO ABOUT 12 YEARS OF AGE.

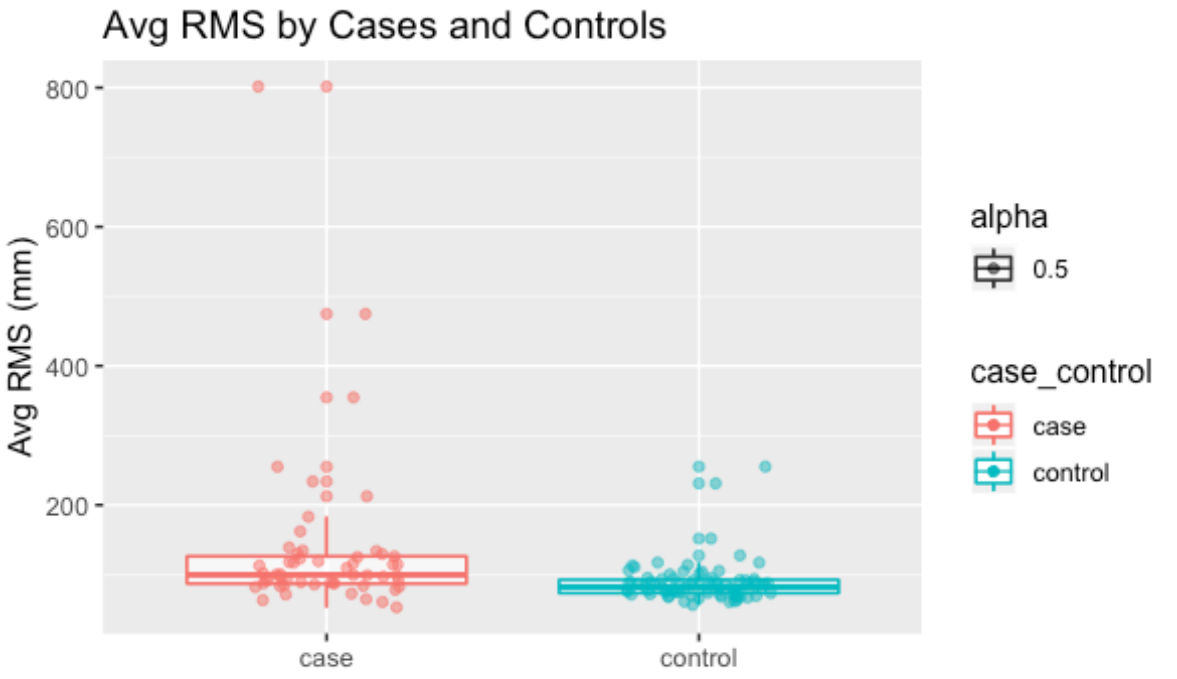

FIGURE 15. AVERAGE RMS SCORES FOR ALL AGES OF CASES (N=53) AND CONTROLS (N=80) COMBINED. CASES HAD SIGNIFICANTLY HIGHER RMS SCORES THAN CONTROLS AS WELL AS A GREATER INTERQUARTILE RANGE.

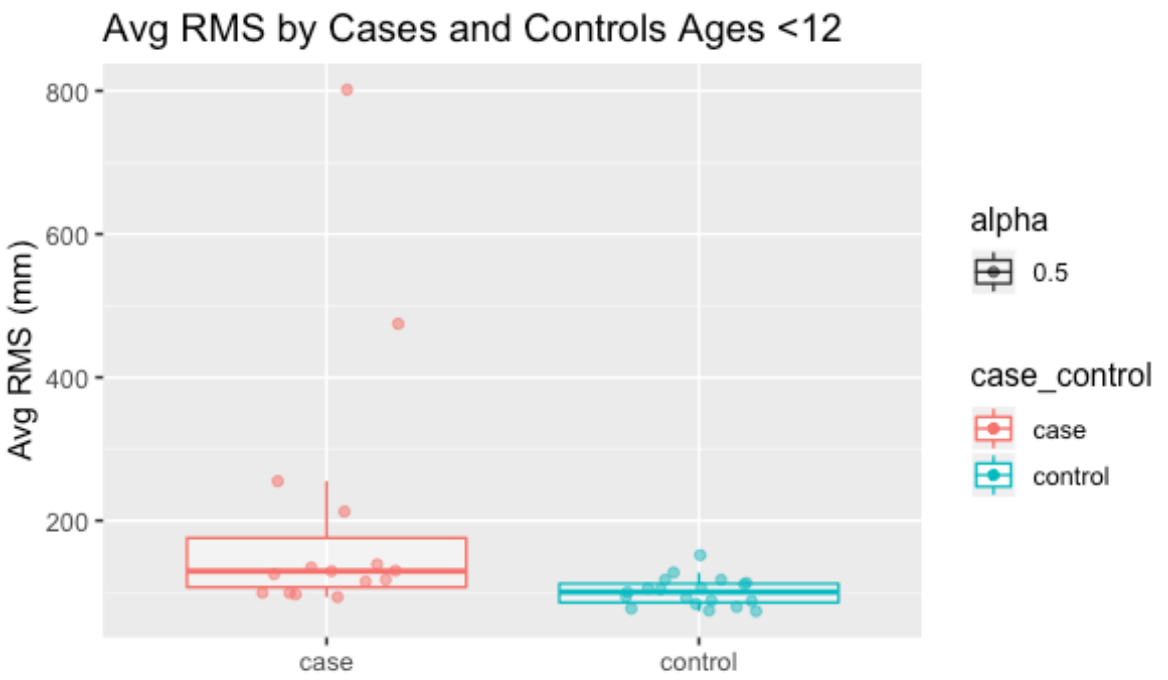

FIGURE 16. AVERAGE RMS SCORES FOR CASES (N=14) AND CONTROLS (N=19) AGE 6 TO < 12. CASES HAD SIGNIFICANTLY HIGHER RMS SCORES THAN CONTROLS AS WELL AS A GREATER INTERQUARTILE RANGE.

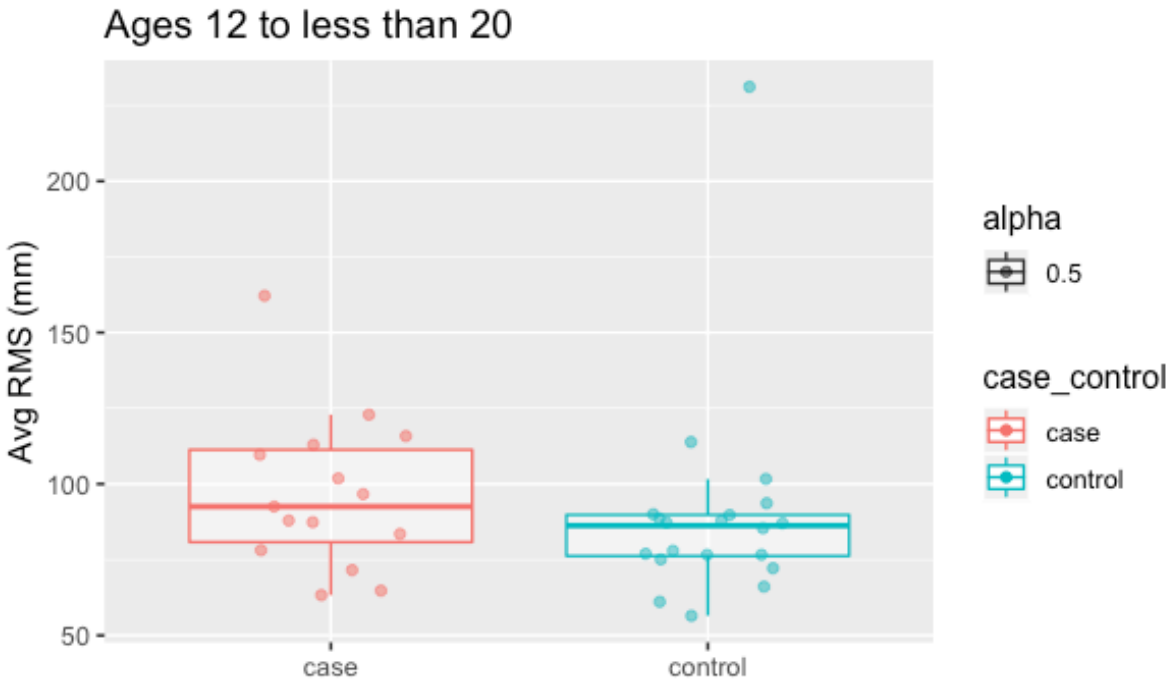

FIGURE 17. AVERAGE RMS SCORES FOR CASES (N=15) AND CONTROLS (N=20) AGE 12 TO <20. CASES HAD SIGNIFICANTLY HIGHER RMS SCORES THAN CONTROLS AS WELL AS A GREATER INTERQUARTILE RANGE.

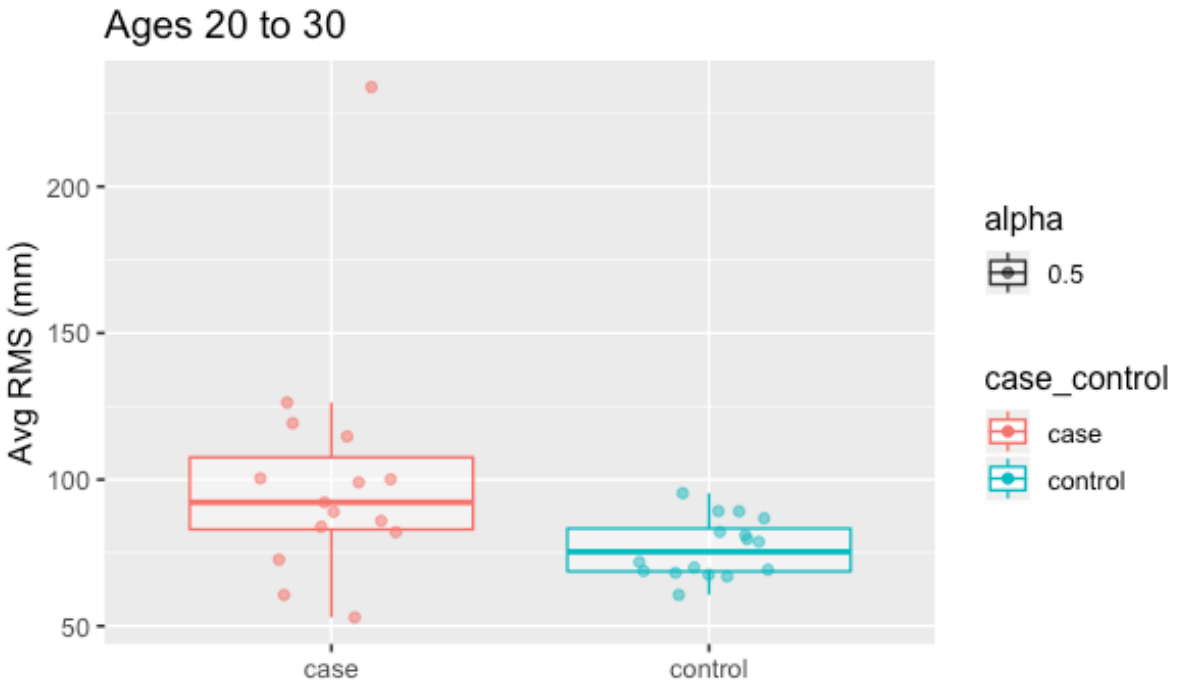

FIGURE 18. AVERAGE RMS SCORES FOR CASES (N=15) AND CONTROLS (N=16) AGE 20 TO <30. CASES HAD SIGNIFICANTLY HIGHER RMS SCORES THAN CONTROLS AS WELL AS A GREATER INTERQUARTILE RANGE.

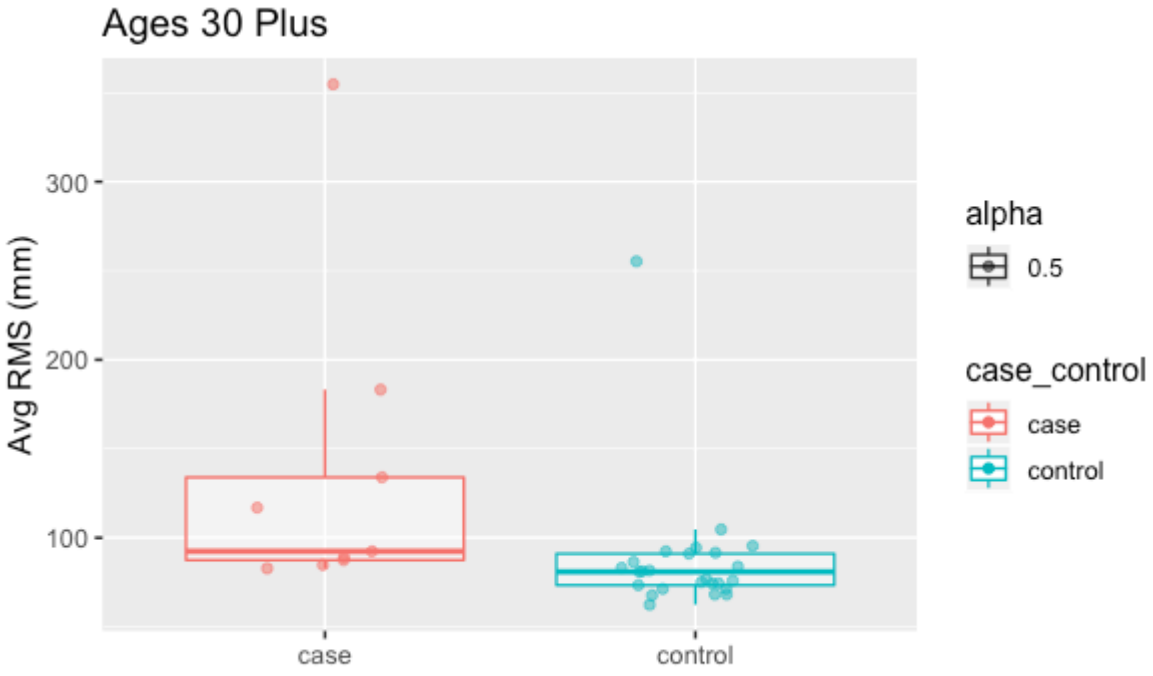

FIGURE 19. AVERAGE RMS SCORES FOR CASES (N=9) AND CONTROLS (N=25) AGE 30 AND OLDER. CASES HAD SIGNIFICANTLY HIGHER RMS SCORES THAN CONTROLS AS WELL AS A GREATER INTERQUARTILE RANGE.

DISCUSSION

Cases and controls were significantly different in every age group and also when combined together. Agreeing with anecdotal expectation, cases and controls below 12 years of age had significantly higher RMS scores than ages 12 and above, indicating that fine motor control isn't fully developed in younger children. In the Kuiper et al. (2018) study, 78.95% (n=19) of adult patients reported that their motor symptoms started before the age of 10 years, 10.53% reported that they started between the ages of 10 and 20, one patient between 20 and 40 years, and 5.26% after the age of 40.[20] This information agrees with the results mentioned above in that motor symptoms were detected in children below 12 years as well as in all age groups above 12 years of age.

It has been an open question in CG whether motor symptoms are progressive, and 36.84% (n=19) of the adults from the Kuiper et al. (2018) study reported that their symptoms worsened with age. This information was collected retrospectively and through open-ended surveys, and no quantitative evidence has confirmed the progressivity of motor symptoms in classic galactosemia. In our study, cases showed no statistical difference in RMS median scores between age groups above 12 years of age, suggesting severity may not increase with age. However, since ours was not a longitudinal study, this does not provide definitive evidence for the lack of progressivity.

The most compelling results of this analysis are summarized in Table 9. Quantifying the proportion of patients who experience motor control problems has been done in a number of ways including expert examination and self-report surveys. Here we report the results of a large

study that uses a method of quantification that does not rely on human subjectivity in diagnosis of patients and can be done without the presence of a neurologist or motor specialist. This technique is convenient, completely non-invasive, and only takes about three minutes per subject to complete. Using this method, we were able to identify about 30 to 36% of CG participants who exhibited problems with hand fine motor control.

CHAPTER 4: NO MOTOR DIFFERENCES FOUND BETWEEN MALES AND FEMALES WITH CLASSIC GALACTOSEMIA

INTRODUCTION

In the general population, males have approximately twice the prevalence of coordination (1.8:1) and speech disorders (2:1) as females.[35] The ratio of male to female patients with CG and coordination disorders is unknown, and it is also unknown whether male CG patients experience worse outcomes than females.[14] Out of 18 patients diagnosed with motor problems by expert examination in the Kuiper et al. (2018) study, 44.44% were male and 55.56% were female, and no significance of association between sex and presence of motor disorders was identified. Of the eight males diagnosed with motor disorders, the average overall Clinical Global Impression (CGI, a 7-point scale with higher scores indicating more severe motor disorders) severity scale score was 2.75. Of the 10 females with a motor disorder, the average overall CGI severity scale score was 3.4; however, the differences in CGI scores were also not significant.[20] We analyzed our NG data to test these conclusions in our own dataset.

METHODS

STATISTICAL METHODS

The Wilcoxon Exact test was used to assess significance between males and females.

RESULTS

We found no significant difference in RMS scores between males and females with all ages combined ($p = 0.36$; Wilcoxon Exact test). Additionally, there was no significant difference between males and females within any of the age groups tested: 6 to < 12 ($p = 0.18$); 12 to < 20 ($p = 0.12$); 20 to < 30 ($p = 0.12$); and 30 plus ($p = 0.25$). See Figure 20. Of the 22 cases found to lie

beyond the 95th percentile of the controls, about 50% were female and 50% were male (Table 10).

Table 10. Cases Outside 95th Percentile Stratified by Sex [n(%)]

		Female	Male	Total
Dominant hand only				
	6 to <12	1	3	4
	12 +	8	4	12
	Total	9 (56.25)	7 (36.84)	16 (100.00)
Both hands combined				
	6 to <12	1	4	10
	12 +	9	5	12
	Total	10 (52.63)	9 (47.37)	19 (100.00)

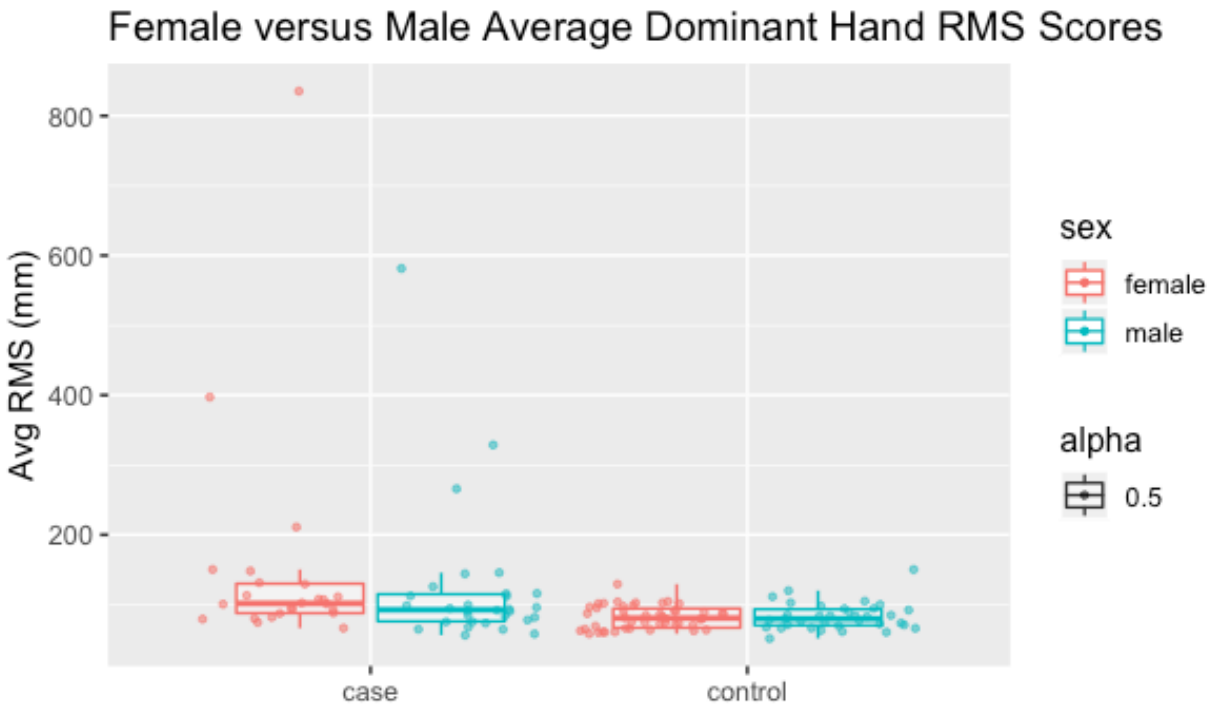

FIGURE 20. NO SIGNIFICANT DIFFERENCE WAS FOUND BETWEEN MALES AND FEMALES IN BOTH CASES AND CONTROLS.

DISCUSSION

No differences in RMS scores between males and females was found. A summary of cases found outside the 95th percentile stratified by sex is presented in Table 8. The Kuiper et al. (2018) study also found no evidence of difference in prevalence of hand fine motor problems between males and females with CG ($p = 0.64$). Although there is a potential for males to be more susceptible to neurodevelopmental problems in general, this does not seem to be the case for neuromuscular problems in classic galactosemia.[35] The mechanism by which the nervous system, and specifically the cerebellum in the case of neuro-motor and speech problems, is damaged in patients with classic galactosemia is unknown, but data suggest that it likely affects males and females similarly.

CHAPTER 5: DISCUSSION AND FUTURE DIRECTIONS

Use of a digitizing tablet and Neuroglyphics software is a quick and effective tool for identifying and quantifying fine motor control outcomes in patients with classic galactosemia. This method does not require the expertise of a neurologist and no special training is necessary. Using NG to quantify motor problems is an attractive option for measuring motor outcomes in future intervention studies. An strength of this study is the large sample size [N (cases) = 55 and N (controls) = 80] which allows us to provide meaningful statistical results. Even though outcomes such as cognitive ability and fertility have been more thoroughly, hand fine motor control problems also impact those living with them. Tasks such as writing your name, buttoning a shirt, or tying a shoe may be difficult components of daily life for those with hand fine motor control problems. The daily challenges that motor problems impart make them an important area for thorough study within classic galactosemia.

Results from this study further our current understanding of motor problems in CG. The lack of dominant hand advantage in many CG patients affected by motor problems is a significant finding (explained in Chapter 2) and puts the experience into perspective for those unaffected by motor control problems. It is as if these patients must go about life using only their non-dominant hand. About 30 to 36% of cases (n=53) fell beyond the 95^{th} percentile determined by controls, and of that 30 to 35%, about 53 to 63% lacked dominant hand advantage (percent ranges come from using data from dominant hand only and both hands combined).

Results from Chapter 3 show that NG can effectively detect differences between cases and controls. Cases and controls age 6 to < 12 were found to have higher RMS scores as

compared to cases and controls above 12 years of age. This is not a surprise, as most children are still learning to write and are still developing neuromotor functions at this age. Even though no statistical difference was found between age groups over 12, qualitative observation of patients and visualization of RMS means shows that motor problems in at least some patients may be progressive with age. Lastly, Chapter 4 provides strong evidence that there is no difference in the prevalence of motor problems between males and females.

LIMITATIONS

A few notable limitations are discussed next. First, it is important to understand that NG can only be used to calculate RMS scores when spirals are drawn approximately along the designated path. Scores for two cases with crudely drawn spirals could not be scored and were thus not included in any of the calculations using RMS scores. However, these spirals were still included in calculations of proportions outside of 95th, 97th, and 100th percentiles. Another limitation is the possible confounding of "amount of focus" for dominant and non-dominant hand spirals. Spiral time was evaluated as a possible measure of "amount of focus," assuming that those who focused harder would take longer to draw their spiral. However, no relationship was found between spiral time and RMS scores, and "amount of focus" is not a variable that we measured directly. For future trials, it may be beneficial to have participants perform another task, such as counting or singing a song. By focusing on the mental task, the participants will not be likely to focus harder with one hand versus the other. The possible drawback to this strategy may be that some participants are better multi-taskers than others, and are thus able to focus on their spiral drawings while simultaneously performing a mental task. Additionally, this mental "distraction" would also be significantly harder or impossible for patients with cognitive disability, and so the attempt to control for focus may then impart a new confounder.

Another limitation of the study is that we were only able to quantify hand fine motor control problems, but patients with CG may also be affected by gross motor problems such as: walking, running, balancing, or catching a ball. This study also does not classify motor problems into specific diagnoses such as ataxia, dystonia, and tremor. Analysis of specific features of spirals that give insight into the specific type of motor problem is a question that will be addressed in the future. Another limitation that will be addressed in the future is the collection of longitudinal data. Collecting data from the same participants over time will give us a much clearer picture of the possible progression of motor problems in CG.

Finally, it is important to recognize that our sample was limited and may not be representative of the classic galactosemia population as a whole. It can be argued that patients who were "more affected" are more likely to seek treatment from a metabolic specialist and more likely to attend the conference from which our study sample derives. In this case, our sample may have an over representation of CG patients who experience more severe outcomes. This is not necessarily true, however, and it may be that patients with more severe outcomes are less likely to attend research events because it is more difficult for them to travel. Whatever the case may be, it is important to recognize that our sample was not chosen randomly, and this makes it difficult to extend our results to the entire CG population.

FUTURE DIRECTIONS

The next steps following this study will focus on testing the concurrence of speech and motor problems in patients with CG. As explained in the introduction, speech is not only found to co-occur in the general population, but has also be qualitatively observed in classic galactosemia. A previous study found no association between motor and speech problems, but with a larger dataset, this question can be asked with more statistical power. Besides investigating the interaction between motor and speech, we have the capacity to look into many outcomes of CG, including: cognitive ability, fertility, weight gain, etc. It may be possible to perform predictive models based on collected data. Further analysis should also

include the effects of genotype on outcome prevalence and severity. Since there are many genotype variations, estimated percent residual activity of GALT should be used as the independent variable in these future analyses.

Another future aim is the use of NG software to further analyze spiral data to detect features that can pinpoint the specific motor disorder affecting the patients. Dystonia was the most frequently observed motor disorder in the Kuiper et al. (2018) study (3 children and 9 adults), tremor was the second most common (1 child and 5 adults), as well as mild myoclonus (4 children), ataxia (1 child 3 adults), ticks, stereotypies, and spasticity.[20] The incorporation of specific mutations/estimated percent activity would also give more insight into the effects of different genotypes and degrees of impairment on long-term outcomes.

An ideal future study would be a collection of longitudinal data on participants over years or decades. This is the best way to measure the potential progressivity of motor control problems over time. If motor problems were found to worsen over time, this would also give more insight into the development of all long-term outcomes of classic galactosemia and the necessity of additional support for older patients living with the disease. The mechanism by which a neuromotor problem develops in classic galactosemia and why this outcome affects only some patients and not others is unknown. Further research into these questions may provide useful information for developing drugs and and/or potential interventions.

REFERENCES

1. Calderon FRO, Phansalkar AR, Crockett DK, Miller M, Mao R. Mutation database for the galactose-1-phosphate uridyltransferase (GALT) gene. *Hum Mutat*. 2007;28(10):939-943. doi:10.1002/humu.20544

2. McCorvie TJ, Kopec J, Pey AL, et al. Molecular basis of classic galactosemia from the structure of human galactose 1-phosphate uridylyltransferase. *Hum Mol Genet*. 2016;25(11):2234-2244. doi:10.1093/hmg/ddw091

3. Robertson A, Singh RH, Guerrero NV, Hundley M, Elsas LJ. Outcomes analysis of verbal dyspraxia in classic galactosemia. *Genet Med Off J Am Coll Med Genet*. 2000;2(2):142-148. doi:10.109700125817-200003000-00005

4. Berry GT. Classic Galactosemia and Clinical Variant Galactosemia. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews®*. Seattle (WA): University of Washington, Seattle; 1993. http://www.ncbi.nlm.nih.gov/books/NBK1518/. Accessed March 30, 2019.

5. Waisbren S, Potter N, Gordon C, et al. The Adult Galactosemic Phenotype. *J Inherit Metab Dis*. 2012;35(2):279-286. doi:10.1007/s10545-011-9372-y

6. Thoden JB, Hegeman AD, Wesenberg G, Chapeau MC, Frey PA, Holden HM. Structural Analysis of UDP-Sugar Binding to UDP-Galactose 4-Epimerase from *Escherichia coli* [†,‡]. *Biochemistry*. 1997;36(21):6294-6304. doi:10.1021/bi970025j

7. Coelho AI, Rubio-Gozalbo ME, Vicente JB, Rivera I. Sweet and sour: an update on classic galactosemia. *J Inherit Metab Dis*. 2017;40(3):325-342. doi:10.1007/s10545-017-0029-3

8. Elsas LJ, Lai K. The molecular biology of galactosemia. *Genet Med Off J Am Coll Med Genet*. 1998;1(1):40-48. doi:10.1097/00125817-199811000-00009

9. Berry GT, Nissim I, Gibson JB, et al. Quantitative assessment of whole body galactose metabolism in galactosemic patients. *Eur J Pediatr*. 1997;156(1):S43-S49. doi:10.1007/PL00014271

10. Berry GT, Moate PJ, Reynolds RA, et al. The rate of de novo galactose synthesis in patients with galactose-1-phosphate uridyltransferase deficiency. *Mol Genet Metab*. 2004;81(1):22-30.

11. Jumbo-Lucioni PP, Garber K, Kiel J, et al. Diversity of approaches to classic galactosemia around the world: a comparison of diagnosis, intervention, and outcomes. *J Inherit Metab Dis*. 2012;35(6):1037-1049. doi:10.1007/s10545-012-9477-y

12. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. *J Inherit Metab Dis*. 1990;13(6):802-818.

13. Rubio-Agusti I, Carecchio M, Bhatia KP, et al. Movement disorders in adult patients with classical galactosemia. *Mov Disord Off J Mov Disord Soc*. 2013;28(6):804-810. doi:10.1002/mds.25348

14. Potter NL, Nievergelt Y, Shriberg LD. Motor and speech disorders in classic galactosemia. *JIMD Rep*. 2013;11:31-41. doi:10.1007/8904_2013_219

15. Berry GT, Walter JH. Disorders of Galactose Metabolism. In: Saudubray J-M, van den Berghe G, Walter JH, eds. *Inborn Metabolic Diseases: Diagnosis and Treatment*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012:141-150. doi:10.1007/978-3-642-15720-2_7

16. Chen J, Yager CT, Reynolds RA, Segal S. Identification of galactitol and galactonate in red blood cells by gas chromatography/mass spectrometry. *Clin Chim Acta Int J Clin Chem*. 2002;322(1-2):37-41.

17. Waisbren SE, Read CY, Ampola M, et al. Newborn screening compared to clinical identification of biochemical genetic disorders. *J Inherit Metab Dis*. 2002;25(7):599-600. doi:10.1023/A:1022003726224

18. Frederick AB, Cutler DJ, Fridovich-Keil JL. Rigor of non-dairy galactose restriction in early childhood, measured by retrospective survey, does not associate with severity of five long-term outcomes quantified in 231 children and adults with classic galactosemia. *J Inherit Metab Dis*. 2017;40(6):813-821. doi:10.1007/s10545-017-0067-x

19. Gitzelmann R. Galactose-1-phosphate in the pathophysiology of galactosemia. *Eur J Pediatr*. 1995;154(7 Suppl 2):S45-49.

20. Kuiper A, Grünewald S, Murphy E, et al. Movement disorders and nonmotor neuropsychological symptoms in children and adults with classical galactosemia. *J Inherit Metab Dis*. 0(0). doi:10.1002/jimd.12054

21. Alty J, Cosgrove J, Thorpe D, Kempster P. How to use pen and paper tasks to aid tremor diagnosis in the clinic. *Pract Neurol*. 2017;17(6):456-463. doi:10.1136/practneurol-2017-001719

22. What is Ataxia? – National Ataxia Foundation. https://ataxia.org/what-is-ataxia/. Accessed April 5, 2019.

23. Dystonias Fact Sheet | National Institute of Neurological Disorders and Stroke. https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Dystonias-Fact-Sheet. Accessed April 5, 2019.

24. Shriberg LD, Potter NL, Strand EA. Prevalence and phenotype of childhood apraxia of speech in youth with galactosemia. *J Speech Lang Hear Res JSLHR*. 2011;54(2):487-519. doi:10.1044/1092-4388(2010/10-0068)

25. Early identification: are speech/language-impaired toddlers at increased risk for Developmental Coordination Disorder? - Gaines - 2007 - Child: Care, Health and Development - Wiley Online Library. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2214.2006.00677.x. Accessed March 30, 2019.

26. Dubroff JG, Ficicioglu C, Segal S, Wintering NA, Alavi A, Newberg AB. FDG-PET findings in patients with galactosaemia. *J Inherit Metab Dis*. 2008;31(4):533-539. doi:10.1007/s10545-008-0806-0

27. Hughes J, Ryan S, Lambert D, et al. Outcomes of siblings with classical galactosemia. *J Pediatr*. 2009;154(5):721-726. doi:10.1016/j.jpeds.2008.11.052

28. Oliveira TG, Soares-Fernandes JP. Brain MRI in a patient with classical galactosemia: acute event of unilateral hemispheric cerebral edema. *Childs Nerv Syst ChNS Off J Int Soc Pediatr Neurosurg*. 2017;33(11):1879-1880. doi:10.1007/s00381-017-3594-8

29. Haubenberger D, Kalowitz D, Nahab FB, et al. Validation of Digital Spiral Analysis as Outcome Parameter for Clinical Trials in Essential Tremor. *Mov Disord Off J Mov Disord Soc*. 2011;26(11):2073-2080. doi:10.1002/mds.23808

30. Elble RJ, Lyons KE, Pahwa R. Levetiracetam is not effective for essential tremor. *Clin Neuropharmacol*. 2007;30(6):350-356. doi:10.1097/WNF.0b013E31807A32C6

31. Feys P, Helsen W, Prinsmel A, Ilsbroukx S, Wang S, Liu X. Digitised spirography as an evaluation tool for intention tremor in multiple sclerosis. *J Neurosci Methods*. 2007;160(2):309-316. doi:10.1016/j.jneumeth.2006.09.019

32. Hess CW, Hsu AW, Yu Q, Ortega R, Pullman SL. Increased variability in spiral drawing in patients with functional (psychogenic) tremor. *Hum Mov Sci*. 2014;38:15-22. doi:10.1016/j.humov.2014.08.007

33. Hsu AW, Piboolnurak PA, Floyd AG, et al. Spiral analysis in Niemann-Pick disease type C. *Mov Disord Off J Mov Disord Soc*. 2009;24(13):1984-1990. doi:10.1002/mds.22744

34. Rudzińska M, Krawczyk M, Wójcik-Pędziwiatr M, Szczudlik A, Tomaszewski T. Tremor in neurodegenerative ataxias, Huntington disease and tic disorder. *Neurol Neurochir Pol*. 2013;47(3):232-240.

35. Pieters S, De Block K, Scheiris J, et al. How common are motor problems in children with a developmental disorder: rule or exception? *Child Care Health Dev*. 2012;38(1):139-145. doi:10.1111/j.1365-2214.2011.01225.x

36. Nugent BM, O'Donnell CM, Epperson CN, Bale TL. Placental H3K27me3 establishes female resilience to prenatal insults. *Nat Commun*. 2018;9(1):2555. doi:10.1038/s41467-018-04992-1