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Movement Disorders in Classic Galactosemia

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Movement disorders in classic galactosemia

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MD and PhD, University of Michigan, 1996

Advisors: Stewart Factor, DO; Judith Fridovich-Keil, PhD

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ABSTRACT

Movement disorders in classic galactosemia

By Claudia M Testa, MD, PhD

More than one hundred infants with classic galactosemia are born each year in the United States alone. In this autosomal recessive disorder, mutations impair galactose metabolism, causing acute complications with high morbidity and mortality as soon as the infant begins a milk-based diet. Removing galactose from the diet is a critical, life-saving intervention; yet this treatment does not prevent multiple long-term complications. Long-term neurological outcomes, particularly movement disorders, have been reported but not well studied. This project was sparked by a request from a parent and galactosemia community organizer for a neurologist to help understand causes and possible treatments of tremor in classic galactosemia. We conducted a cross sectional observational study of 45 classic galactosemia patients, including both children and adults, and patients diagnosed with newborn screening or after initial symptoms. We report a range of movement disorders, including action and postural tremor, dystonia and limb ataxia, in children and adults. Bradykinesia was also common, but other parkinsonian features were not observed. Chorea was unusual, as was gait impairment. Most affected areas were limbs, and neck. Gender did not associate with neurological outcome; however, there were trends towards older age at time of neurological exam and symptomatic at diagnosis associating with affected neurological outcome. We used our own laboratory assays to determine genotype and predicted residual enzyme activity for each subject. As in other largely Caucasian classic galactosemia cohorts, the Q188R mutation was by far the most common. There was no clear association between genotype and neurological outcome. Results for GALT enzyme activity and age at diet intervention had weak trends opposite of biological theories in the field; in both cases outliers had a large impact on the results. The pilot results will prove very useful in designing and conducting a larger, longitudinal, multi-center study.

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INTRODUCTION

Classic or type 1 galactosemia is an autosomal recessive disorder resulting from profound loss of galactose-1P uridylyltransferase (GALT) activity. Causal mutations impair expression or function of the GALT gene leading to impaired galactose metabolism via the Leloir pathway. This, in turn, leads to a buildup of metabolic intermediates including galactose-1-phosphate (gal-1P), normally a GALT substrate. Classic galactosemia affects close to 1 in every 60,000 infants; more than 100 affected infants are born each year in the US alone [1]. Neurological complications experienced by patients with classic galactosemia have received relatively little clinical or research attention in the past, but are emerging as a potentially common and in some cases debilitating feature of the disorder.

Patients with classic galactosemia are born with no apparent clinical abnormalities, but shortly after beginning to nurse or drink a milk-based formula these infants develop symptoms that escalate in days to weeks from cataracts, vomiting, and diarrhea, to hepatomegaly, *E. coli* sepsis, and death. Removal of galactose from the diet prevents or resolves the acute symptoms. Early detection and treatment of galactosemia has been hugely successful in preventing catastrophic and often fatal early symptoms [1]. Case reports still describe severe neurological complications, such as seizures and hypotonia, in patients with delayed treatment onset [2].

Once early screening and diet modification programs were in place in the US and Europe, galactosemia prognosis was portrayed as excellent. However, starting in the mid-1960's, unexpected reports of complications in older galactosemic patients prompted some investigators to re-examine the idea of diet-controlled galactosemia as benign and easily manageable [3, 4]. Subsequent studies firmly established the idea of

long-term complications among well-treated patients with galactosemia, with a wide range of symptoms and severities [5-9]. Complications include delayed puberty and/or ovarian dysfunction in at least 80-90% of girls and women, delayed growth and bone mineral density problems in many patients, cognitive, behavioral, and speech abnormalities in at least half of all patients, and a variety of other neurological outcomes [1, 5-11].

Most striking was the clear presence of long-term complications in the majority of well-treated classic galactosemics, regardless of age at diagnosis or success of diet modification [3, 9, 12]. This disparity between intervention and long-term outcome was a key motivation for this project: we believe that better understanding of long-term symptoms in galactosemia can and will improve our ability to provide more accurate prognosis and more effective intervention.

Another key motivation was to understand a community need. This project started when a parent of a galactosemic child with severe tremor advertised for tremor neurologists to come learn about galactosemia, and hopefully start working on answers for classic galactosemia patients with movement disorders (Appendix). We did not conduct a formal needs survey, or structure this project as true community engaged research. However the original parent identified other parents with the same concerns, and introduced the idea for an initial pilot project to the research team.

BACKGROUND

Long-term neurological complications in classic galactosemia

The most studied long-term neurological sequelae of galactosemia are impaired cognitive function, measured as developmental delay, IQ scores or detailed neuropsychological testing; and speech abnormalities such as dyspraxia [3, 5-10]. Several groups have also reported other neurological symptoms related to movement. This study focuses on such movement disorders.

Movement disorders are clinical diagnoses, meaning there is no gold standard test for them beyond expert clinical opinion. Both published and self-reported labels like "tremor" may be highly inaccurate. Why does this matter? Accurate labeling of movement abnormalities is critical for directing treatment choices, and for understanding disease mechanisms in classic galactosemia.

Specific neurological signs can strongly imply involvement of particular brain regions. Descriptions of neurological outcomes in galactosemia point to two areas: the cerebellum, and the basal ganglia. The cerebellum is particularly involved in targeted movements and learned motor programs. For example, when you reach for an object, the cerebellum helps make constant corrections so that you correctly and smoothly move your hand to the object, without overshoots or wild movements back and forth (dysmetria). Disorders that damage the cerebellum cause ataxia, meaning an abnormal regulation of movement speed, force, and direction. In the limbs this translates to poor targeting, and poor rhythmicity of movement. In the trunk this can create gait ataxia and poor balance. Ataxia is often reported in galactosemia [13], although limb versus gait ataxia is often not specified. Kinetic tremor is also often reported in galactosemia [13]. Kinetic tremor, meaning tremor that occurs with active voluntary movement like writing

or lifting a cup to drink, is the cardinal feature of essential tremor (ET) [14]. ET-like tremors are thought to originate in cerebellar pathways [15]. There is some overlap between tremor and ataxia: a form of action tremor, intention tremor, in on a continuum with limb ataxia.

There is some evidence for basal ganglia involvement in ET as well [16, 17]. The basal ganglia are interrelated nerve cell groups that are intimately involved in dictating or controlling movements. Reports of poor coordination or "clumsiness" experienced by some patients with classic galactosemia may reflect abnormally slow movements (bradykinesia), poor fine motor coordination, and stiffness (rigidity), all key features of basal ganglia dysfunction, as seen in Parkinson disease (PD). Dystonia is an abnormal involuntary twisting posture. It is a key motor feature of disorders that impact the basal ganglia, such as PD and Huntington disease (HD). New research suggests that the cerebellum also contributes to generating dystonia [18]. While there is little in the galactosemia literature on dystonia [19], dystonia can causes “tremor”, and dystonia is a common feature of inborn errors of metabolism disorders.

Chorea, a writhing or fidgeting involuntary movement characteristic of HD, has occasionally been reported in both untreated [2] and diet restricted galactosemia patients [20].

Prior reports are largely retrospective, lack detailed neurological exams, do not include video data of movements observed, and do not include comments from a movement disorders specialist. The most detail is found in small case studies. Four case studies, each describing two affected siblings (in one study identical twins), reported progressive neurological symptoms in subjects treated from birth or within the first week of life [20-

23]. Neurological symptoms described included ataxia, dysmetria, postural and kinetic tremors, and low IQ. In addition, the twins developed chorea and ballistic movements [20]. Two sets of siblings demonstrated relatively late onset of neurological symptoms; one set presented with ataxia and chorea at 9 years [20], and another set presented with neurological symptoms and seizures after age 30 [23].

The early galactosemia literature has some direct and indirect references to movement disorders. For example, Komrower et al [3], described a cohort of galactosemic subjects in Great Britain; they did not conduct neurological exams, but their report included Bender-Gestalt drawing tests. Distorted figures were produced by 18 of 23 subjects (78%) [3]. This result could reflect a primary visuospatial (cognitive) deficit, or could indicate dystonia or ataxia. Schweitzer et al [8], reporting on a cohort of galactosemia cases in Germany, conducted neurological exams on 73 of 134 cases and found that 12 showed “severe clumsiness”, 11 intention tremor, 3 mild ataxia and 3 severe ataxia (including the twins described in the case study). Kaufman et al [9] conducted neurological exams on all 45 galactosemic patients in a Los Angeles cohort: 12 subjects demonstrated tremor, ataxia, or dysmetria [9]. Waggoner et al [7] collected retrospective survey data from 29 centers in the US, Germany, England, France and Scotland, thus including findings from the single center/country studies described above. Out of 206 patients older than 3.5 years with adequate survey data, 26 had problems with coordination, 14 had gait abnormalities, 9 had “fine motor tremors”, and 2 (the German twins) had severe ataxia [7].

Published data concerning neurological outcomes in adult patients are relatively scarce. A presentation at the PGC 2010 meeting on adults noted several cases of tremor in adults. This work was recently published (after our pilot study data collection was

completed) [19]. Thirty-three adults (16 women) ages 18 to 59 years underwent various exams aimed at covering a wide range of physical, neurological, and endocrine outcomes in classic galactosemia. Although not stated explicitly in the results, most if not all of the subjects were not diagnosed by newborn screening, based on their ages; this implies that the vast majority were symptomatic at time of diagnosis. A neurologist conducted exams; details of the exam i.e. any validated scales used are not available, and there are no published videos of the exams. Postural and/or intention tremor was noted in fifteen subjects; ataxia (presumably limb ataxia but not specified) was noted in 5 subjects, 4 of whom also had intention tremor. Dystonia was noted in two subjects; the body area affected was not noted.

Building on our understanding of galactosemia:

forward progress and significance

All published reports of neurological complications including movement disorders generally involve subjects born prior to the onset of newborn screening for galactosemia in their communities, or do not detail newborn screening information. Our work includes both children and adults, the majority of whom were diagnosed by newborn screening. This is an important distinction from previous studies, as our proposal speaks to long-term consequences in promptly diagnosed and treated classic galactosemia. Ours is also the first study to focus movement disorders expertise on classic galactosemia patients. This study will therefore help to describe, in quantitative terms, the spectrum of neurological complications experienced across age groups.

Exam features may be challenging to elicit for non-experts. For example, dystonia and chorea are relatively rare and are often missed by doctors, including neurologists. Clinical labels may be overlapping or honestly disputed: limb “ataxia” may be tremor

alone, or tremor and ataxia may both be present. Tremor is under-reported by both subjects and physicians [16, 24, 25]. Direct examination of subjects by a movement disorders neurologist is critical for discerning target neurological symptoms. Published reports on neurological outcomes in classic galactosemia to date do not include video data, or assessments by movement disorders specialists, two key aspects of our work.

The neurological phenotype in classic galactosemia provides clues to disease mechanism, such as what areas of the brain are affected? There are multiple other unanswered questions in the field. Why are patients with classic galactosemia at risk for neurological complications? Further, what are the factors that distinguish those galactosemia patients who experience neurological complications from those who do not? Despite decades of research the answers to these questions remain unknown, though it is clear that neither differences in *GALT* genotype nor timing of treatment onset [3, 8, 26] can fully explain the breadth of patient outcome severity, neurological or otherwise. Movement disorders may track with speech and cognitive symptoms, but it is unclear if they track with any of the non-neurological consequences of galactomsemia, such as ovarian dysfunction [7, 8] There was no clear correlation between cognitive function as measured by IQ testing and tremor in the recent adult classic galactosemia phenotype study [19].

In summary, long-term complications do occur in classic galactosemia despite early diagnosis and excellent diet control. Prior studies are fairly low in detail on movement disorders, although there are clues to which movements and therefore which brain areas and treatment possibilities may apply in classic galactosemia. More information on accurate movement disorder diagnosis, range of symptoms and severity, and better quality data are all needed. There is at least some specific interest in the community in addressing these questions. Therefore, the goals of this study were to take a first step

toward characterizing the nature and severity of movement disorders complications in children and adults with classic galactosemia, and to explore potential mediators of this outcome. Investigating possible associations between neurological outcome and other biological or environmental parameters helps expand understanding of what may contribute to the presence or absence of specific neurological complications. This information could enable more accurate prognosis, point the way toward future improvements in prevention or treatment, and inform basic studies such as animal model work on disease mechanism.

METHODS

Hypothesis 1: There are a variety of different movement disorders present in classic galactosemia.

Specific Aim 1: We analyzed neurological questionnaire and physical exam data in order to establish accurate diagnoses of study volunteer neurological outcomes. Parameters evaluated included: tremor, dystonia, ataxia, fine motor coordination, gait. We used descriptive statistical techniques to better understand neurological exam outcomes.

Hypothesis 2: Long-term neurological outcomes in classic galactosemia patients correlate with other biological or environmental parameters.

Specific Aim 2: We set rules for a neurological outcome variable based on expert movement disorder review of the exam data from specific aim 1. We leveraged study participation in an existing galactosemia cohort, and gathered any required missing genetic, biochemical, and other data. Parameters tested for possible association with neurological outcome included: age and gender of the volunteer, diagnosis type (newborn screening, once symptomatic), GALT enzyme activity level, *GALT* genotype.

Study type

We conducted a cross-sectional, single time point cohort study in a sample of convenience.

Subjects

We took advantage of Dr. Judith Fridovich-Keil's existing classic galactosemia project, amending the protocol (Emory IRB Protocol # 618-99) and adding consents to include new (not previously studied) neurological work by Dr. Testa. Dr. Fridovich-Keil's study had focused primarily on scholastic and behavioral outcomes in pediatric volunteers, and

on ovarian function in girls and women (the latter with MSCR graduate Dr. Jessica Spencer) [11, 27]. Note that the parent study did not include any work on movement disorders outcomes, or any direct neurological exams. Neurologically relevant data are confined to scholastic achievement surveys as a cognitive measure in a subset of subjects, and limited data on speech difficulties in a subset of subjects. This allowed us to construct a new project on neurological outcomes, while leveraging a large existing dataset, IRB-approved structure, and existing and engaged volunteer population.

We were therefore able to get up and running rapidly, and work with a relatively large number of subjects for a rare disease. One consequence was working with a sample of convenience: we conducted neurological interviews and exams on 44 pediatric and adult consented volunteers with classic galactosemia at the 2010 Parents of Galactosemic Children (PGC) meeting in Bloomington, MN. We were able to examine one more subject at a later date in Atlanta, for a total of 45 subjects. Thus participation was limited to people able and willing to travel to the PGC meeting (or Atlanta), and be in the overall Fridovich-Keil study.

We offered the add-on neurological interview and exam to all participants in the Fridovich-Keil study, regardless of neurological status. We did not attempt to select participants with self-reported or observed movement disorders symptoms. Dr. Testa was not informed of any research team or family observations on subjects prior to conducting the exams. We did not attempt to select subjects by gender, ethinicity, or age, again offering participation to all willing and available subjects, with the exception of children under age 5 years: an informative exam and (parent) interview would be significantly different in very young subjects, and may require more pediatric expertise to both design and interpret. All subjects had previously diagnosed classic galactosemia.

Specific Aim 1

The purpose of aim 1 is to accurately delineate neurological signs, particularly movement disorders, in participating volunteers. Data were gathered using semi-structured interviews with subjects and parents, based on research questionnaires developed for other movement disorders studies by Dr. Testa [25, 28, 29]. Questions were designed to capture information that may impact genetic risk factors; medical history; medications particularly agents that can cause movement disorders; family history. Prior to the 2010 PGC meeting, questionnaires were modified specifically for classic galactosemia; for example, any history of seizures. Any demographic information not already captured in existing Fridovich-Keil study questionnaires was also added. Subjects or their parents were asked to self-report any tremor or other movement disorders (prior to the neurological exam).

We used an exam modified from Dr. Testa's essential tremor phenotyping work, which covers tremor items from the Tremor Research Group, parkinsonism symptoms from the United Parkinson Disease Rating Scale motor section, and gait from multiple published scales [25, 28, 30-32]. We added exam items for dystonia, based on consensus exam scales from the Dystonia Coalition effort (in consultation with Dr. Hydar Jinnah, Dystonia Coalition PI, at Emory University) and exam items for ataxia based on a published scale [33, 34]. Validated scales in wide use were exploited whenever possible. We examined all available and consented volunteers, without any prior information about whether subjects had any level of neurological complications of galactosemia.

All tests were videotaped (with specific written consent) for later independent review by movement disorders experts. Subtle forms of dystonia, chorea, and other movements

may be missed on exams. Clinical labels may be overlapping or honestly disputed as discussed above. Videotaping exams for independent evaluation by different specialists is critical to overcoming possible external label-based rather than outcome-based differences in reporting between investigators. Videotaping exams retains the true primary data rather than relying on one examiner's opinion as all the data. Finally, it allows scoring an exam for various overall features.

The exam items used were from scales with attached scores, for example finger tapping impairment 0-4 on the United Parkinson Disease Rating Scale, or level of tremor 0-4 on reaching for a target on Tremor Assessment Scale. However, one exam item, such as reaching for a target, may bring out multiple different movement disorders – in this example, tremor, ataxia, or dystonia. As our goal was to discern the type and range of movement disorders, we made an overall exam scoring sheet to capture this information, rather than use individual exam item scores (Figure 1). This was modeled after Dystonia Coalition exam scoring efforts. Our exam scoring sheet references five categories of movement disorder (tremor, ataxia, dystonia, bradykinesia, and other) against a full range of body areas (face areas, neck, trunk, each limb). This yields four basic score groups: each cell 0-10 with 10 most severe and 5 moderate; each movement disorder category total (possible range 0-130); each body area total (possible range 0-50); and overall total (possible range 0-650). General gait and voice scores were also included. The scoring sheet was used by Dr. Testa and an independent movement disorders expert exam rater, Dr. Stewart Factor (Emory Neurology) to review all videotaped exams. Dr. Testa conducted 39 of the exams in person with another research staff member videotaping; 7 exams were conducted by an MD research team member using the same exam script when Dr. Testa was not available at the PGC meeting. Thus Dr. Factor had no information beyond the videotapes to influence his scoring, while Dr. Testa observed

most subjects outside of the videotaping time and in person during exam time.

Exam score data were used to create a neurological outcome variable (see below). For purposes of clinical labeling and this study, and given our sample size, we pre-determined using a dichotomous outcome variable i.e. unaffected / affected, rather than categorical i.e. unaffected / possible / probable / definite affected.

We piloted digitized spiral analysis, a rapid, easy for subjects, portable, and noninvasive instrument [35]. Subjects draw a series of free-hand spirals using a pen that simultaneously writes on paper and "writes" on a digitizing tablet beneath the paper. Spirals are drawn with each hand. Data captured include motion in three dimensions (Z = pressure into the digitizing tablet), speed, and spiral shape. The associated analysis software is then used to "unwind" the spirals, determining several variables such as axis and frequency of any tremor. The task has been used in a different inherited metabolic disorder, Niemann-Pick disease [36], but not in classic galactosemia, and in elderly and adults more than children. Our purpose was to see if subjects of many ages could handle the task, so that we could expand to analyzable spirals with age-matched controls at a later date.

Specific Aim 2

We investigated connections between the neurological data and biological and environmental data. For all subjects, we attempted to gather: age (at time of neurological exam); gender; demographics, family and medical history particularly as related to genetics and movement disorders clinical research; whether diagnosis was by newborn screening (presymptomatic) versus in response to clinical symptoms; and age at first intervention. Information was from family / subject recall, and medical record review.

Direct sequencing of patients' *GALT* loci to define genotype and assays using a yeast model system [37] to define the functional significance (predicted residual GALT enzyme activity level) of genetic variants were conducted in Dr. Fridovich-Keil's lab as part of this project. Briefly, *GALT* genotype was used to build a yeast construct expressing each specific mutation [37]. Clinical GALT enzyme testing on human samples is done across many labs all with different assays, and has a severe floor effect rendering nearly all samples as zero activity. The yeast system, while not a direct test on human cells, is more sensitive, reproducible, and comparable between subject samples. It provides more of a range of activity points than the clinical lab tests. For genetically heterozygous subjects, the average of the two predicted activities was used. We were able to use some existing data from prior work by Dr. Fridovich-Keil. Still, when Dr. Testa's specific study was launched, of the 45 subjects 33% were missing *GALT* genotype data, 76% needed GALT activity level, and 60% review of diagnosis and first treatment data points. We also attempted but were unable to obtain initial (untreated) and treated gal-1P levels on the majority of subjects.

Predictor variables acquired under specific aim 2 included:

Age of subject in years = age at time of neurological exam

Gender, self reported, male or female

Age at initial intervention in days; zero was used for prenatal diet intervention

Diagnosis status: not symptomatic at diagnosis (newborn screening / other),
symptomatic at diagnosis

Predicted residual GALT enzyme activity in
enzyme units as per yeast construct system

GALT genotype coded as number of Q188R alleles (0, 1, 2)

The main hypotheses were that predicted GALT residual enzyme activity and age at intervention associate with neurological outcome status. Secondary hypotheses were that GALT genotype, diagnosis status, age, and gender are not associated with neurological outcome.

Data analysis:

Basic summary and descriptive analyses were used for the demographics, medical questionnaire, and exam data under specific aim 1. Analyses were done using JMP 9.0 software.

We used rules based on expert opinion of the two independent exam raters to create a dichotomous neurological outcome variable. The outcome variable was defined as affected if:

Any one cell (exam features / body area combination) >= 3

Any one of the five exam feature totals >= 4

The overall total >= 5

For discordant assignments between the two exam raters: if discordant and one rater total = 0, then subject was rated as unknown. Otherwise subject was rated as affected.

We compared the bimodal neurological outcome to each specific predictor variable. The null hypotheses for all individual tests were structured as: the predictor variable is not associated with neurological outcome. Individual logistic regression tests were used for age at neurological exam, predicted GALT enzyme activity, age at intervention, and *GALT* genotype. Fisher exact tests were used for gender and diagnosis status. A logistical

regression model approach was used to investigate any interactions between predictor variables not uncovered in the individual association tests. We fit a variety of logistical regression models using source data for all six of the predictor variables. We fit models using both untransformed and transformed values of the predictor variables. Transformations consisted of simple categorizations.

RESULTS

Cohort and demographics

The mean subject age (years) was 22.6 +/- 12.3, median 19.2, with a range of 5.7 to 60.1. Sixty-four percent of subjects were female (Figure 2).

Of the 43 subjects self-reporting ethnicity, 39 stated Caucasian, and 4 either Hispanic or Hispanic / Caucasian. Four subjects also reported Ashkenazic Jewish heritage from at least one parent. For one subject, both parents were Amish. The majority reported European origins, mainly western and northern areas. Five subjects reported partial Native American (US or Mayan/Mexican) ancestry, and two reported Mexican and Spanish ancestry. There was no reported Asian or African ancestry.

Specific Aim 1: Characterizing long-term neurological outcome in classic galactosemia

Two of the subjects were left-handed, three did not report handedness. Three subjects had a medical history of seizures: one during neonatal pre-diagnosis galactosemia illness, the other two as adults (initial seizure ages 22 and 50 years). There were no subjects with a family history of seizures.

Seventeen subjects (38%) self-reported tremor; none self-reported other movement disorders diagnoses. Of the 17 subjects, 6 reported some variation of “always”, “forever” or “birth” for age of tremor onset, otherwise reported tremor onset ranged from age 5 to 32 years. In 9 cases tremor was in both hands, otherwise was in one hand. There was one report of vocal tremor not confirmed on exam, otherwise no tremor reported outside of hands. All cases had exam findings per both exam raters: all but one had tremor, while many also had dystonia or ataxia in addition to tremor.

Only one subject had a clear family history of tremor, in an autosomal dominant multi-generational pattern; thus tremor in this subject could well be essential tremor, a very common action tremor usually associated with an autosomal dominant inheritance pattern, rather than a classic galactosemia complication. This subject did have tremor on exam per both exam raters. There were no other subjects with potentially confounding family history or medication use i.e. medications that could cause a movement disorder side effect.

There was considerable but far from complete agreement between the two independent exam raters on initial exploration of the data (Figure 3). Overall, very low exam feature total scores (0, 1) were common for all five categories, with a left shifted score distribution in all categories. The main features were tremor, dystonia, and ataxia (Figure 4). Tremor was always characterized as action, sometimes also postural, never rest tremor. Of note on the in person exams there was no rigidity in limbs or neck; this exam item cannot be judged by video. “Other” was all chorea, comparatively mild and less common than the other categories. Most affected body parts were limbs and neck (Figure 5). Upper extremities were much more likely to be affected than lower extremities. This is reflected in the low number of low scores under gait (Figure 5). Trunk was rarely affected, and face unaffected.

Individual cell scores maximum was 6, a rare finding. Our cohort was split between not affected (overall total zero), possibly or mildly affected, and moderately affected by expert clinical opinion. Per subject, none of the five exam feature totals got above 13% of the possible maximum total of 130. Subjects with scores across multiple exam features were much more common than “pure” tremor, dystonia, etc.

The exam features that most contributed to variability in the exam scoring data were dystonia, tremor and bradykinesia for CMT, and dystonia, tremor and ataxia for SAF (Figure 6). Comparing the five exam feature totals to each other, the highest correlations were between tremor and dystonia, followed by tremor and bradykinesia, tremor and ataxia, dystonia and ataxia (Table 1).

Specific Aim 2: Potential predictors of long-term neurological outcome in classic galactosemia

Neurological exam score distributions were left skewed, with no clear cut-offs or peaks in the data for the affected / unaffected outcome rules. Therefore expert opinion from the exam raters was used to make the cut-offs, as above. Using the affected / unaffected dichotomous outcome variable, there were 9 discordant pairs between the two exam raters: 6 rated affected by CMT, and 3 rated affected by SAF. Of the 9, 3 had one affected rating and the other examiner overall total score of zero. The rest just missed the affected cut-offs for the second examiner. Overall, 19 of the 45 subjects were classified as affected (42%).

There were only 8 subjects with predicted residual GALT activity greater than zero. In addition, 8 more subjects were missing data for this variable. Some cases did not give blood for genetic and biochemical assays. Some had a *GALT* mutation type such as a non-coding intronic change that could not be modeled in the yeast GALT activity system. The one subject with predicted GALT enzyme activity almost an order of magnitude higher than any other subject was in the affected neurological outcome group (Figure 7). Logistic regression using residual GALT enzyme activity as the single predictor against neurological outcome status did not yield sufficient evidence to reject the null hypothesis

(Table 2). *GALT* genotype was also not predictive of neurological outcome in a single variable logistic regression analysis (Table 2).

Age at diet intervention yielded a similar result (Table 2). Again there was one extreme outlier, with a very late age at intervention, this time in the unaffected outcome group (Figure 7). This outlier represents the opposite of the presumed biological effect wherein delayed intervention is thought to lead to worse outcome.

The most common *GALT* mutation genotype was a point mutation, Q188R, as reported in other studies with this ethnicity mix. Most other mutations were single occurrences in our cohort, with few exceptions. The four subjects with Ashkenazim Jewish heritage were the only ones with a 5kb deletion mutation: the three reporting Ashkenazim heritage in both parents were heterozygous, and the one reporting one parent as Ashkenazim was homozygous for this mutation. There was not enough evidence to reject the null hypothesis that *GALT* genotype (as Q188R or other) is not associated with neurological outcome (Table 3).

On average affected individuals were 5.6 years older than unaffected, but the difference was not significant (Table 2). In a sample twice as large a difference this size would be mildly significant. There was no association between gender and neurological outcome (Table 3).

Two subjects were diagnosed before symptoms although not with newborn screening, for example prenatal diagnosis. Two subjects with a false negative newborn screen who were subsequently re-tested after symptoms occurred were considered symptomatic at diagnosis. Three subjects became symptomatic before their initial newborn screening

results were available, within the first week of life; these were also considered symptomatic at diagnosis. There was a non-significant trend towards symptomatic at diagnosis more likely to be affected (Table 3).

Some logistical regression models using multiple predictor variables, either untransformed or transformed, did not converge. This is likely due to the fact that there were no affected individuals in some of the categories for some of the categorical variables; the problematic categories contained almost no individuals. We attempted to resolve issues of convergence by collapsing predictor categories; for example, changing newborn screening to two categories, pre- and post-symptomatic diagnosis (Table 3, top two categories collapse into one). No logistical regression models achieved statistical significance. The single best yet still non-significant predictor was the dichotomized diagnosis timing variable, asymptomatic versus symptomatic at diagnosis (p value 0.1475).

DISCUSSION

The goals of this research are to delineate the spectrum of neurological complications among pediatric and adult patients with classic galactosemia, to explore issues of mechanism by testing the statistical significance of potential relationships between neurological outcomes and specific biological or environmental parameters, and to respond community needs in this area. This project included key features novel to galactosemia research: movement disorders expertise, videotaped exam review, quantifiable measures of movements, data on both pediatric and adult subjects particularly patients diagnosed with newborn screening, and a motivation directly from the galactosemia community. The long-term goals of this research were to better predict outcome severity and to facilitate the development of symptomatic, responsive, and preventive treatments for long-term consequences of galactosemia. Improved understanding of specific neurological outcomes in galactosemia will provide patients and families information they may need to understand prognosis and seek appropriate interventions.

Community

We began addressing concerns from parents involved with the PGC regarding tremor and other movement disorders in classic galactosemia. Results from the current study will be reflected back to the galactosemia community through scientific publication, through written and oral presentations at the 2012 PGC meeting, and through PGC newsletters.

Neurological Outcomes

We observed a range of different movement disorders in 42% of subjects in a classic galactosemia cohort. Unaffected and affected subjects were split nearly evenly across

both male and female genders. Movement disorders were observed across the full subject age range. We saw mainly mild to moderately affected individuals, as well as unaffected individuals. Given the limitations of our pilot study, it is unclear how broadly generalizable the prevalence, severity, and type of movement disorders results are to the larger classic galactosemia population. Possible secondary causes of abnormal movements were unusual: one subject had a strong family history of tremor, implying potential essential tremor in that case. Thus the vast majority of our observations are likely related to classic galactosemia in this population.

Both movement disorders specialists observed tremor and dystonia, followed by ataxia and bradykinesia, as well as rare very mild chorea. Chorea scores were low enough that they may not hold up on review by different exam raters, or re-review of a larger cohort, but this abnormal movement has been reported in classic galactosemia by others [2, 20]. No other movements such as tics or myoclonus, were observed. Some particular types of movement disorders, for example action and postural tremor but not rest tremor, did stand out. The types of movements observed imply involvement of cerebellar more so than basal ganglia pathways in classic galactosemia pathology. There may be mechanistic links to primary forms of action tremor, dystonia, and limb ataxia. Of note, there were no firm parkinsonian signs such as rest tremor or rigidity, distancing classic galactosemia from Parkinson disease and other parkinsonisms. Gait impairment was rare with no gait ataxia observed; limb ataxia implies involvement of particular cerebellar areas and pathways, distinct from gait ataxia pathways. There was a mixed picture with most subjects having two or more exam features, with no clear phenotype subcategories. Symptomatic treatment approaches in dystonia and action-based tremor may be particularly applicable to classic galactosemia.

The specific aim 1 exams were (nearly all) confined to data gathered at the 2010 PGC meeting. This represents a single time point with ascertainment bias. For example, we may be missing subjects with higher severity symptoms who cannot travel to meetings. Or, we may see more movement disorders than is typical because those subjects are motivated to get a neurological exam while at the meeting. In terms of forwarding research in galactosemia, even the very low end of severity is interesting. In future studies we hope to get a wider perspective on the severity and types of movement disorders in galactosemia, including larger community input.

We chose an outcome variable based on high sensitivity i.e. capturing even mildly affected subjects as affected, starting at zero and working up the scores to low cut-off values. This is a common line of reasoning in the field for determining clinically defined outcomes. An equally valid clinical approach is starting at the high total score end and working back, capturing only strongly affected subjects as affected and rating all others unknown. This would be more specific, i.e. more likely to discard false negatives. In a small cohort, with no prior knowledge of what we would detect for movement disorders, we pre-specified the high sensitivity approach. In a larger future cohort, based on our pilot experience, it may be more informative to pre-specify a more robust high specificity approach to defining the neurological outcome variable. With a larger sample size, we may be able to use a favored approach in this clinical area, assigning categories such as unaffected, mild, moderate, severe or unaffected, possible, probable, definite affected.

Predictors of Neurological Outcomes

While timing of diagnosis and initial diet intervention have a large impact on severe neurological outcomes such as neonatal seizures and immediate severe cognitive and motor impairments, in contrast long-term movement disorders outcome is not solely

driven by diagnosis or diet intervention timing [3, 8, 26]. We were able to detect movement disorders across pediatric and adult populations, and in patients diagnosed before or after symptom onset. We did not observe any significant associations between the predictor variables and neurological outcome. There were non-significant trends of older age at time of exam and symptomatic at time of diagnosis associating with affected neurological outcome.

Residual GALT enzyme activity level was not associated with neurological outcome status. Indeed, there was a weak positive trend (beta = .0262) (Table 2). This is the opposite of the presumed biological effect wherein higher residual GALT enzyme activity would be protective, and lead to better neurological outcome. The observed effect may be heavily influenced by one outlier with very high residual GALT activity yet affected neurological status (Figure 7). Note this subject was one of the 9 discordant between neurological exam raters, and by our rules set prior to specific aim 2 analyses still considered affected. This is an example of a borderline case other expert raters may well consider unaffected or unknown creating a large impact in the final results. It illustrates how pre-specifying a more stringent set of affected outcome criteria in a larger future cohort may have an impact. Also, discounting a GALT enzyme activity outlier in our cohort brings us down to only 7 subjects with detectible enzyme activity. A much larger cohort is needed to re-address this analysis.

One of the weaknesses of the yeast model system for determining residual GALT activity is that some mutations, like intronic ones, cannot be modeled, creating missing data points. Also, GALT is a dimeric enzyme. Heterozygous mutations may not create an enzyme activity level that is the average of the homozygous mutation situations. The Fridovich-Keil lab continues to explore ways to better determine functional impact of

GALT mutation combinations.

GALT mutation itself was not predictive of neurological outcome in our analysis. This result may be influenced by the choice to code *GALT* mutation based on Q188R status, discarding information from less common mutations. Creating a greater number of meaningful mutation categories will require a larger sample size. Classic galactosemia occurs across many ethnicities, including ones not represented at all in our cohort. A larger cohort with a wider range of ethnic backgrounds could produce a very different distribution of genotypes.

Age at diet intervention was generally very well documented. Delayed intervention, reflected in greater age at diet intervention, clearly contributes to short-term consequences of classic galactosemia. The impact on long-term consequences is less clear, but presumed to be similar: older age at intervention is thought to increase risk for long-term complications. Again we observed a weak trend in the opposite direction, likely driven by one extreme outlier, this time in the unaffected outcome group (Table 2, Figure 7).

The best although by all analyses not significant predictor of neurological outcome was diagnosis status as symptomatic versus non-symptomatic. Newborn screening, only recently mandated in many parts of the US, accounts for the vast majority of pre-symptomatic classic galactosemia diagnoses. A few families knowing the status of siblings opt for even earlier methods of diagnosis detection. False negatives do occur. In some areas it may take several days to obtain newborn screening results, by which time some infants are already acutely symptomatic. Both phenomena contribute to symptomatic diagnoses despite newborn screening. In all, diagnosis prior to acute

symptoms may make a difference in long-term as well as short-term outcome, a key hypothesis to test in future larger studies.

Future Studies

This project started when a parent of a galactosemic child with severe tremor advertised for tremor neurologists to come learn about galactosemia, and hopefully start working on answers for classic galactosemia patients with movement disorders. Most reports of neurological features do not list subjective disability or other severity measures (ex: tremor amplitude). In the general population, kinetic tremors that negatively impact quality of life are often underreported and undertreated [16, 38], and both mild and severe tremor by clinical standards can significantly impact quality of life [39, 40]. There is a dearth of information on the galactosemia community's attitudes about neurological complications. Anecdotal reports suggest that neurological complications of galactosemia range from mild to severe. The full range of severity is informative for research questions on causes of symptoms and prediction of outcome. Surveys can capture patient and family perspectives on symptom severity, disability, and quality of life. We know from our preliminary data that surveys are not designed to capture all cases of mild symptoms. However, they can help us understand the perceived need for interventions. What kind of treatment trials might best serve the community? Are there enough subjects to power a certain type of trial? Quality of life and preference information gathered from the patient community enables us to develop future studies around community goals. Survey data may upend research assumptions about patient and family experiences of symptoms. While survey data clearly miss crucial details captured in direct exams, long-distance surveys offer the advantage of being accessible to a much larger group of subjects, particularly people who cannot travel to PGC meetings.

We gathered initial information on self-reported tremor and impact of tremor from the pilot study participants. We plan to use our pilot data to design a survey for all Fridovich-Keil classic galactosemia study participants (currently 168 individuals) to begin to better understand which neurological complications, if any, present the greatest challenges to quality of life for children and adults with classic galactosemia. We will review questions already used in movement disorders research to best identify motor symptoms like dystonia in lay terms [41-43]. Based on our pilot data, identifying non-tremor symptoms may be more challenging than tremor. We will include questions about impact of motor symptoms on activities, for example illegible handwriting, or spilling food when eating [28, 31]. We will also review quality of life questionnaires that have been applied to galactosemia [44]. We would aim to improve our survey instruments and then survey the larger galactosemia community, outside of the Emory-based study.

We intend to obtain a second set of neurological exam data at the 2012 PGC meeting, creating longitudinal neurological data on current subjects and expanding subject number. We will be able to adjust our approach based on our pilot findings. For example, we will adjust the scope of the exam and videotaping protocol to better capture features difficult to see on the initial videos, and eliminate exam items that did not prove informative. We will add a third exam rater, so that at least two raters will have the same level of information (video exam only) impacting their scores. We will add spiral analysis with an age cut-off (likely 7 years) and age-matched controls based on how pilot subjects handled the task.

This pilot represents an extension of an ongoing study. We therefore have the ability to conduct further biochemical and genetic studies on the same subjects, and connect data

to the movement disorders data. We will compare movement disorders outcome with other long-term outcomes such as ovarian function and cognitive testing. We also intend to use our work to generate future larger scale prospective longitudinal collaborative studies, particularly critical in understanding rare diseases [8, 12]. We observed a trend towards older age at time of exam more likely to be affected. This could simply indicate that people who were not diagnosed pre-symptomatically with newborn screening are more likely to be affected, another trend we observed. Or, long-term neurological symptoms may gradually progress with age, similar to essential tremor. Longitudinal studies will help elucidate if movement disorders progress with age, indicating a neurodegenerative process rather than a static process. The distinction has major implications for the timing and type of interventions that could alter disease progression.

Conclusions

There is a range of long-term movement disorders outcomes in classic galactosemia. Movement disorders were observed equally in female and male subjects, and across the full age range of our cohort. While no firm predictors emerged from this pilot study, trends indicate older subject age and symptom status at diagnosis contributing to affected outcome. Results for GALT enzyme activity and age at diet intervention had weak trends opposite of biological theories in the field; in both cases outliers had a large impact on the results. The pilot results will prove very useful in conducting a larger, hopefully longitudinal next effort.

This project was designed to generate hypotheses about mechanisms behind long-term complications in galactosemia, and thus approaches to discovering new treatments distinct to galactosemia. New hypotheses may come out of the range and type of neurological outcomes as analyzed under aim 1, correlations between neurological and

other features of galactosemia as explored in aim 2, or galactosemia patient and family perspectives on symptom impact on quality of life. Novel, ideally neuroprotective treatments that prevent or slow down long-term complications of galactosemia are the ultimate future goal.

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FIGURE 1: Reproduction of exam scoring sheet for rating galactosemia study videos.

Subject: Rater: Date rated:

Scores = 0-10; zero is none; 5 is moderate Other: chorea, tics, ballism, myoclonus… More comments? Turn page over

Body region	Dystonia		Tremor		Ataxia		parkinsonism		Other		Comments
Face (upper and lower)											
Jaw											
Tongue											
Neck											
Trunk											
Shoulder & proximal arm	L	R	L	R	L	R	L	R	L	R	
Elbow, distal arm, hand	L	R	L	R	L	R	L	R	L	R	
Pelvis & upper leg	L	R	L	R	L	R	L	R	L	R	
Distal leg & foot	L	R	L	R	L	R	L	R	L	R	
Voice											
Gait											

Any specific exam items useful in this subject i.e. pass pointing, arms out posture, tandem gait, bradykinesia:

FIGURE 2

Age distribution (years, Y axis) for the 29 females, right side plot, and 16 males, left side plot. Within the box plots, triangles indicate the mean and 95% confidence limits. The bracketed red ranges indicate the most concentrated 50% of the data.

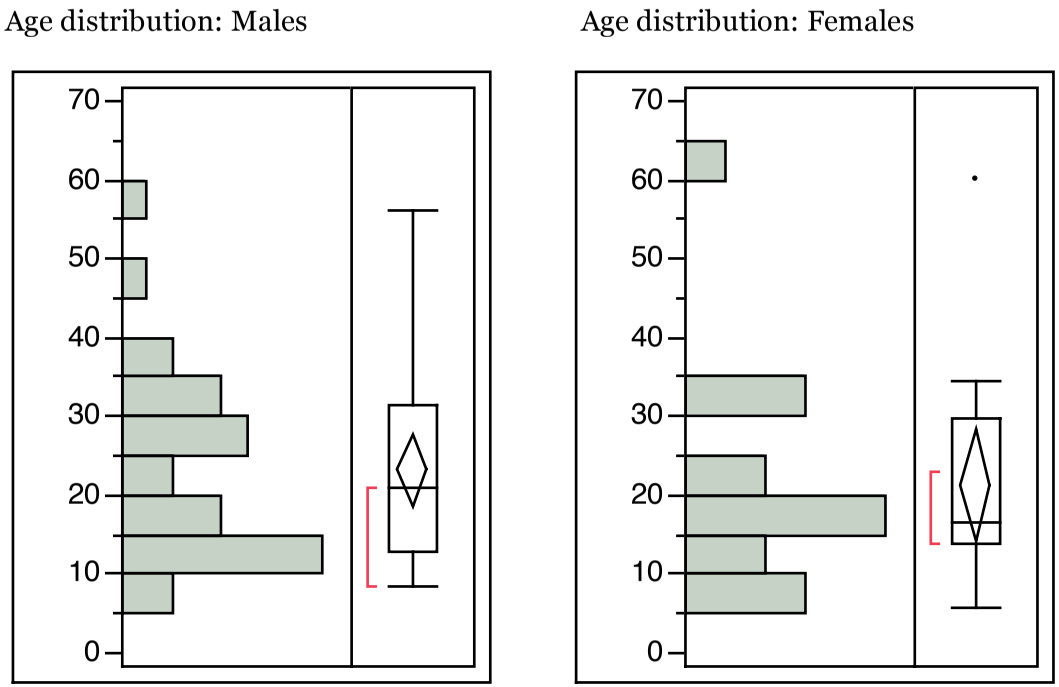

FIGURE 3

Cell plots of exam scores from CMT (upper plot) and SAF (lower plot). Columns are individual cells x exam feature (ataxia, dystonia, tremor, bradykinsia, other) x body part. Rows are subjects. Columns with all zeros are whited out. Shading scale is blue as zero, grading through light blue to light red to red as largest score per column. CMT scored more tremor and less dystonia, with the greatest visual difference in "other" columns to the far left, but there is considerable agreement in most exam features and body areas on this gross visual comparison.

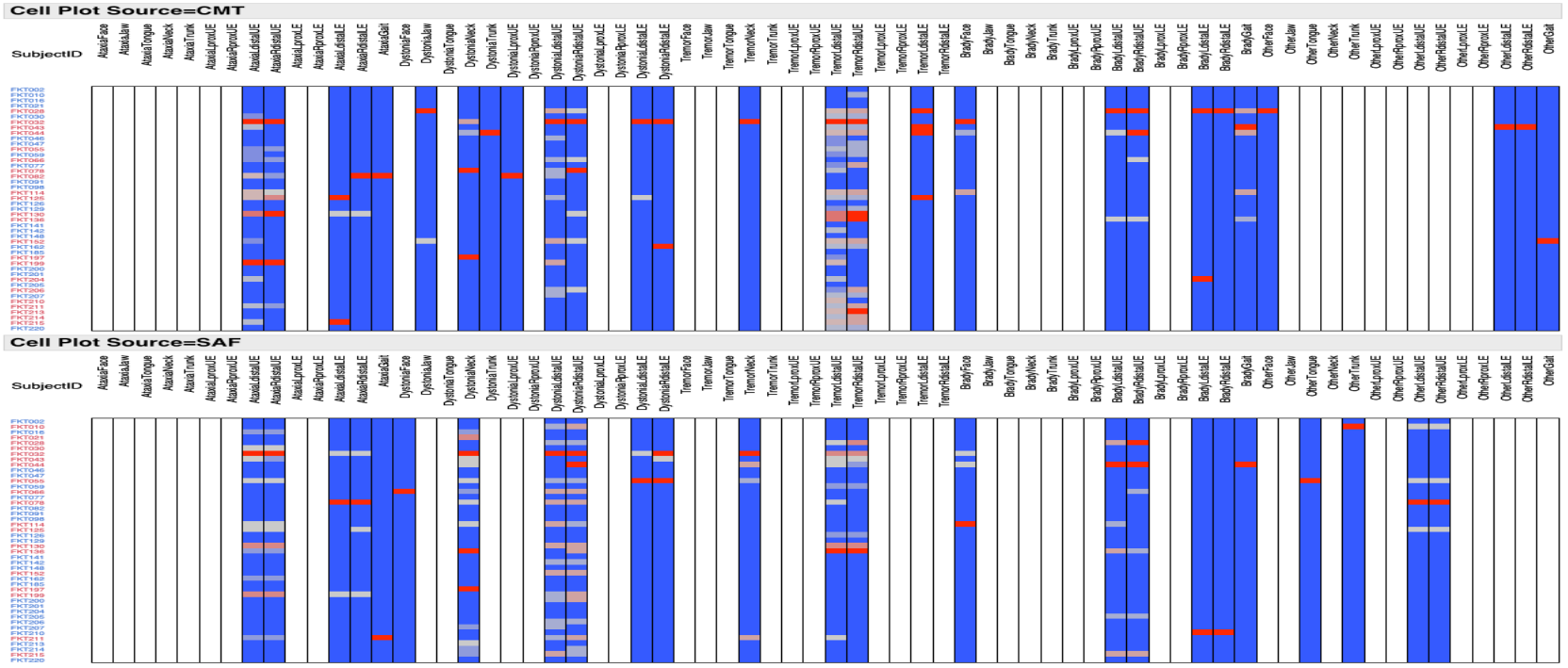

FIGURE 4

Exam feature distribution in exam ratings. Totals for each of the five exam features are displayed with box plots. Within the box plots, triangles indicate the mean and 95% confidence limits. The bracketed red ranges indicate the most concentrated 50% of the data. Upper row of plots are scores from Dr. Testa (CMT), lower row are scores from Dr. Factor (SAF).

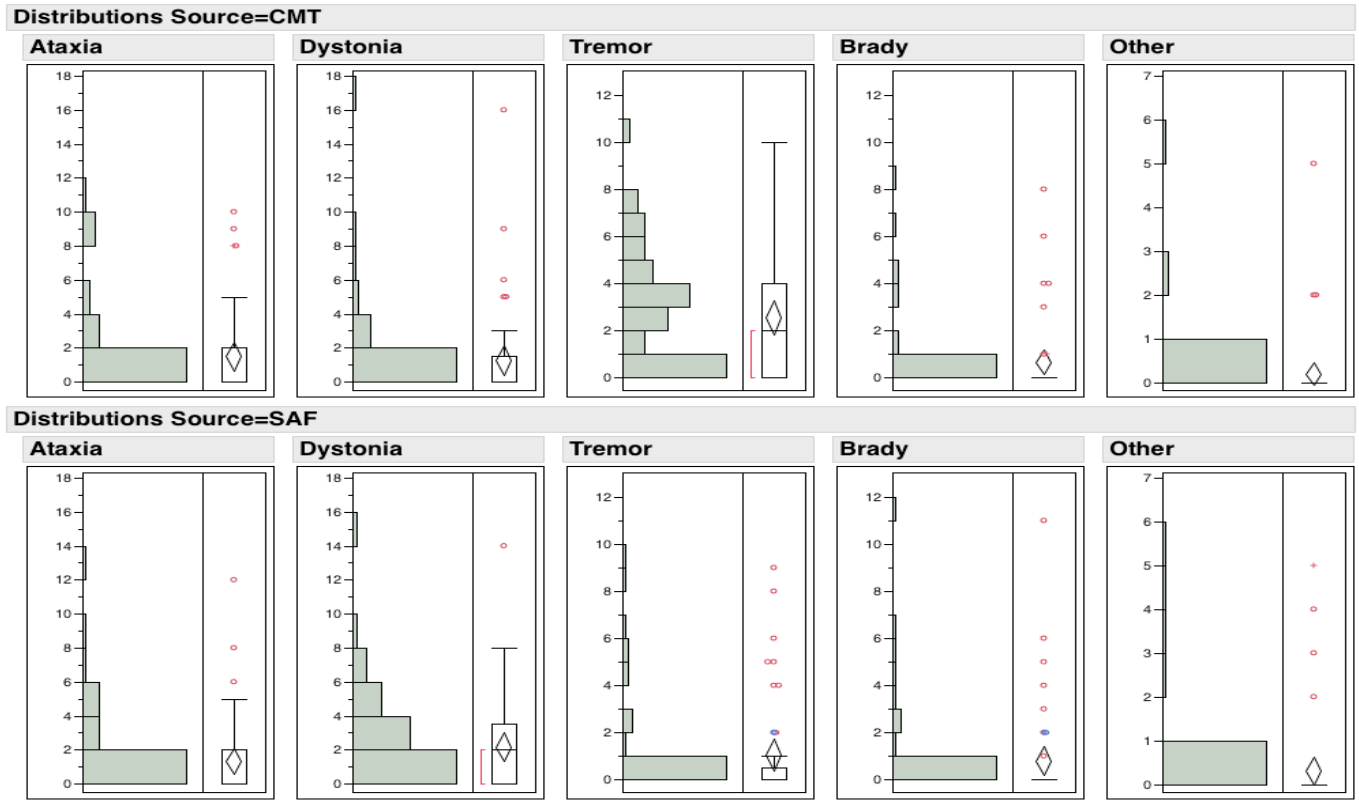

FIGURE 5

Body area distribution in exam ratings. Totals for select body regions displayed with box plots. Within the box plots, triangles indicate the mean and 95% confidence limits. The bracketed red ranges indicate the most concentrated 50% of the data. Upper row of plots are scores from Dr. Testa (CMT), lower row are scores from Dr. Factor (SAF).

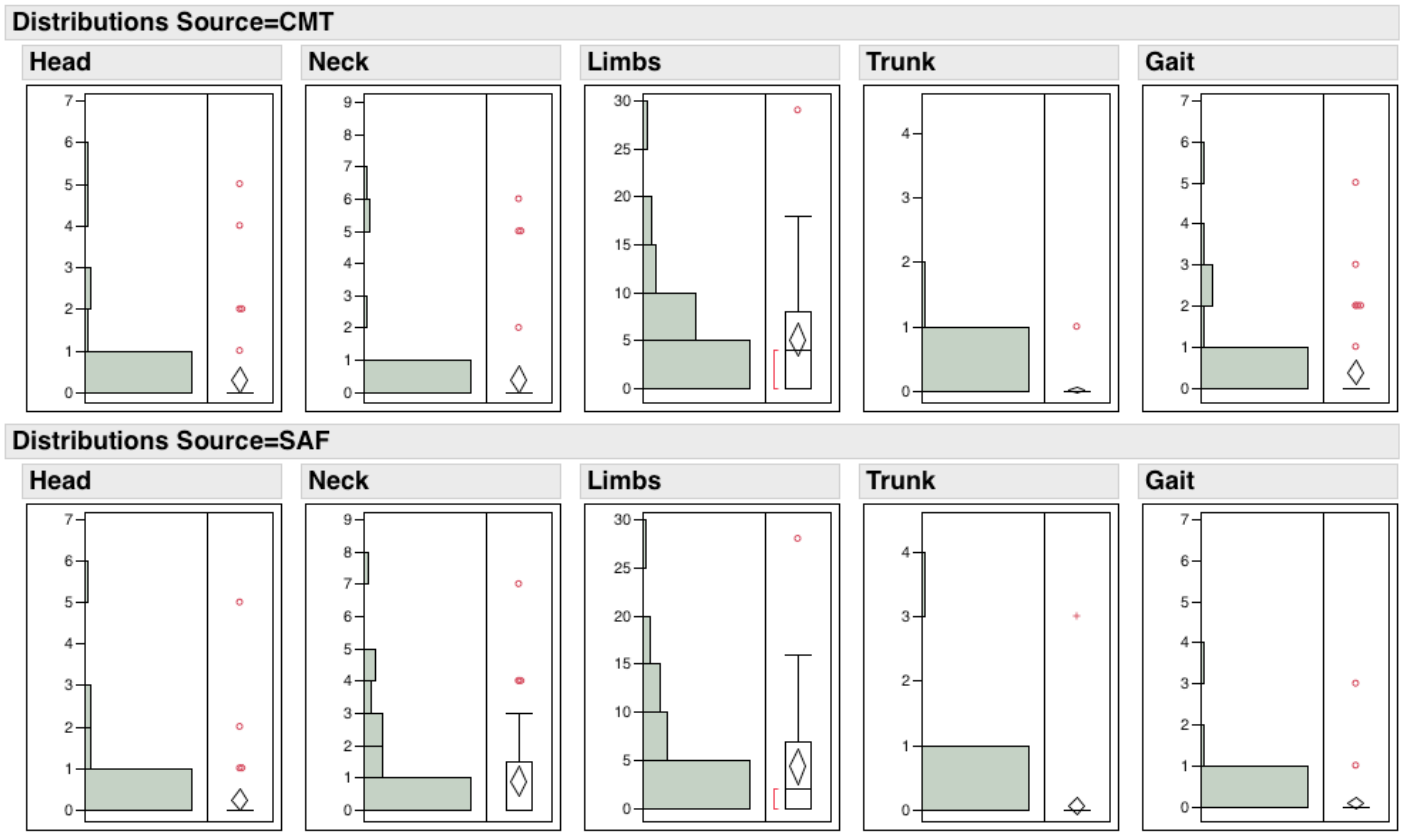

FIGURE 6

Principle components analysis of exam data by exam feature totals. Top row summarizes Dr Testa's exam scores data. Bottom row summarizes Dr. Factor scores. Eigenvalues are displayed in the far left tables. In the central plots, red data points are affected neurological outcome subjects, blue unaffected; circles are concordant neurological outcome assignment between two exam raters, and crosses are discordant neurological outcome assignments. The far right plots show PCA 1 on the X axes, and PCA 2 on the Y axes.

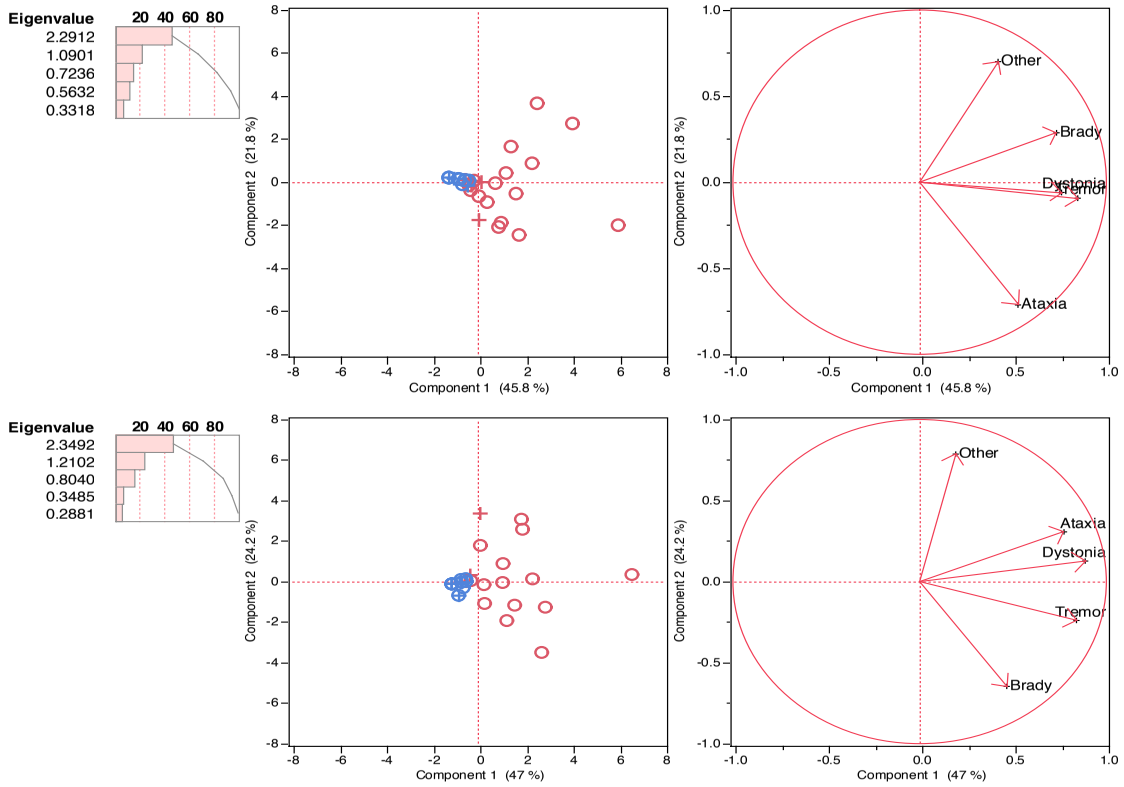

FIGURE 7

Predictor variables by affected status. X axes are not affected on left, affected on right for neurological outcome variable. Left plot is predicted residual GALT enzyme activity; note few subjects have any predicted activity above zero, and there is one outlier with relatively high residual enzyme activity in the affected group. Right plot is age at diet intervention in days; note there is one outlier of relatively late intervention age in the unaffected group.

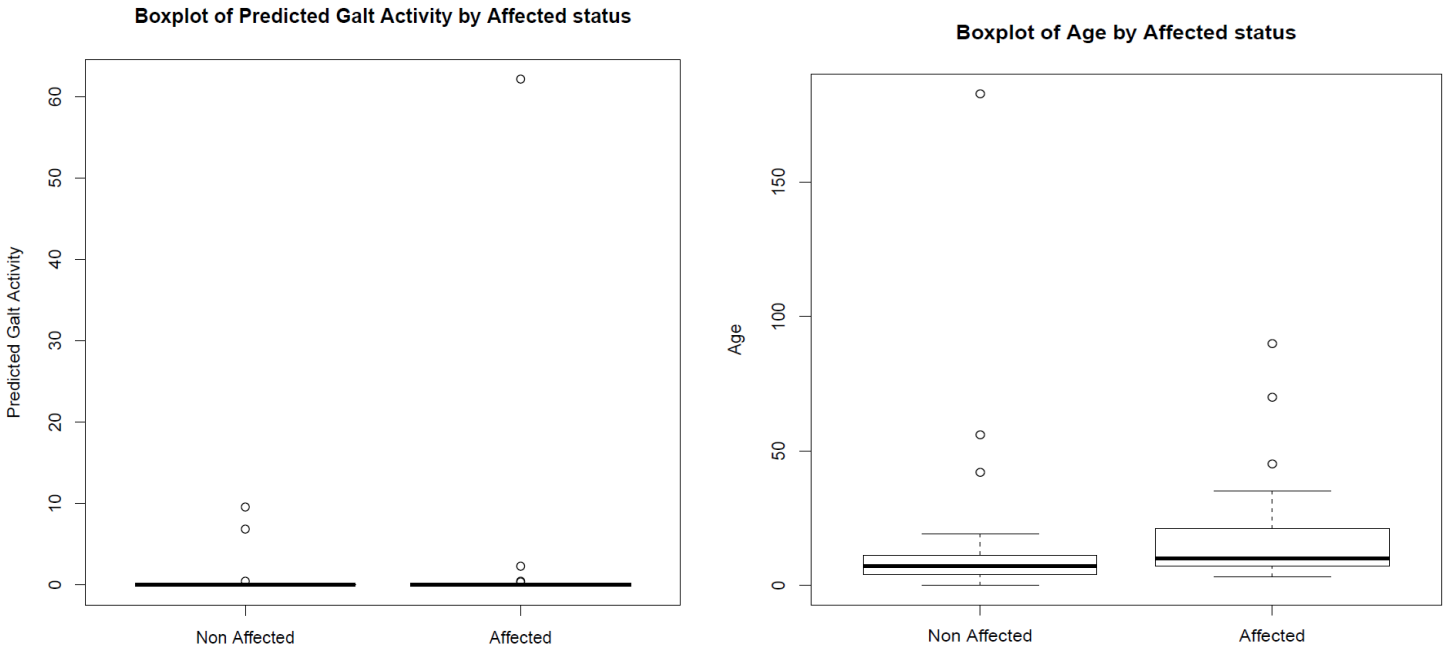

TABLE 1

Correlation coefficients between exam feature totals, using the average of rater scores per exam feature. P-values are in parentheses.

	Dystonia	**Tremor**	**Ataxia**	**Bradykinesia**	**Other**
Dystonia	1	0.65	0.55	0.38	0.30
Tremor	0.65 (< 0.01)	1	0.53	0.58	0.08
Ataxia	0.55 (< 0.01)	0.53 (.00017)	1	0.10	0.04
Bradykinesia	0.38 (.0097)	0.58 (< 0.01)	0.10 (0.513)	1	-0.01
Other	0.30 (0.0464)	0.08 (0.586)	0.04 (0.812)	-0.01 (0.957)	1

TABLE 2

Logistic regression tests for association between individual predictor variables and neurological outcome affected / unaffected. Each row is a single logistic regression test.

Predictor Variable	**Estimated Effect (beta)**	**Z-statistic**	**P-value**
Predicted Galt Activity	0.0262	0.575	0.565
Age at intervention	-0.001	-0.097	0.923
Genotype	-0.478	-0.909	0.364
Age at neurological exam	-0.297	-1.09	0.2745

TABLE 3

Fisher exact tests for association between predictor variables and neurological outcome affected / unaffected.

Newborn screening (NBS): Two subjects were diagnosed prior to newborn screening and prior to symptoms. All subjects diagnosed when symptomatic (no NBS, false negative NBS, etc) were considered diagnosed with symptoms.

Predictor Variable	**Affected**	**Unaffected**
Gender (p-value=.75)		
Male	7	7
Female	15	12
NBS (p-value=0.06187)		
Before Symptoms	0	2
NBS (symptoms unknown)	6	9
With Symptoms	16	8

APPENDIX

The following is copied verbatim from Volume 5, Issue 2 of the Parents of Galactosemic Children, Inc (PGC) newsletter, available for PDF download from www.galactosemia.org. PGC was founded in 1985. This newsletter came out in 2010. The article was brought to the attention of Dr. Fridovich-Keil by its author. Dr. Fridovich-Keil then brought it to Dr. Testa who used it as a motivation for this thesis project.

Neurological Complications of GALACTOSEMIA

Neurologist for 2010 Conference in Minnesota

My assignment for the past two PGC Conferences has been to find a speaker to present information on the neurological issues that about 20% of children and young adults with Galactosemia face. If you have attended the last two conferences, you know that my efforts have yet to come to fruition!

Through my efforts over the past four years, I have compiled a fairly impressive list of Neurologists—unfortunately, only one of them has ever seen someone with Galactosemia. The one thing that I have learned since Adam developed his mild hand tremors 20 years ago when he was 4, is that the neurological issues that Galactosemics exhibit are not of the "textbook" variety— I've heard things like, "Well, it's unusual to exhibit all three types of tremors (functional, kinetic, and intentional)"; or "I've never seen a case like this." I've spoken to many parents who have heard these same words.

As I watch Adam's tremors get worse, he's now developed a slight head tremor, my frustration only worsens. At the last conference, after the GG Luncheon with the Doctors, Adam went up to Dr. Levy and asked in his stilted speech, "Can you help my tremors go away?"

We've tried a number of medications over the years and there really is no "magic pill." Not to mention that some of those we tried had to be composed by a compound pharmacist because the prescribed medication contained lactose.

My dream is to of course find one Neurologist who will agree to "specialize" in the "Galactosemia neurological issues." I'm not one to give up, it may take a while but I do believe that this is possible. After all, I was told that Adam would never

read—so I hired a private tutor and he reads on an 8th Grade level. I was told Adam would never swim— and I let air out of his swimmies slowly until he didn't realize that he was "swimming" without any air in them. And as many of you have read before, when Adam was born and I asked the physician if there were other families who I could talk to, I was told other families don't need to talk to one another, they need to love their children—that was when I founded Parents of Galactosemic Children!

Any parent whose child has neurological issues can understand my frustration.

We are looking for a Neurologist who specializes in Cerebullar Ataxia, Tremors, and/or Movement Disorders specifically as exhibited in those with Galactosemia.

This call goes out to physicians and families who may know of a Neurologist:

• Has your child seen a Neurologist who you would recommend to speak at the PGC Conference?

• Do you as a physician know of Neurologist who can address the families about the neurological issues that Galactosemics face?

If so, please contact me—maybe my dream will come true (or at least I'll accomplish my assignment to find a Neurologist to speak at the 2010 PGC Conference).

Linda Manis Lmscript1@aol.com 954-610-3739