A Statistical Approach for Small Sample Clinical Trials to Improve Individualized Care for Patients with Neurodevelopmental Disorders

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Abstract

Despite the many recent, replicable findings in genetic studies of neurodevelopmental disorders (e.g., autism spectrum disorders, intellectual disability, schizophrenia, bipolar disorder), these discoveries have not translated into improved clinical care. One reason is that the effect sizes of individual common variants identified through genome-wide association studies are small (odds ratios near 1). However, these illnesses also have an increased mutational burden for rare structural genomic variants, which have much larger effect sizes (odds ratios between 2 and 60) than those associated with common genetic variants and may therefore be more amenable to targeting the biological effects of individual mutations. One such rare variant, involving a triplication (four copies instead of the usual two) of the gene encoding glycine decarboxylase, *GLDC*, was identified in a mother and son, each of whom had a diagnosis of a psychotic disorder. Triplication of *GLDC* would be expected to increase the breakdown of glycine and D-serine, resulting in low brain levels of these co-agonists at the N-methyl-D-aspartate receptor (NMDAR) and NMDAR hypofunction. Augmentation of usual psychotropic drug regimen with glycine, a full agonist at the glycine modulatory site (GMS) or d-cycloserine (DCS), a partial agonist at low doses at the GMS, could potentially normalize NMDAR function.

The purpose of this project was to determine whether administering glycine and DCS in individuals who have the gene triplication of *GLDC* can influence the slope of the absorption of glycine with the goal of changing the slope from positive to flat. We also wanted to find if glycine and DCS treatment had any effect on brain bioenergetic variables and event-related potential variables, which have previously been shown to be connected to psychotic disorders such as schizophrenia and bipolar disorder. Positive, negative, and flat slopes are determined by the 50% confidence intervals. In addition to the carrier mother and son, the glycine absorption was collected over 120 minutes for 7 healthy controls, and 2 non-carrier relatives for glycine absorption data. The event-related potential (ERP) data was collected for 170 bipolar patients, 170 healthy controls, and 176 schizophrenic patients. The brain bioenergetics data consists of 26 controls and 26 schizophrenic patients. The glycine absorption levels were collected for the two carriers before treatment (Baseline), after DCS (Post-DCS), and after Glycine (Post-Glycine). A linear fit of each participant's glycine level or score defines a trajectory with two features: the intercept or initial glycine level and the slope or rate of glycine absorption. For each brain bioenergetics and ERP auditory variable scores were recorded before and after treatment. The mean and standard deviation for each group were found and a z-score was computed to determine extreme values before and after treatment. A z score above |2| was considered

extreme. Boxplots were also created to compare the mother, son, and non-carrier siblings' sample.

We found that carriers' glycine levels were considered flat after glycine treatment for both carriers, however only one carrier showed changes in trajectory after DCS treatment. Glycine appears to be better treatment for both, but one carrier could benefit from either treatment. Glycine and DCS appeared to be equally effective in improving brain bioenergetic and ERP deficits. This is precisely the goal of most ``n-of-1" trials - to pinpoint research and treatment for individualized clinical care.

Background

Large randomized clinical trials have been considered the gold standard for improving clinical care since their beginning in the 18th century and has since been spurred on by advancements in statistical analyses and computing power. But can a large clinical trial with thousands of participants provide specialized treatment for each member of the population? With each person's unique genetic coding and make-up, it would seem almost impossible to expect a single treatment (drug, nutrient, supplement, behavioral change, etc.) to be beneficial for thousands of unique individuals. In fact, despite its gold standard level, large cohort randomized clinical trials have been found lacking in certain cases where some individuals have difficulty finding a treatment that works for them, despite it showing outstanding results from a treatment group in a clinical trial.[1] This limitation has led to the desire to conduct extremely small clinical trials in order to specialize treatment for an individual versus an entire group. These trials known as ``n-of-1" trials are usually performed on a single individual, or a handful of individuals with similar qualities such as family members, compared to healthy controls to determine effective specialized treatments and improve clinical care at the individual level.

One of the main reasons large sample randomized clinical trials are so popular is because of their statistical properties. With a large sample comes improved reliability of results, higher statistical power, and availability of more statistical testing. However, with these advantages there are also disadvantages, such as reduced individualized care, no personalized plan of treatments, and treatment failures for individuals under unique circumstances. Clinical trials must get smaller to properly treat the individual as opposed to the general population. Despite similar population characteristics, two individuals are extremely different at the genetic level no matter how many demographic similarities they possess, and to improve clinical care clinical trials must shrink to an individual level.

A copy number variation (CNV) is genetic phenomenon that occurs when sections of the genome are repeated and the number of repeats in the genome varies between individuals in the human population.[2] Even the most recurrent account for only ~1% of ASD and schizophrenia, and most are only found in a few individuals or are unique to that individual.[3] As a result, sample sizes will invariably be small, even limited to one or a few families, and thus most studies will be variations of``n-of-1" trials.[4] Indeed, if specific mutations represent a molecular subtype, therapeutic benefit from pathway-defined treatments may not be limited to individual mutations,[5] resulting in potentially larger sample sizes.

The study of rare structural variants such as copy number variants (CNV) in individuals with psychosis has been performed in recent years to develop targeted treatment. It has previously been found that individuals with a copy number variant containing a genomic triplication of the glycine decarboxylase gene accounts for an increased mutational burden for

schizophrenia and other neurodevelopmental disorders (e.g. autism spectrum disorders, intellectual disability, epilepsy, bipolar disorder) .[6]

Our work is a continuation of a previous study that found glycine and D-cycloserine to be treatments that improved psychotic and mood symptoms in a placebo-controlled trial. Since the previous study reported improvement on symptoms but did not provide precise monitoring of levels of glycine and DCS over time we felt that a trajectory analysis was appropriate to provide further information on the effects of the treatments. We have previously identified two carriers of a triplication of the gene encoding glycine decarboxylase, *GLDC*, who have been diagnosed with a psychotic disorder (mother and son). The novelty of this mutation means that the sample we used to compare to our healthy controls and other psychotic disorder patients is two, which for a clinical trial is uncommonly small. However, it is believed that to perform precision medicine, treatment that focuses on the individual and not the general population, a different type of clinical trial must be conducted. Well-designed individual sample clinical trials may be ideal for rare diseases and in this case a rare copy number variant; it also allows us to explore options for individuals with rare genomic variants.[1]

Further interest beyond glycine absorption in this same population was also conducted as part of this research. Brain energy metabolism is critical for supporting synaptic function and information processing. There is evidence that suggests abnormalities in brain bioenergetics and psychiatric disorders, including bipolar disorder (BD) and schizophrenia, are related.[7] A study previously found that redox dysregulation and brain bioenergetic anomalies have been implicated in the pathophysiology of psychotic disorders.[8] Previous studies have also identified abnormalities in brain bioenergetics in psychotic disorders using ^{31}P magnetic resonance spectroscopy (MRS). ^{31}P MRS allows the measurement of high energy phosphate (HEP) metabolite levels, including phosphocreatine (PCr) and adenosine triphosphate (ATP) as well as inorganic phosphate (Pi).[7, 8] ^{31}P magnetic resonance spectroscopy provides a noninvasive window into these processes in vivo. It has also been previously found that using a ^{31}P magnetization transfer approach revealed new evidence of dysfunctional brain bioenergetics, specifically, a significant reduction in the forward rate constant of a critical enzyme involved in energy metabolism, creatine kinase.[7] In this research it was used to measure the forward rate constant (k_f) of the creatine kinase enzyme in the frontal lobe, the phosphate to beta-ATP ratio ($PCr/\beta\text{-}ATP$), and the inorganic phosphate to beta-ATP ratio ($Pi/\beta\text{-}ATP$) .[7]

Similar to brain bioenergetics, there are several auditory brain responses that are abnormal in patients with schizophrenia [9], and can be used as indicators of disease progression. The auditory steady-state response (ASSR) has been widely used to assess neural synchrony and the integrity of auditory pathways within and between cortical regions in patients with schizophrenia. The ASSR also reflects neural synchrony in the gamma band (30-100 Hz) and is abnormal in patients with schizophrenia.[9] However, previous studies have shown that not only schizophrenia patients show deficits in gamma ASSRs; patients with bipolar disorder also exhibit deficits.[9]

Another brain function that lends insight into the effects of psychotic disorders in the brain is event-related potential. Event-related potentials (ERPs) are very small voltages generated in the brain structures in response to specific events or stimuli. Electroencephalography (EEG) provides an excellent medium to understand neurobiological dysregulation. Another term for event-related potential is time-locked EEG, and it helps capture neural activity related to both sensory and cognitive processes.[10] There are some waveforms of ERP whose levels, specifically deficits, are related to schizophrenia and bipolar disorder. These waveforms include

P50 wave, N1 wave, P2 wave, N2 wave, and P3 wave.[10] The N2 wave has three components: N2a/Mismatch Negativity (MMN), N2b, and N2c. MMN is a negative component which is elicited by any discriminable change in a repetitive background of auditory stimulation.[10] There is decreased MMN amplitude as well as abnormal MMN topographical distribution in treatment-refractory patients with schizophrenia.[11] The P3 wave consists of two parts: P3 latency and P3 amplitude. P3 latency was found to be increased in schizophrenic patients, but not in their first-degree relatives. P3 Amplitude is sensitive to fluctuations in the severity of symptoms, independent of medication, and to the enduring level of negative symptom severity. Sensory gating is the process of filtering out irrelevant stimuli (for example, the sound of the air conditioning running) from meaningful ones (e.g. your phone ringing). For example, P50 event-related potential sensory gating deficit, a failure to inhibit responses to repeated stimuli, is a robust finding and leading endophenotype for schizophrenia.[11] Sensory gating is crucial to an individual's ability to selectively attend to important stimuli and ignore redundant, repetitive, or trivial information which protects the brain from overflow. The study of the P50 waveform as a test of sensory gating. N1 and P2 waveforms may reflect the sensation-seeking behavior of an individual. The N1 wave is an orienting response that matches a stimulus with previously experienced stimuli.[11]

The many recent replicable findings in genetic studies of neurodevelopmental disorders (e.g., autism spectrum disorders, intellectual disability, schizophrenia, bipolar disorder) have not translated into improved clinical care. Effect sizes of individual common variants identified through genome-wide association studies are small. However, these illnesses have an increased mutational burden for rare structural genomic variants, which have much larger effect sizes than those associated with common genetic variants and may therefore be more amenable to targeting the biological effects of individual mutations. The gene encoding glycine decarboxylase, *GLDC*, is believed to reduce availability of the N-Methyl-D-aspartate receptor coagnoists glycine, D-serine and N-methyl-D-aspartate receptor hypofunction.[6] Triplication of *GLDC* would therefore be expected to increase the slope of absorption of glycine and D-serine, resulting in low brain levels of these co-agonists at the N-methyl-D-aspartate receptor (NMDAR) and NMDAR hypofunction. Augmentation of usual psychotropic drug regimen with glycine, a full agonist at the glycine modulatory site (GMS) or d-cycloserine (DCS), a partial agonist at low doses at the GMS, could potentially normalize NMDAR function, brain bioenergetic responses, and ERP sensory gating deficits.

Methods

Two double-blind, placebo-controlled clinical trial of psychotropic drug treatment in two individuals were previously conducted. In the first trial glycine was used, and in the second trial D-cycloserine was used as the treatment. The data examining two carriers - the proband (Subject 3363) and his mother (Subject 5459) - of the CNV consists of 7 healthy controls, and two non-carrier siblings (one half- and one full-sibling). Both carriers presented with DSM-IV diagnoses of a psychotic disorder. The variable of interest is glycine level collected at baseline, 60 minutes, 80 minutes, 100 minutes, and 120 minutes. Glycine crystals were dissolved in a juice and dose adjusted for the participant's body weight. The dose was administered orally at 0.4g/kg with a maximum set to 30g to avoid gastrointestinal distress. The participants were given 10 minutes to consume the dose and magnetic resonance spectroscopy (MRS) scanning resumed 30 minutes after the dose was administered. In a separate study, each carrier was administered 50 mg of

DCS. We were interested in the slope of glycine absorption upon treatment over time and measured glycine levels over two hours to determine the trajectory of glycine in the brain over time post-treatment.[6]

In order to determine the estimated slope of absorption for each group, glycine and DCS were regressed on time after each dose was administered and the Kruskal-Wallis test was used to determine median slope differences across groups. Because this is a trajectory analysis, we chose to focus on the change in slope post-treatment as opposed to simply reporting p values from a statistical test. Post-treatment results were collected for the two carriers. The slopes are categorized as flat, positive, or negative based on the 50% confidence interval of the slope. If the confidence interval contained zero, then we classified the group as having a flat slope; above zero indicates positive slope and below zero indicates negative slope. All groups can be classified as either decreasing in glycine absorption, staying about the same, or increasing in absorption over time.[12] The 50% confidence interval is commonly used in trajectory analysis and to classify slopes of trajectories.[12] A previous study using trajectory analysis in post-operative patient pain measurements employed the 50% confidence interval in order to determine slope categories. The pain trajectory used the initial pain measurement as the intercept and the change over time as the slope and pain trajectory was over a short period of time (7 days). Due to the similarities in the analysis, we chose to also use a 50% confidence interval for our slope. Other trajectory analysis research has used 95% confidence intervals, suggesting that a confidence interval range (50% - 90%) is considered useful in the categorization of slopes for trajectory analysis.[13] The aim was to analyze the trajectories of glycine for each group across the 120 minute time-frame, and compare the carriers to their non-carrier relatives and healthy controls. Determining whether this treatment is beneficial to those with an increased mutational burden and therefore improve quality of care for these individuals, and to determine a reliable statistical analysis that is applicable in determining significant differences using small samples were the main goals.

Another previous study measured ERP sensory gating deficit as an endophenotype for schizophrenia and bipolar disorder.[10] The data from this study included 170 healthy controls, 176 individuals with schizophrenia, and 170 individuals with bipolar disorder; 170 individuals with bipolar disorder consists of bipolar I and bipolar II. The 176 individuals with schizophrenia consist of all subtypes schizophrenic, schizoaffective, schizophreniform, and psychosis not otherwise specified. The auditory measurements included are ASSR 20, ASSR 30, ASSR 40, Mismatched Negativity (MMN) Amplitude, N1 Amplitude, P 50, P2 Amplitude, P3 Amplitude, and P3 Latency. A paired-stimulus paradigm, in which two identical auditory stimuli are presented 500 milliseconds apart, was used to evaluate sensory gating. A z-score for the paired-stimulus paradigm for each ERP variable for the carriers was computed using the mean and standard deviation of the healthy controls for each measurement, post-glycine, and post-DCS. A z-score with an absolute value greater than 2 is considered an extreme value.

The brain bioenergetics data included 26 healthy controls and 26 individuals with schizophrenia. The brain bioenergetic response the measurements included are k_f, $PCr/\beta\text{-}ATP$, and $Pi/\beta\text{-}ATP$. As with the ERP data, z-scores for the carriers were computed using the mean and standard deviation of the healthy controls.

Results

Tables 1 and 2 provide the 50% confidence intervals for the slope of glycine levels post-treatment. After glycine was administered the slope for both carriers changed from positive (50% CI is positive) to flat (50% CI includes 0). After DCS was administered, Subject 3363 had a slope change from positive to flat (50% CI: -0.0197 to 0.0366), while Subject 5459's slope remained positive (0.0067 to 0.1064). Figures 1 and 2 show the pre-treatment and post-treatment slope of glycine absorption over the 120-minutes period. At baseline, the median slope of absorption was not statistically significantly different by group ($p = 0.0508$). Carriers at baseline did not significantly differ from non-carrier relatives ($p = 0.333$); carriers at baseline did not have a statistically different median slope than the controls ($p = 0.056$). Non-carrier relatives did not significantly differ from the controls ($p = 0.222$).

Table 1a: Median Estimated Slopes by Group at Baseline and Post-Glycine and Post-DCS

Group	ID	Slope	Baseline(50% CI)	Median(IQR)
Carriers	5459	0.0857	(0.054, 0.118)	0.094 (N/A)
	3363	0.1018	(0.067, 0.137)	
Non-Carriers	6463	0.0724	(0.024, 0.120)	0.0740 (N/A)
	5754	0.0755	(0.065, 0.086)	
Controls	1001	-0.0419	(-0.057, -0.026)	(-0.055, -0.031)
	1002	0.0043	(-0.018, 0.027)	
	1003	-0.0400	(-0.028, 0.020)	
	1004	0.0837	(0.034, 0.133)	
	1005	-0.0719	(-0.100, -0.043)	
	1006	0.0313	(0.005, .0058)	
	1007	-0.0549	(-0.089, -0.021)	

Table 1b: Median Estimated Slopes by Group at Baseline and Post-Glycine and Post-DCS

Group	ID	Slope	Post-Glycine(50% CI)	Median(IQR)	Slope	Post-DCS(50%)	Median(IQR)
Carriers	5459	-0.0269	(-0.065, 0.012)	-0.0087 (N/A)	0.0868	(0.0067, 0.1064)	0.0391(N/A)
	3363	0.0096	(-0.016, 0.035)		0.0085	(-0.0197, 0.0366)	

Table 2: Slopes by Category at Baseline and Post-Glycine and Post-DCS

Group	ID	Baseline (SlopeCategory)	Post-Glycine (Slope Category)	Post-DCS (SlopeCategory)
Carriers	5459	Positive	Flat	Positive
	3363	Positive	Flat	Flat
Non-Carriers	6463	Positive		
	5754	Positive		
Controls	1001	Negative		
	1002	Flat		
	1003	Flat		
	1004	Positive		
	1005	Negative		
	1006	Positive		
	1007	Negative		

Table 3: Summary Statistics for ERP Data Overall and by Groups, N=516

Variable	Overall	Healthy Controls N = 170	Schizophrenia N = 176	Bipolar Disorder N = 170	P value*
Age Mean(SD)	37.64 (13.74)	31.98 (12.56)	42.89 (12.87)	38.44 (13.79)	**<0.0001**
ASSR 20 Mean (SD)	0.07 (0.07)	0.08 (0.08)	0.05 (0.07)	0.05 (0.06)	**0.0038**
ASSR 30 Mean (SD)	0.11 (0.10)	0.14 (0.11)	0.09 (0.10)	0.09 (0.08)	**<0.0001**
ASSR 40 Mean (SD)	0.18 (0.14)	0.25 (0.14)	0.14 (0.14)	0.15 (0.13)	**<0.0001**
MMN Amplitude Mean (SD)	-2.06 (2.09)	-3.15 (1.99)	-1.28 (2.06)	-2.11 (1.71)	**<0.0001**
N1 Amplitude Mean (SD)	-3.50 (2.91)	-4.00 (2.89)	-3.08 (2.82)	-3.45 (3.03)	**0.0243**
P 50 Mean (SD)	59.57 (41.34)	39.57 (27.06)	74.81 (44.80)	66.12 (42.37)	**<0.0001**
P2 Amplitude Mean (SD)	4.47 (3.42)	5.18 (3.70)	3.78 (2.97)	4.46 (3.48)	**0.002**
P3 Amplitude Mean (SD)	369.66 (77.22)	366.27 (49.35)	415.58 (88.04)	412.94 (79.37)	**<0.0001**
P3 Latency Mean (SD)	10.17 (5.54)	13.00 (5.62)	8.07 (4.57)	9.27 (5.13)	**<0.0001**
Sex					
Male, N (%)	271 (52.23)	68 (25.09)	115 (42.44)	84 (50.00)	
Female, N(%)	247(47.77)	102 (41.30)	60 (24.29)	84 (50.00)	**<0.0001**

Schizophrenia includes subtypes Schizoaffective, Schizophreniform, and psychosis not otherwise specified. Bipolar disorder includes types I and II.
Bold pvalues denote statistically significant differences.

Table 4: Summary of Frequencies (%) of Diagnosis for ERP Data

Diagnosis	Frequency	Percent
Bipolar	170	32.63
-Bipolar-I	168	32.25
-Bipolar-II	2	0.38
Controls	170	32.63
PsyNOS	4	0.76
Schizoaffective	95	18.23
-Schizoaffective	5	0.96
-Schizoaffective, bipolar type	58	11.13
-Schizoaffective, depressive type	31	5.95
-Schizoaffective, unspecified	1	0.19
Schizophrenia	79	15.16
-Schizophrenia	16	3.07
-Schizophrenia, disorganized	5	0.96
-Schizophrenia, paranoid	32	6.14
-Schizophrenia, residual	3	0.58
-Schizophrenia, undifferentiated	22	4.22
-Schizophrenia, unspecified	1	0.19
Schizophreniform	1	0.19

PsyNOS denotes psychosis otherwise not specified.

Figure 1: Pre-Treatment Measures

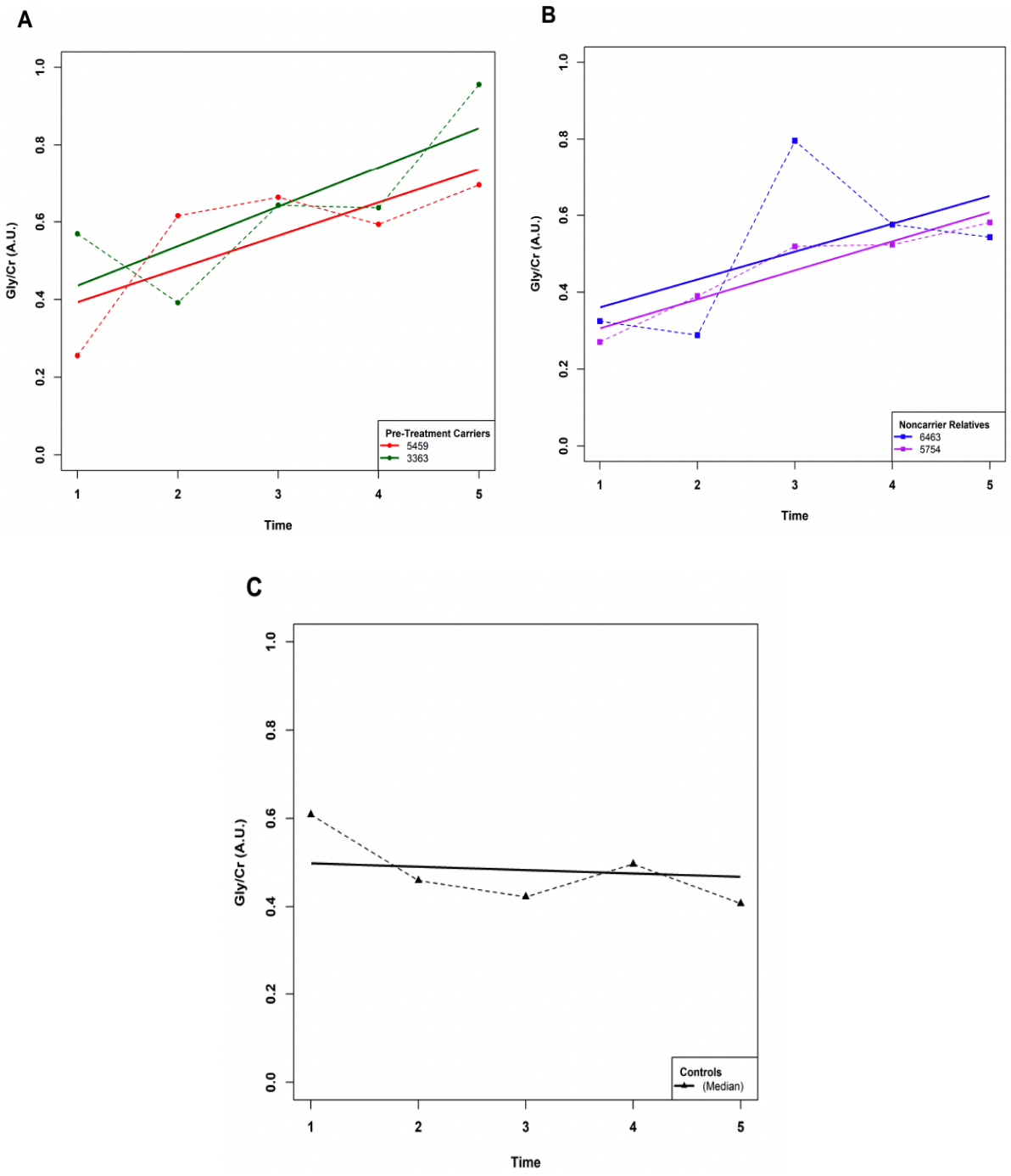


Figure 2: Post-Treatment Measures

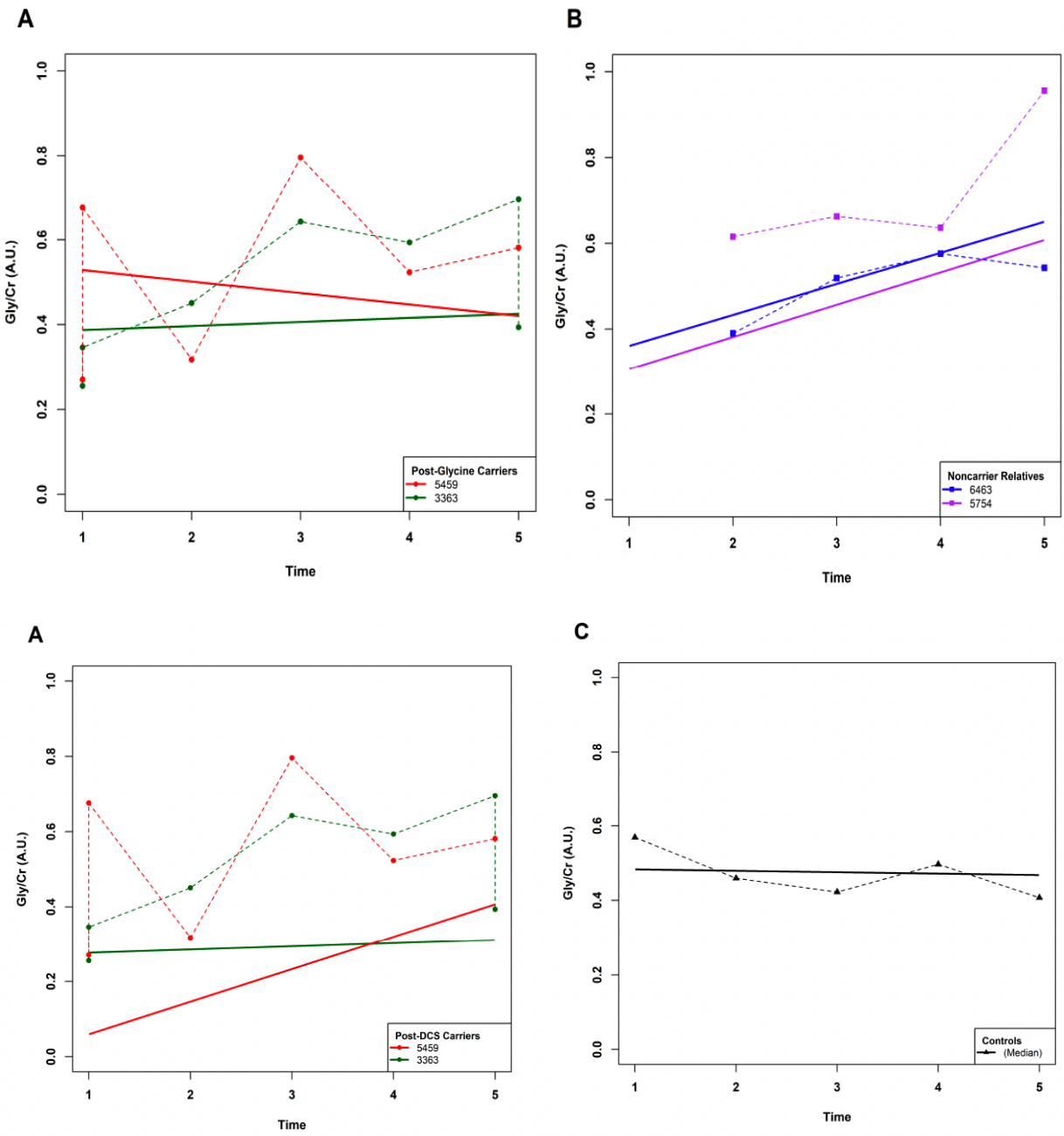


Table 5 provides ERP mean and standard deviation data for the healthy controls and carriers as well as the computed z-scores for the carriers post-treatment. Carrier baseline z-scores do not include any values that are outside ±2, which indicates that even before treatment the carriers ERP scores are not considered extreme in comparison to healthy controls. However, after glycine was administered z-scores for ASSR 20, ASSR 30, ASSR 40, P 50, P2 Amplitude, P3 Amplitude, and P3 Latency all improved (i.e., were closer to the median value for healthy controls). After DCS was administered z-scores for ASSR 30, ASSR 40, MMN Amplitude, P2 Amplitude, P3 Amplitude, and P3 Latency improved. We have found that glycine and DCS treatment may have some effect on the event-related potential variables that have been shown to have connection to psychotic disorders such as schizophrenia.

Table 5a: ERP Data Z scores for the Carriers

Variable	Healthy Controls Mean(SD)	Carrier Baseline	Carrier Baseline (Z-score)
ASSR 20	0.08 (0.08)	0.02	-0.75
ASSR 30	0.14 (0.11)	0.18	0.36
ASSR 40	0.25 (0.14)	0.14	-0.79
MMN Amplitude	-3.15 (1.99)	-3.36	-0.11
N1 Amplitude	-4.00 (2.89)	-3.93	0.02
P 50	39.57 (27.06)	44.52	0.18
P2 Amplitude	5.18 (3.70)	1.66	-0.95
P3 Amplitude	366.27 (49.35)	279.3	-1.76
P3 Latency	13.00 (5.62)	5.34	-1.36

The Z-Score for the carriers is computed by taking the mean of the healthy controls Minus the carrier mean and divide by the standard deviation.

Table 5b: ERP Data Z scores for the Carriers

Variable	Carrier Glycine	Z Score Glycine	Carrier DCS	Z Score DCS
ASSR 20	0.11	0.34	0.01	-0.88
ASSR 30	0.18	0.34	0.17	0.25
ASSR 40	0.26	0.04	0.34	0.67
MMN Amplitude	-1.00	1.08	-3.33	-0.09
N1 Amplitude	-4.71	-0.25	-3.26	0.26
P 50	35.67	-0.14	30.00	-0.35
P2 Amplitude	6.29	0.30	8.20	0.82
P3 Amplitude	292.97	-1.49	294.00	-1.46
P3 Latency	5.57	-1.32	6.62	-1.14

The Z-Score for the carriers is computed by taking the mean of the healthy controls minus the carrier mean and divide by the standard deviation.

Table 6: Summary Statistics for Brain Bioenergetics Data Overall and By Groups, N = 58

Variable	Overall	HC, N = 26	SZ, N = 26	P value
Age	33.21 (8.42)	34.5 (8.38)	31.92 (8.43)	0.2743
K_f	0.24 (0.07)	0.22 (0.07)	0.27 (0.06)	**0.0049**
PCr/β-ATP	1.39 (0.22)	1.66 (0.17)	1.36 (0.17)	0.9261
Pi/β-ATP	0.43 (0.08)	0.40 (0.11)	0.42 (0.07)	0.353
Sex				
Male	27(51.92)	13 (50.00)	14 (53.85)	
Female	25(48.08)	13(50.00)	12 (46.15)	0.7817

Variables are reported as mean(SD) and frequency(%) were applicable.

Table 7: Summary of Frequencies(%) of Diagnosis for Brain Bioenergetics Data

Diagnosis	Frequency	Percent
Controls	26	50
Schizophrenia	26	50
-Schizophrenia	12	23.08
-Schizohrenia* in Major Depressive Episodes	2	3.85
-Schizoaffective	2	3.85
-Schizoaffective-Bipolar type	4	7.69
-Schizoaffective-Depressive type	5	9.62
-Schizoaffective-Depressive type* in Major Major Depressive Episodes	1	1.92

Figures 3, 4, 5, and 6 provide images of the boxplots of the distribution of the ERP data. We illustrate side-by-side the single values for the carriers, at baseline and post-treatment, and non-carrier siblings. Glycine appears to have brought values closer to that of median healthy control levels for ASSR 20, ASSR 30, ASSR 40, P3 Amplitude, P 50, N1 Amplitude, and P2 Amplitude. DCS appears to have brought values closer to that of the median healthy control levels for MMN Amplitude, P 50, P3 Latency, P3 Amplitude, ASSR 40, and ASSR 30.

Figure 3: Boxplots of Auditory Frequency Measures Across Subgroups for ERP Data

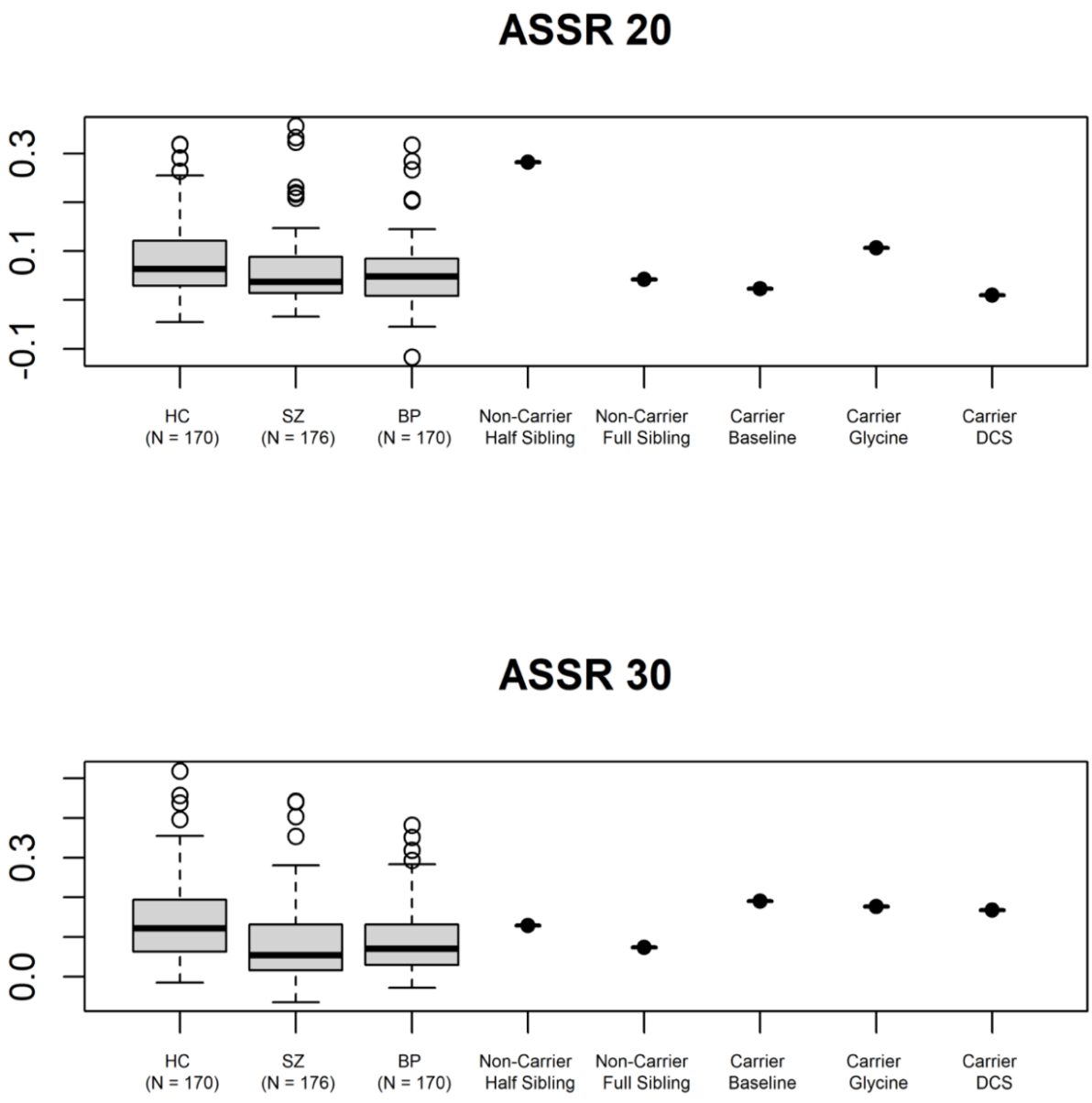
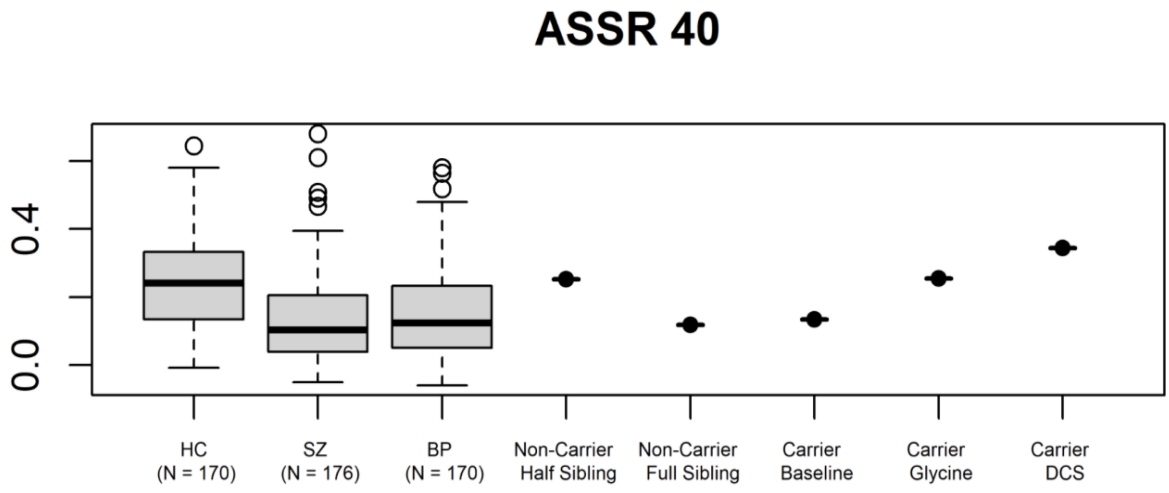


Figure 4: Boxplots of Auditory Frequency Measures Across Subgroups for ERP Data



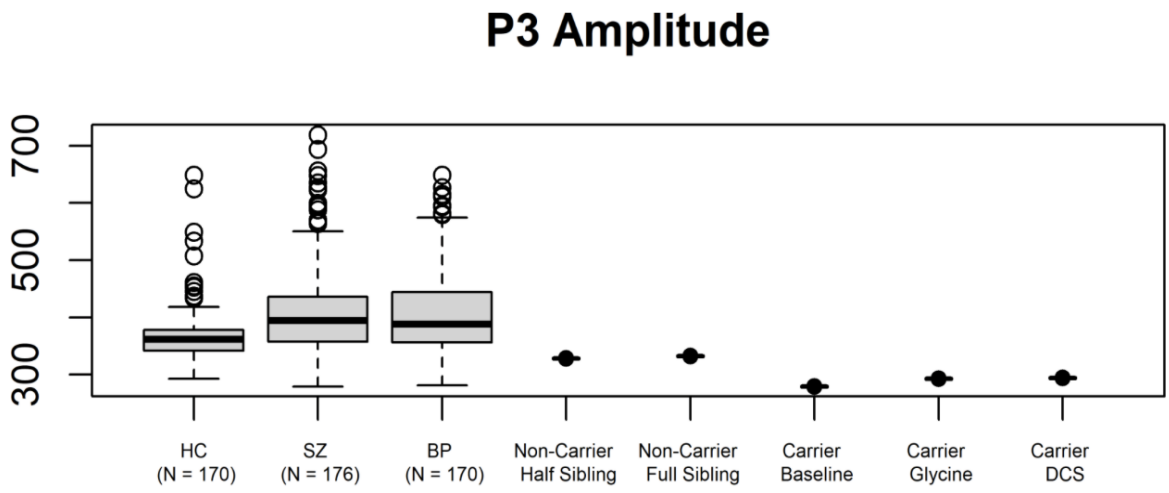


Figure 5: Boxplots of Auditory Frequency Measures Across Subgroups for ERP Data

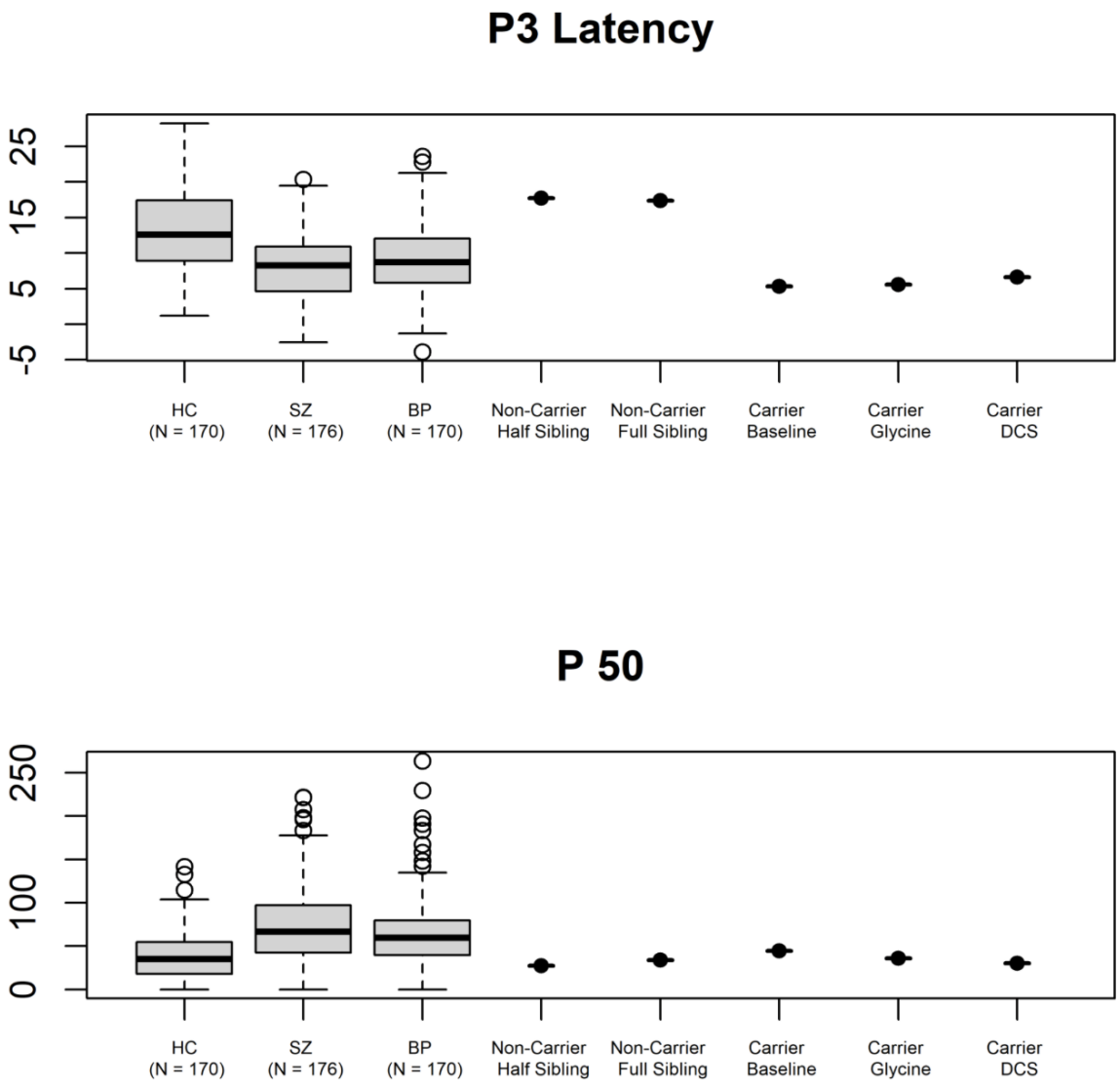


Figure 6: Boxplots of Auditory Frequency Measures Across Subgroups for ERP Data

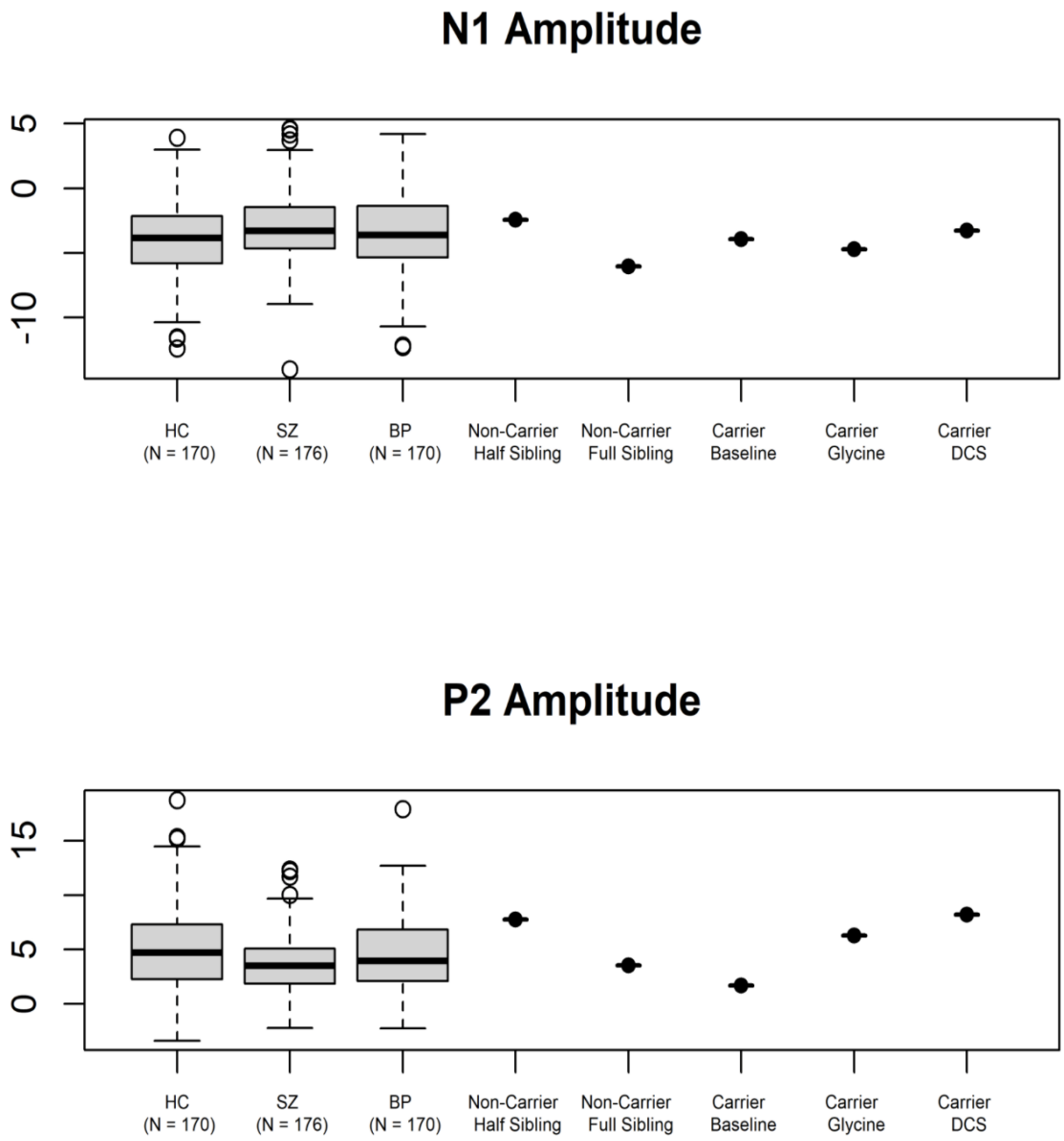


Figure 7: Boxplots of Auditory Frequency Measures Across Subgroups for ERP Data

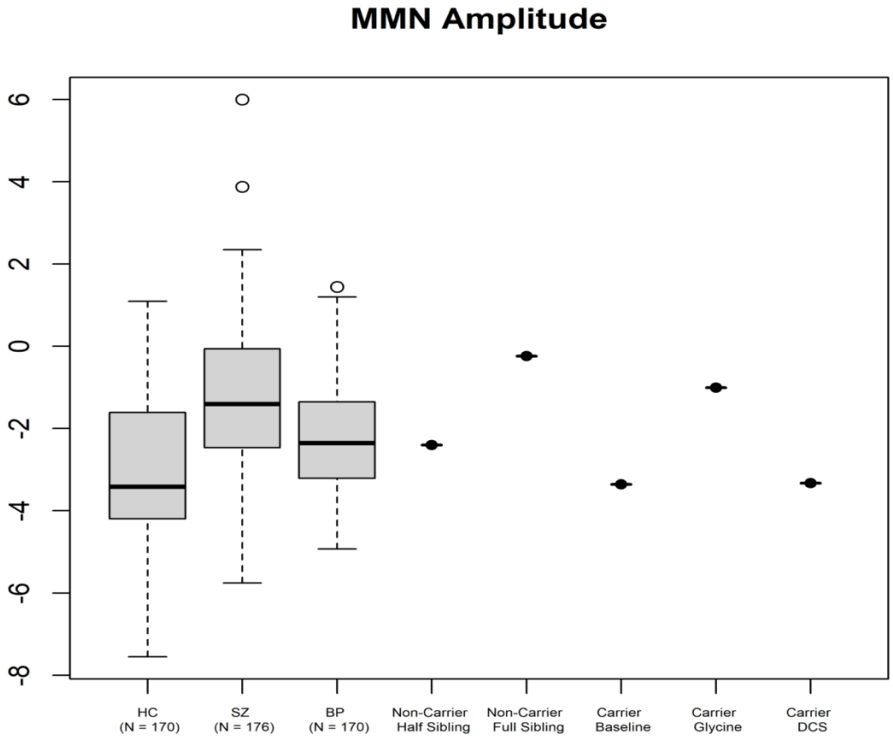


Table 8 provides brain biochemistry mean and standard deviation for the healthy controls and carriers as well as the computed z-scores for the carriers post-glycine. For Subject 5459, $PCr/\beta\text{-}ATP$ showed improved z-scores, and for Subject 3363 k_f showed improved z-scores. Figures 8 and 9 provide images of the boxplots of the distribution of the ERP data. We illustrate side-by-side the single values for the carriers, at baseline and post-treatment, and non-carrier siblings. Glycine appears to have brought values closer to that of median healthy control levels for Subject 5549 for $PCr/\beta\text{-}ATP$ and $Pi/\beta\text{-}ATP$.

The lack of significance difference may be due to the lack of flat slopes in the control groups. Table 2 shows that only two of the controls had a flat slope; three had negative slopes and two had positive. Since the control group was to be a standard comparison, it was expected for more if not all the controls would have a flat slope. Lack of consistency in the control group caused the non-significant differences despite expectations for a significant difference.

Table 8a: Brain Bioenergetics data Z scores for the Carriers

Variable	HealthyControls Mean(SD)	Carrier 1 Baseline	Carrier 1 Z Score	Carrier 2 Baseline	Carrier 2 Z Score
K_f	0.22 (0.07)	0.22	0.00	0.18	-0.57
PCr/β-ATP	1.66 (0.17)	1.44	-1.29	1.85	1.12
Pi/β-ATP	0.40 (0.11)	0.31	-0.82	0.43	0.27

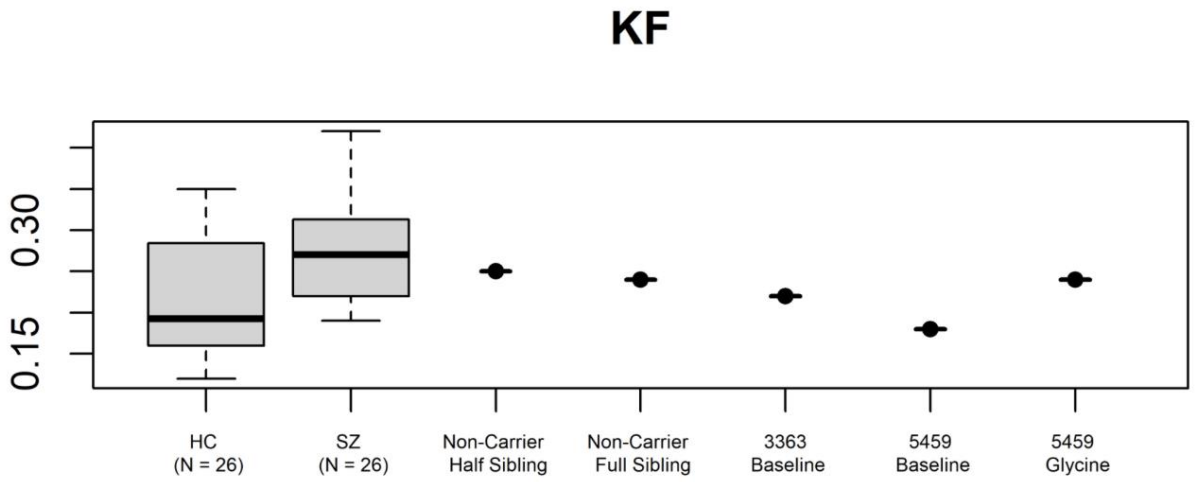
The Z-Score for the carriers is computed by taking the mean of the healthy controlsminus the carrier mean and divide by the standard deviation.

Table 8b: Brain Bioenergetics data Z scores for the Carriers

Variable	Carrier 1 Glycine	Carrier 1 Z Score	Carrier 2 Glycine	Carrier 2 Z Score
K_f	N/A	N/A	0.24	0.29
PCr/β-ATP	1.63	-0.17	0.31	-7.94
Pi/β-ATP	1.70	11.82	0.53	1.22

The Z-Score for the carriers is computed by taking the mean of the healthy controls minus the carrier mean and divide by the standard deviation.

Figure 8: Boxplot of Brain Bioenergetics Data Across Subgroups



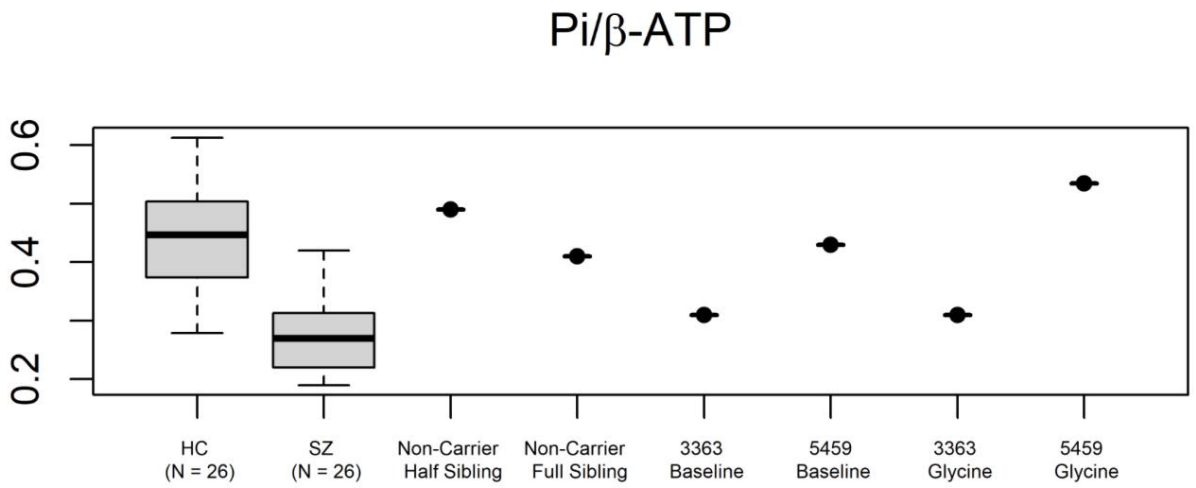
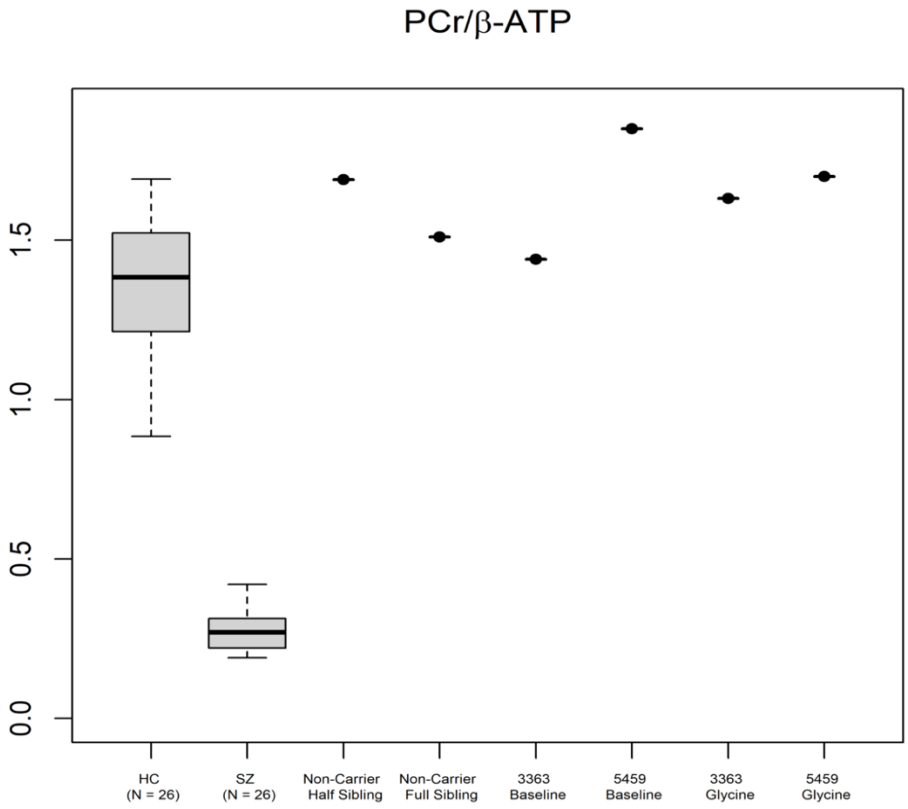


Figure 9: Boxplot of Brain Bioenergetics Data Across Subgroups



Discussion

For these carriers an individual treatment can now be prescribed to them based on a small sample clinical trial. Instead of having broad conclusion about a large population, our conclusion statements can only be made about a small number of individuals. This could be a reason why ``n-of-1" trials are not conducted more because the purpose is to treat and cure as many individuals as possible in the same amount of time. Is it truly feasible to conduct enough small sample clinical trials to perform individualized care for every person on the planet? Probably not. But in rare cases such as gene variants in individuals with neurodevelopmental disorders it may be useful. The purpose here is not to do away with the practice of large randomized clinical trials, but to bring awareness to the idea that in some cases an individual trial may be more appropriate. There is no single answer to creating treatment that addresses all individual issues at their personal disease and treatment level. Other methods should be investigated and encouraged where deemed useful, instead of immediately opting for a large trial. As shown here, there are benefits to conducting clinical trials for one or two individuals. Their treatment can be personalized and improvements in clinical care should be made on a personal level.

One statistical limitation of the analysis is, as expected, the small sample size. Although it is ideal for individualized care, it still presents many challenges when attempting to develop models that best fit small data. More data points could provide a better picture of the overall shape of the relationship between glycine absorption and time. Due to our small data set, only a

linear relationship could be validly investigated. However, there could be an underlying quadratic, cubic, or root relationship that is simply not seen because there aren't enough data points to fit an appropriate model. The trajectory analysis provides these findings: glycine absorption on average in healthy individuals with no gene mutations is negative, but near zero on average. Individuals with the *GLDC* triplication have significantly increased glycine absorption which leads to worsening of psychotic symptoms, and relatives of individuals with *GLDC* triplication tend to absorb glycine in a similar accelerated rate. Administering glycine has shown to improve the glycine absorption in individuals with the *GLDC* triplication from rapid absorption (positive trajectory) to more level absorption rate over time (flat slope). However, the administration of DCS only improved glycine absorption of the son (Subject 3363) in this study, but not the mother (Subject 5459). We speculate that this discrepancy could be due to age or progression of disease. DCS may not be as effective as glycine on individuals who are older or who have had the disease longer. For these individuals it may be more effective to administer glycine, a full agonist at the glycine modulatory site (GMS) as opposed to d-cycloserine (DCS), a partial agonist at low doses at the GMS.

This is the purpose of "n-of-1" trials; to determine which treatment works best for which individual. Because of the rarity of the *GLDC* triplication, it is not feasible to conduct a large sample clinical trial with an inclusion criterion of this genome deformity. However, it has been found that for Subject 3363 glycine or DCS slows the amount of glycine absorption in the brain to that of normal levels, but for Subject 5459 only glycine has been shown to improve absorption. Each patient can then be given treatment based on their personal outcomes.

Randomized controlled clinical trials that aim to investigate genomic abnormalities based on chemical absorption in the brain can use trajectory analysis as an outcome to measure the improvement of certain treatments. With this approach it is possible to develop targeted therapies for individuals that work exclusively for them or members of their family who may suffer from similar genetic abnormalities. In turn, this will increase the effectiveness of treatment and personalization of care for individuals with neurodevelopmental disorders.

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