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Rare copy number deletions predict individual variation in human brain metabolite concentrations in individuals with alcohol use disorders

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Abstract

Background—Although variations in neurometabolite concentrations occur in diverse neuropsychiatric and neurodegenerative disorders, very little is known about the nature of underlying genetic influences. The current study investigated the importance of a specific type of genetic mutation, copy number variation (CNV), for neurometabolite concentrations in a bilateral anterior cingulate voxel.

Methods—These neurometabolic signals were quantified using proton magnetic resonance spectroscopy (¹H-MRS): N-acetyl aspartate (NAA), creatine-phosphocreatine (Cr), glutamate/glutamine (Glx), myoinositol (mI), and phosphorylcholine-glycerol phosphorylcholine (Cho). Genetic data was collected using the Illumina 1M DuoBeadChip Array from a sample adults with alcohol use disorders (N = 146).

Results—The number of base pairs lost through rare copy number deletions (occurring in less than 5% of our sample) predicted lower NAA, Cr, mI, and Glx. More total rare deletions also predicted lower NAA, Cr, and Glx. Principal components analyses of the five neurometabolites identified two correlated components, the first comprised of NAA, Glx, and Cr, and the second comprised of Cho, mI, and to a lesser extent, Cr. The number and length of rare deletions were correlated with the first component, capturing approximately 10% of phenotypic variance, but not the second component.

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Conclusions—These results suggest that mutation load affects neurometabolite concentrations, potentially increasing risk for neuropsychiatric disorders. The greater effect of CVNs on NAA, Glx, and Cre may reflect a greater sensitivity to the effects of mutations, i.e., reduced canalization, for neurometabolites related to metabolic activity and cellular energetics, due to extensive recent selection pressure on these phenotypes in the human lineage.

Keywords

copy number variations; magnetic resonance spectroscopy; mutations; genetic; neurometabolites; schizophrenia

Introduction

Variations in brain neurometabolite concentrations measured by proton magnetic resonance spectroscopy (^1H -MRS) have been linked with diagnoses and traits for which there exists substantial heritability. Schizophrenia, mood disorders, and Alzheimer's Disease, each of which is heritable, have been linked with important neurometabolites. Similarly, several studies have demonstrated an association between neurometabolite concentrations and heritable cognitive skills [1]. Yet very little is known about the nature or magnitude of genetic contributions to variation in the concentration of neurometabolite complexes.

No biometric genetic studies have yet been conducted estimating the heritability of these neurometabolites. A single ^1H -MRS study partly implemented the standard behavior genetic twin design, comparing similarity of neurometabolite concentrations in monozygotic twin pairs with the similarity observed in unrelated control pairs [2]. Frontal and temporal single-voxel studies quantified NAA, Cre, Cho, Glx, and mI. The pattern suggesting heritability was statistically significant in only two of the 20 statistical comparisons. The purpose of the present study was to evaluate the importance of a specific type of genetic abnormality, copy number variations (CNVs), for variation in the concentration of neurometabolites in a sample of individuals with alcohol use disorder.

^1H -MRS Neurometabolites

N-acetyl-aspartate (NAA) is found at exceptionally high concentrations in the human brain, though its functions are the subject of much debate [3]. As NAA is found almost exclusively within neurons, reductions have been interpreted as reflecting loss of neurons, as in studies of traumatic brain injury [4] and Alzheimer's Disease [5]. However, reduced metabolic activity can also lead to lower NAA concentration [6], and much evidence links NAA to mitochondrial energetics, as NAA is produced in neuronal mitochondria [3]. Very little is known about the genetic determinants of NAA concentration. Canavan Disease is a genetic disorder characterized by high levels of NAA leukodystrophy, and motor and cognitive deficits [7]. Several mutations have been identified in this disorder [8, 9]. Several studies have found that Val/Met allelic variation of the brain-derived neurotrophic factor (BDNF) gene is linked with NAA [10–12]. Other possibly important genetic variants include variations in DISC1 [13], GRM3 [14], and HLA-DRB1 [15] genes. Finally, increased paternal age, itself positively associated with de novo germline mutations, is associated with lower NAA/Cre ratios in schizophrenics, independent of maternal age [16].

Other commonly quantified spectroscopic signals are Cre and Cho. Creatine and phosphocreatine exist in equilibrium and help maintain the cell's ATP level [17]. Cre levels are reduced in Alzheimer's Disease [18]. The Val/Met genotype affects Cre as well as NAA [10] and in non-demented elderly individuals the APOE4 allele has been linked with decreased Cre [19]. The Cho signal primarily reflects phosphorylcholine and glycerol phosphorylcholine, two membrane lipid metabolites. Higher levels reflect breakdown of

myelin and membranes and/or increased concentrations of neurometabolites needed for repair processes, such as occur in traumatic brain injury [20–23]. Elevated Cho has been reported in white matter diseases [24, 25], frontotemporal dementia [26], mood disorders [27, 28], and in normal individuals with low positive affect [29]. We are aware of no studies linking Cho concentration with specific genetic variants.

Relatively fewer studies have quantified glutamate, glutamine, or their combined signal (Glx), despite the central importance of glutamate as a neurotransmitter and its role in excitotoxic brain injury [30]. In gray matter, glutamate serves as an excitatory neurotransmitter at approximately 80 – 90% of all synapses and local glutamate flux is linked to local brain activity [30, 31]. A recent genome wide screening study of multiple sclerosis patients identified several single nucleotide polymorphisms (SNPs) predicting glutamate concentration [32].

Myoinositol (mI), which is largely present in glial cells, has been most frequently studied in relation to aging and dementia. Many studies have reported increased mI or mI/Cr ratios in Alzheimer's disease [33]. Further, mI/Cr ratios in patients with Alzheimer's Disease predict the Braak stage of Alzheimer's disease upon post-mortem neuropathology investigation [34]. Hence, like Cho, mI is associated with the processes of inflammation and maintenance of neuronal integrity.

Copy Number Variations

Genetic variations include SNPs and CNVs. A substantial portion of the genome is subject to copy number variation—differences across individuals in number of copies of a chromosomal segment at least 1000 bases long. Several thousand such sequences have been found in the human genome, comprising about 30% of it [35]. Variation across individuals, then, consists not only of differences at single nucleotide sites, but also in number of copies of particular DNA strands (structural variation). Ultimately, CNVs originate as mutational events [35]. Like point mutations, most are probably mildly deleterious. That is particularly true of deletions, and especially ones maintained at low frequencies (<5%, [36]). Duplications appear to be less deleterious, and some CNVs may exist at high relative frequencies due to relaxed selection. CNVs have a de novo mutation rate 10–1000 times as great as point mutations [35, 37]. Point mutations affect a single nucleotide base, but CNVs affect many, with multiple genes sometimes affected by a single CNV. As a result, CNVs may account for more total inter-individual genetic variation than all single nucleotide variants combined [38]. Recent studies have revealed an elevated incidence of rare deletions in schizophrenia, bipolar disorder, autism, and mental retardation [39–44]. We have recently reported that the number of base pairs found in rare or infrequent deletions (i.e., those occurring in less than 5% of the population) is negatively correlated with psychometric intelligence [45].

Our specific hypotheses derive from the expectation that mutational events are apt to have adverse health outcomes. Hence, greater numbers of rare CNVs and greater total length of rare CNVs may adversely affect neuronal integrity, which in turn is reflected by alterations in neurometabolite concentrations. As greater NAA and reduced mI and Cho are clearly associated with greater neuronal integrity, we predict that more (and larger) rare CNVs may be associated with reduced NAA and elevated mI and Cho. The relationships between Cr and Glx concentrations and neural integrity are less clear, but we hypothesize they will be negatively correlated with rare CNVs and affected in the same direction as NAA, in light of their collective involvement in cell energetics. Our ¹H-MRS protocol investigated bilateral anterior cingulate gray matter, a region commonly studied due to high signal/noise characteristics, measurement reliability [46], and relevance for schizophrenia [44] and mood disorders [47].

Materials and Methods

Subjects

Subjects were recruited from the general community for a study designed to investigate genetic correlates of alcohol use disorders and related phenotypes. Hence, this represents a convenience sample with respect to the major hypotheses. Inclusion criteria were (1) age between 21 and 55; (2) within 21 days of their last drink; (3) drinking at least 5 or more drinks per drinking occasion for men (4 or more for women) at least 5 times in the past month; (4) negative drug screen for opiates, cocaine, or amphetamine; (5) have a blood alcohol level of 0 at the time of assessment; and (6) must have a Clinical Institute Withdrawal Assessment (CIWA) [48] score of less than 8, indicating no need for medical detoxification. Alcohol dependence was measured with the Alcohol Dependence Scale (ADS) [49] and consumption by the average number of drinks per drinking day. The Beck Depression Inventory [50], and the average number of cigarettes smoked per smoking day were obtained for all participants. The Wechsler Abbreviated Scale of Intelligence (WASI) [51] was obtained in a representative subsample (N = 72).

Genetic Analyses

Participants provided at least 5 ml of saliva into a sterile 50 ml conical centrifuge tube. DNA was extracted, purified, and hybridized. Detection of 1,199,187 SNP and CNV markers across the entire genome, based on the reference genome_build 36.2, was performed using the Illumina Human 1M Duo BeadChip Array, according to the manufacturer's instructions. We did not analyze DNA from the X and Y-chromosomes, reducing the number of loci to 1,147,842. The data were further scanned and 10,272 loci with missing measurements were removed. The median distance between adjacent markers was approximately 2.5kbp.

Details of the series of procedures used to quantify CNVs are described in Chen et al. [52]. Briefly, principal component analysis (PCA) was performed to minimize noise effects and remove extraneous sources of variance, including batch effects and, as well as variances related to GC percentage. Next, samples were eliminated if they appeared to be outliers as determined by the standard deviation of the Log R Ratio larger than 0.28 (see [53]). The preprocessed data were segmented independently using two methods, the circular binary segmentation (CBS) algorithm implemented in MATLAB and a hidden Markov model (HMM) algorithm implemented in PennCNV [54]. To be counted as a CNV, a segment needed to be identified by both approaches. This is a conservative approach to CNV identification designed to increase reliability of detection. We classified rare or infrequent CNVs as those occurring in 5% or less of the sample (though we also performed supplementary analyses based on more strict criteria of rarity, 3% and 1%; see results). For both rare deletions and duplications we calculated the total number of CNVs, as well as the total number of base pairs included in each type of abnormality.

The observed frequency distribution of CNV number was markedly skewed, as a few participants had extremely high numbers of CNVs. As oversensitivity of CNV detection for these individuals likely led to unrealistically high values, we eliminated extreme outliers, ones exceeding Tukey's criterion of the third quartile value plus three times the inter-quartile range [55]. Application of this criterion resulted in discarding 13 cases (8.1% of the sample), resulting in a final data set of 146 participants.

MRI and MRS Data Acquisition

MR imaging and spectroscopy were performed on a Siemens 3-tesla TrioTIM MR scanner using the 12-channel radiofrequency head coil. T1-weighted images were collected in the sagittal plane using a five-echo 3D MPRAGE sequence (TR/TE/TI = 2530/1.64, 3.5, 5.36,

7.22, 9.08/1200 ms, flip angle = 7°, field of view (FOV) = 256 × 256 mm, matrix = 256 × 256, 1 mm thick slice, 192 slices, GRAPPA acceleration factor = 2). Using these images, a single ¹H-MRS voxel was positioned in the bilateral medial cortex directly superior to the corpus callosum (see Figure 1). A PRESS (point-resolved spectroscopy) sequence (TR/TE = 1.5 s/40 ms, voxel size = 20 × 30 × 20 mm, averages = 192) was collected, using an echo time (TE) of 40 ms for improved detection of Glu [46]. Based on the LCModel analysis, Glu on average comprises approximately 80% of the Glx signal in this study. An unsuppressed water sequence was used as a concentration reference and eddy current correction in postprocessing was collected with 16 averages and otherwise identical parameters for each single voxel spectrum.

MRS Data Analysis

Raw time-domain ¹H-MRS data from 4.0 – 1.0 ppm in the spectral dimension were analyzed using LCModel [56] with the unsuppressed water scan as a concentration reference. As a quality-assurance measure, LCModel produces a Cramer-Rao lower bound (CRLB) of the fit to the peak of interest. If this value was greater than 20%, the fit was deemed unreliable and excluded from analysis. Analyses utilized two different dwell times (1 ms or 0.625 ms). Metabolite concentrations were computed for total creatine plus phosphocreatine (Cre), total choline containing compounds (Cho), total N-acetyl-aspartate plus N-acetyl-aspartylglutamate (NAA), and the combined Glu plus Gln signal (Glx). T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid using SPM5. Metabolite concentrations were then computed, correcting for partial-volume and T1 and T2 relaxation effects, based on relaxation rates derived from reports by others and methods described previously [57]. Figure 1 shows a representative spectrum.

Results

Descriptive Statistics and Data Analytic Strategy

Table 1 provides descriptive statistics. One outlier was identified for total length of rare deletions (2,234,214 base pairs). To reduce the impact of this case, it was replaced with the next highest value (i.e., winsorized), though all significant associations we report remained robust when this value was left unaltered.

Our primary statistical analyses were correlational in nature. First, we computed correlations between the four CNV variables and the five neurometabolites. Second, because neurometabolites covaried with one another, we extracted broader dimensions underlying them through principal components analysis (see below) and computed correlations between the four CNV variables and these broader dimensions. Based on both theoretical and empirical considerations (see above), our predictions pertaining to associations with rare CNV number and length were *a priori*. Hence, we did not adjust for multiple comparisons and applied a two-tailed alpha level of .05 to these associations. For rare duplication number and length, we had no *a priori* predictions. Hence, we adjusted for multiple comparisons through Bonferroni adjustment within a set of associations for a given metabolite, thereby applying an alpha level of $.05/2 = .025$. (As it happened, however, no associations with these variables were statistically robust even at alpha of .05 and, therefore, Bonferroni adjustment had no impact on conclusions.)

In all analyses, we statistically controlled for dwell time and age, as each of these variables significantly predicted one or more of the neurometabolites. ADS did not significantly predict any neurometabolite (all $r < .14$, $p > .10$), though in additional analyses we did control for this variable as well (see below).

All analyses were performed using PASW 17.0 (Predictive Analytics SoftWare; also known as SPSS).

Partial Correlations with Individual Metabolites

Table 2 provides partial correlations between the four CNV variables and the five neurometabolites. Greater number and length of rare deletions predicted decreased NAA, Glx, and Cre concentrations. Lower levels of mI were predicted by deletion length. Correlations of deletion length and number with Cho fell just short of statistical significance. None of these correlations was appreciably changed or rendered non-significant if ADS total score, the average number of drinks per drinking day, cigarettes per smoking day, Beck Depression Inventory score, or sex were included as covariates. Further, none of the four CNV variables was related to any tissue parameter of the spectroscopy voxel (percent gray matter, white matter, or CSF, all p values $> .08$).

Partial Correlations with Broader Dimensions Underlying Neurometabolites

The neurometabolites covaried with one another. To identify broader dimensions underlying them, we conducted a principal components analysis on 273 cases for whom we had complete ^1H -MRS data, recruited through the same research protocol as the subsample described here for which genetic data was available. The eigenvalue scree suggested two systematic factors (eigenvalues = 2.89, .95, .50, .36, .30). We hence extracted and obliquely rotated (using direct oblimin) two components. The pattern matrix is presented in Table 3. Component 1 reflected variation in the three neurometabolites most clearly associated with metabolism and cellular energetics, Glx, NAA, and (more moderately) Cre. Component 2 reflected variation in two neurometabolites involved in cellular inflammation and repair, mI and Cho, as well as, moderately, Cre. These two components covaried at $r = .40$, $p < .001$.

For each individual, we computed scores for each of the two components (based on weighted composites of metabolites yielding components; PASW 17.0). We then performed correlational analyses on these scores, partialling out age and dwell time. Results are presented in Table 4. The Glx/NAA/Cre component was predicted by length and number of rare deletions. The mI/Cho/Cre component was not significantly predicted by any measure of rare CNVs. When the second component in addition to age and dwell time was partialled out, deletion length and number remained significantly correlated with the Glx/NAA/Cre component (see Table 4.) Once again, results were not altered when clinical measures, sex, or tissue parameters of the spectroscopy voxel were also controlled.

Our definition of “rareness” was somewhat arbitrary, though consistent with other recent studies’ treatment of non-common variants [58]. To ensure that our results did not reflect idiosyncrasies of this specific definition, we also examined correlates of rare deletion number and length using more stringent criteria of 3% and 1% incidence rates. Use of these criteria did not change results; every significant effect remained significant with both of these criteria and no additional significant effects were noted.

Though our primary interest was in rare CNVs, we also examined associations between neurometabolites and common CNV variations (with representation in the sample of $> 5\%$). In these analyses, neither the length nor the number of common deletions predicted any neurometabolite. By contrast, number of duplications positively covaried with levels of Cre, Cho, and mI, and total duplication length predicted greater levels of Glx. Correlations of the Glx/NAA/Cre component with duplication length and number, as well as the mI/Cho/Cre component with duplication number fell short of significance (all $p \leq .069$). None of these correlations, however, remained significant once Bonferroni corrections were applied to

control for multiple comparisons within metabolite ($\alpha = .05/4 = .0125$). Hence, we draw no firm conclusions regarding associations between common CNVs and neurometabolites.

Discussion

The numbers of base pairs and segments lost through rare copy number deletions were associated with lower NAA, Cre, and Glx concentrations in the anterior cingulate region. Cho and mI had less robust associations with deletions. Rare duplications did not reliably predict neurometabolite concentrations. These results demonstrate substantial genetic influence on brain neurometabolite concentrations. The observed proportions of variance accounted for (approximately 10%) may well underestimate true effect sizes, as our assessment of CNVs is almost certainly incomplete. Smaller CNVs and those in regions not as well mapped by the reference genome may well have gone undetected.

As our data were obtained in a sample of individuals with alcohol use disorders, it is important to consider the extent to which our findings generalize to other populations, though a clear answer awaits replication. No specific observations suggest that the observed relationships are unique to the current sample. When we performed analyses partialling out clinical variables, thereby statistically controlling for variation related to chronic alcohol use, depression, and smoking, we observed nearly identical results.

The overall impact of greater number and size of rare CNVs must be considered adverse. This is most clearly the case for NAA, a marker of neural integrity, as reductions in NAA are seen after diverse forms of brain injury [4, 5, 59]. Lower NAA also correlates with reduced functional skills [1, 60]. Rare deletions may thus represent a vulnerability factor for neuropsychiatric disorders characterized by lower NAA and lower cognitive ability. The health impact of lower Glx and Cre are less clear, in part due to the specific nature of these spectroscopic signals. The Glx measure does not distinguish between glutamate, the brain's major excitatory neurotransmitter, and glutamine, which is metabolically coupled to glutamate at glutamatergic synapses [31]. Additionally, lower glutamate levels might confer less vulnerability to excitotoxic brain injury such as occurs in stroke or traumatic brain injury [30]. Interpretation of Cre is similarly ambiguous, as the Cre signal measured by ^1H -MRS at 3T arises from both creatine and phosphocreatine in both glial and neuronal compartments.

Our principal components analysis (PCA) may shed some light on the nature of the effects of rare deletions on neurometabolites and brain function. One major component emerging from our PCA reflected variations in Glx, NAA, and Cre. These neurometabolites serve diverse functions, but all may demonstrate increased concentration with increased metabolic activity. They may also impact cell energetics. The role of Cre in this regard is well known, but glutamate and acetate (from NAA metabolism) can each be oxidized by neurons [61]. Moreover, increased rates of glutamine-glutamate cycling are associated with increased glucose utilization, with glutamate in particular stimulating glial uptake of glucose [62]. This first component, then, may reflect important variations in cell energetics. The second major component emerging reflected variations in mI, Cho, and Cre. Increased concentrations of each may be seen with neuronal damage, as each may be involved in inflammation and repair processes. The only truly robust associations with rare deletions we observed were ones with neurometabolites reflecting the first component. The associations we observed between rare deletion number and length and this component were particularly strong. They suggest that rare deletions primarily affect neurometabolites by altering brain cell energetics and metabolic activity.

The rare CNVs we tabulated were scattered across the genome, and by definition occurred at a given locus in less than 5% of the sample. This has two important implications. First, the deletions and duplications are in different places in different people, and any two individuals are very unlikely to share a given CNV. Thus, our results likely do not reflect perturbation of the synthesis or regulation of a specific protein. Second and relatedly, many different individual CNVs must have common effects on neurometabolite concentrations, with no one CNV possessing any more than a very small effect. Why, then, would deletions at many places in the genome affect neurometabolite concentrations? We offer two considerations. First, there may exist massive pleiotropy [63]. A given gene likely contributes to many different metabolic and developmental pathways, so loss or gain of genetic material at a particular locus may have widespread effects. Hence, even genes with primary functions pertaining to outcomes other (or broader) than neurometabolite synthesis or regulation may affect their concentration. Second, a great many genes contribute to brain function; in the mouse, as many as 80% of all genes are expressed in the brain [64]. As a result, randomly placed CNVs may be more apt to have a deleterious effect on brain function than not.

The effects of mutations on human phenotypes might be expected to be a joint function of both the severity of genetic abnormality and the degree of canalization of the phenotype. That is, some CNVs are worse than others, and some traits are better buffered against environmental and genetic perturbations than others. There appears to be much stronger selection pressures against deletions than duplications, and the effects of deletions are relatively more dependent on their length [65]. Thus, longer rare deletions would be expected to have especially severe consequences. With respect to phenotypes, much evidence suggests relatively recent selection pressure in humans for genetic variations related to energy [66] and glucose metabolism [67] allocated to brain function. Traits under recent selection pressure are less apt to be well canalized [68].

As noted above, the major effects of rare deletions on neurometabolites may occur through impacts on cell energetics and metabolism. At this point, we can only offer speculations as to why accumulations of deletions across the genome particularly affect energetics. One possibility is that, though a wide variety of specific phenotypic effects (e.g., inefficiencies in processing of nutrients, excessive immunological demands, relatively poor vasculature and oxygen delivery, poorly regulated energy allocation; see [69] a major downstream effect of many deletions is diminished global metabolic efficiency. Decrements in global metabolic efficiency may then affect the amount of energy (glucose) that can be allocated specifically to brain function, with implications for overall brain metabolic rate. A second, related possibility is that rare deletions specifically affect nuclear DNA regulation of mitochondrial processes such as oxygen phosphorylation (e.g., the electron transport chain), causing increased rates of the creation of reactive oxygen species (ROS) and potential oxidative damage. Synaptic activity requires large amounts of energy and, hence, brains are particularly vulnerable to overproduction of ROS and oxidative damage [70]. Individuals with large numbers of rare deletions who are prone to oxidative damage may adaptively downregulate energy utilization in the brain, perhaps directly through ROS signaling (e.g., [71, 72]). It is noteworthy that the two neurometabolic signals most highly associated with rare deletions in this study, NAA and glutamate (as part of Glx), have both been found to increase production of ROS [73, 74]. Future research may investigate these and other possible mechanisms whereby rare deletions come to covary with neurometabolites.

We have recently reported that greater length of rare deletions predicts lower intellectual functioning [45], and relatively lower intellectual ability is commonly observed in studies of schizophrenia [75]. So too is lower NAA (e.g., [76]) and an increased incidence of rare deletions (e.g., [42]). Taken together, these observations suggest that rare CNVs are a risk factor for the extended phenotype of schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Location of the MRS voxel in the anterior cingulate region and a representative spectrum from LCModel.

Table 1

Descriptive statistics on demographic, genetic, and MRS data (N males = 105, N females = 41)

	<u>N</u>	<u>Mean</u>	<u>SD</u>	<u>Range</u>
Age	146	32.11	9.68	21–55
Education	146	14.24	2.30	8 – 22
Alcohol Dependence Scale	146	13.10	7.78	2–43
Beck Depression Inventory	146	11.80	9.18	0–40
Avg. drinks per drinking day	146	7.37	4.26	1.94 – 39
Avg. cigarettes per smoking day	146	4.52	7.15	0 – 40
WASI Intelligence Quotient	72	98.29	13.71	71 – 135
Number rare deletions	146	11.89	7.96	1–69
Length rare deletions (bp)	146	231,690	211,171	8,670-2,110,967
Number rare duplications	146	7.68	11.24	0–82
Length rare duplications (bp)	146	462,941	754,516	0–4,432,756
NAA	138	18.12	1.94	13.71–23.67
Cre	140	13.80	1.27	10.87–16.89
Glx	140	18.82	2.57	13.01–25.41
Cho	143	3.34	.47	2.41–4.52
Ins	146	13.38	1.91	8.08–18.10

Table 2

Partial correlations between rare CNV variables and neurometabolite concentrations, partialing dwell time, and age

	Number Rare Deletions	Length Rare Deletions	Number Rare Duplications	Length Rare Duplications
NAA	-.29 ***	-.27 ***	.03	.07
Cre	-.18 *	-.23 **	-.13	.07
Glx	-.28 ***	-.30 ***	.04	.11
Cho	-.16 ⁺	-.16 ⁺	.10	.03
mI	-.13	-.17 *	.04	.06

⁺
p < .10

*
p < .05

**
p < .01

p < .001

Note: N ranges from 140 to 146.

Table 3

Principal components analysis; Pattern matrix loadings of rotated components

<u>Neurometabolite</u>	<u>Component 1</u>	<u>Component 2</u>
NAA	.81	.13
Cre	.50	.52
Glx	.97	-.13
Cho	.21	.71
mI	-.15	.96

Note: The two components correlated .40.

Table 4

Partial correlations between rare CNV variables and neurometabolite components, controlling for dwell time and age (N = 133)

	Number Rare Deletions	Length Rare Deletions	Number Rare Duplications	Length Rare Duplications
Glx/NAA/Cre comp	-.31 ***	-.31 ***	.04	.09
mI/Cho/Cre comp	-.14	-.10	.08	.06
with other component controlled:				
Glx/NAA/Cre comp	-.28 ***	-.30 ***	.00	.06
mI/Cho/Cre comp	.02	.06	.07	.02

*
p < .05

**
p < .01

p < .001