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Research paper

Acylcarnitine metabolomic profiles inform clinically-defined major depressive phenotypes

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ABSTRACT

Background: Acylcarnitines have important functions in mitochondrial energetics and β-oxidation, and have been implicated to play a significant role in metabolic functions of the brain. This retrospective study examined whether plasma acylcarnitine profiles can help biochemically distinguish the three phenotypic subtypes of major depressive disorder (MDD): core depression (CD+), anxious depression (ANX+), and neurovegetative symptoms of melancholia (NVSM+).

Methods: Depressed outpatients (n = 240) from the Mayo Clinic Pharmacogenomics Research Network were treated with citalopram or escitalopram for eight weeks. Plasma samples collected at baseline and after eight weeks of treatment with citalopram or escitalopram were profiled for short-, medium- and long-chain acylcarnitine levels using AbsoluteIDQ®p180-Kit and LC-MS. Linear mixed effects models were used to examine whether acylcarnitine levels discriminated the clinical phenotypes at baseline or eight weeks post-treatment, and whether temporal changes in acylcarnitine profiles differed between groups.

Results: Compared to ANX+, CD+ and NVSM+ had significantly lower concentrations of short- and long-chain acylcarnitines at both baseline and week 8. In NVSM+, the medium- and long-chain acylcarnitines were also

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significantly lower in NVSM+ compared to ANX+. Short-chain acylcarnitine levels increased significantly from baseline to week 8 in CD+ and ANX+, whereas medium- and long-chain acylcarnitines significantly decreased in CD+ and NVSM+.

Conclusions: In depressed patients treated with SSRIs, β-oxidation and mitochondrial energetics as evaluated by levels and changes in acylcarnitines may provide the biochemical basis of the clinical heterogeneity of MDD, especially when combined with clinical characteristics.

1. Introduction

Treatment response in patients with major depressive disorder (MDD) is only partially predicted by clinical symptom profiles, and depressive symptoms alone fail to account for the majority of biological heterogeneity in treatment response (Carragher et al., 2009; Cassano et al., 2009; Charney and Drevets, 2002; Harald and Gordon, 2012). Recent efforts to better parse this heterogeneity have included molecular, physiological, imaging, and neuropsychological measures combined with clinical data, with the aim of facilitating more personalized patient care (Rush and Ibrahim, 2018). To support biomarker identification in MDD, we recently proposed three depressive phenotypes based summed scores on items from the 17-item Hamilton Rating Scale for Depression ($HRSD_{17}$) (Hamilton, 1960; Ahmed et al., 2018) that may reflect distinct neural processes aligned with the Research Domain Criteria (RDoC) proposed by the National Institute of Mental Health (Insel et al., 2010). These phenotypes, with corresponding question numbers on the $HRSD_{17}$, were categorized as: (1) core depression (CD+: depressed mood #1, work and activities #7); (2) neurovegetative symptoms of melancholia (NVSM+: late insomnia #6, somatic symptoms gastrointestinal #12); and (3) anxious features (ANX+: agitation #9, psychological anxiety #10, somatic anxiety #11, and hypochondriasis #15).

This report focuses on acylcarnitines due to their emerging role in the central nervous system and relevance to MDD (Chen et al., 2014). Acylcarnitines are involved in mitochondrial function and energy metabolism, anti-oxidative functions and membrane stability, gene expression, neurotransmission, and neuroprotection (Calabrese et al., 2006; Nałęcz et al., 2004; Pettegrew et al., 2000; Virmani and Binienda, 2004; Zanelli et al., 2005). Carnitine is present in the brain (Bresolin et al., 1982; Shug et al., 1982), where it transfers acetyl groups formed during β-oxidation to the cytosol, and it assists with the transfer of fatty acids from cytosol to mitochondria for energy production (Fritz and Mcewen, 1959; Jones et al., 2010).

Elevated medium- and long-chain acylcarnitine concentrations in blood have been associated with incomplete β-oxidation of fatty acids in a rat model of depression (Chen et al., 2014). In clinical studies, patients with a lifetime diagnosis of MDD demonstrated altered mitochondrial function or reduced ATP production (Beasley et al., 2006; Gardner et al., 2003; Suomalainen et al., 1992). Further, patients with mitochondrial disorders frequently have depressive symptoms or a history of MDD (Fattal et al., 2007; Kato, 2001; Manji et al., 2012). Acetyl-L-carnitine levels appear to be lower in treatment-resistent MDD patients ($n = 71$) compared to healthy controls ($n = 45$) (Nasca et al., 2018). Moreover, patients undergoing hemodialysis who were administered L-carnitine have increased total, free, and acylcarnitine levels in plasma and an associated decrease in depression rating scales scores (Tashiro et al., 2017). To date, we are unaware of any studies that link acylcarnitine levels or their changes under antidepressant treatment with clinical phenotypes in patients with MDD.

The present study was undertaken to determine whether peripheral levels of acylcarnitines at could discriminate between three clinical phenotypes of MDD. The following specific questions were addressed:

1 Are there unique patterns of acylcarnitines that differentiate each pure MDD phenotype (CD+; NVSM+; ANX+) from the others at baseline?
2 Are there unique acylcarnitine patterns that differentiate each pure phenotype after eight weeks of treatment with an SSRI?
3 Are there unique changes in acylcarnitine profiles over eight weeks of SSRI treatment that differentiate the three phenotypes?

2. Materials and methods

2.1. Study design and participants

The sample comprised 240 MDD participants from the Mayo Clinic NIH-Pharmacogenomics Research Network - Antidepressant Medication Pharmacogenomics Study (PGRN-AMPS) (Mrazek et al., 2014). Participant selection, symptomatic evaluation, and blood sample collection for the PGRN-AMPS clinical trial have been described in our previous work (Gupta et al., 2016; Ji et al., 2014; Mrazek et al., 2014; Schiepers et al., 2005). MDD symptoms were assessed using the $HRSD_{17}$ at baseline and after eight weeks of treatment with citalopram or escitalopram. Blood samples for metabolomics were collected at these same time points. The data extraction protocol followed the STROBE guidelines (von Elm et al., 2007).

The study was approved and monitored by the Institutional Review Boards of Mayo Clinic and conformed to the standards of the Declaration of Helsinki. All participants provided written informed consent prior to participation in this study.

2.2. Identifying subtypes of major depression

We previously defined three clinical phenotypes that aligned with RDoC conceptual models (Insel et al., 2010) using the individual $HRSD_{17}$ item scores from participants with MDD (Ahmed et al., 2018). The CD+ phenotype represents the RDoC domain of negative valence systems, and the constructs of loss and reward learning. We defined it based on the summed scores of items #1 (depressed mood) and #7 (work and activities) on the $HRSD_{17}$ (Hieronymus et al., 2016). The NVSM+ phenotype represents the RDoC domain of arousal and regulatory systems, and the construct of sleep-wakefulness. We defined it based on the summed scores severity of items #6 (late insomnia) and #12 (somatic symptoms gastrointestinal) on the $HRSD_{17}$. The ANX+ phenotype reflects the RDoC domain of negative valence systems, and the construct of potential threat ("Anxiety"). We defined it based on the summed scores of items #9 (agitation), #10 (psychological anxiety), #11(somatic anxiety), and #15 (hypochondriasis) on the $HRSD_{17}$ (Shafer, 2006) (Cleary and Guy, 1977). **Supplemental Table 1,** Fig. 1**).**

2.3. Metabolomic profiling

Metabolites were measured with a targeted metabolomics approach using the AbsoluteIDQ® p180 Kit (BIOCRATES Life Science AG, Innsbruck, Austria), with an ultra-performance liquid chromatography with tandem mass spectrometry (UPLC)/MS/MS system [Acquity UPLC (Waters), TQ-S triple quadrupole MS/MS (Waters)], which provides semi-quantitative measurements of up to 23 endogenous acylcarnitines as well as multiple analytes from various other classes including amino acids, biogenic amines, phophatidylcholines, and sphingomyelins. It should be noted that the acylcarnitines and lipids were analyzed by flow-injection MS/MS (i.e. without UPLC separation).

In this report, we focused solely on acylcarnitines. The

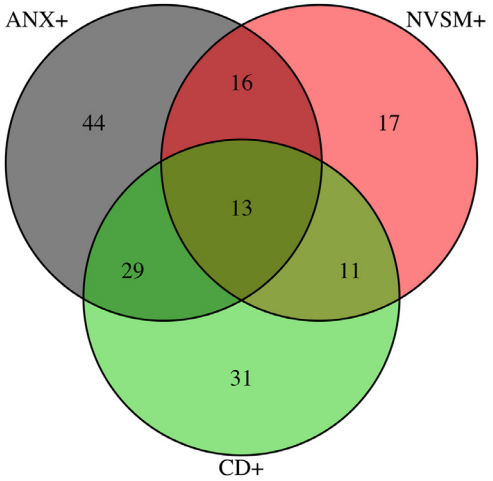

Fig. 1. Venn Diagram of the Overlap between Positive MDD Phenotype [Core Depression (CD+), Neurovegetative Symptoms of Melancholia (NVSM+), and Anxiety (ANX+)].
Circles represent the group in each phenotype (CD+, NVSM+, and ANX+). The numbers inside overlapping circles represent participants who met criteria for two, or all three, of the phenotypes. The total number of participants with each pure phenotype was CD+ (n = 31), NVSM+ (n = 17) and ANX+ (n = 44).

AbsoluteIDQ® p180 kit has been fully validated according to European Medicine Agency guidelines. Additionally, the kit plates include an automated technical validation protocol to approve the validity of the run and provide verification of the actual performance of the applied quantitative procedure including instrumental analysis. The technical validation of each analyzed kit plate was performed using MetIDQ® software based on results obtained, and defined acceptance criteria for blank, zero samples, calibration standards and curves, low/medium/high-level quality control (QC) samples, and measured signal intensity of internal standards over the plate. This platform has been used in numerous publications, including several studies of MDD (Moaddel et al., 2018; Baranyi et al., 2018). De-identified samples were analyzed following the manufacturer's protocol, with the metabolomics labs blinded to diagnosis and clinical data. The list of acylcarnitines reported in the present study, which does not include several in the p180 kit deemed to be irrelevant to mitochondrial metabolism, is shown in **Supplemental Table 2.**

2.4. Data analyses

Preprocessing All metabolite data were first checked for missing values (< limit of detection [<LOD]), and metabolites with >40% missing values were excluded from subsequent analysis. Each assay plate included a set of duplicates obtained by combining approximately 10 µl from the first 76 samples in the study (QC pool duplicates) to enable appropriate inter-plate abundance scaling based specifically on this cohort of samples. To adjust for batch effects, a correction factor for each metabolite in a specific plate was obtained by dividing the metabolite's statistical process quality control (SPQC) global average by the SPQC average within the plate. <LOD values were imputed using each metabolite's LOD/2 value followed by log2 transformation. We checked for the presence of multivariate outlier samples by evaluating the squared Mahalanobis distance of samples in each platform. Samples with Mahalanobis distances that exceeded the upper 0.05/n (with n: number of samples to adjust for multiple comparisons by Bonferroni correction) critical value of the Chi-squared distribution with m degrees of freedom, in which m is the number of metabolites in each platform, were flagged as outliers. An additional 15 samples were removed after they were determined to be multivariate outliers. This resulted in an analysis data set containing 240 participants, 537 samples (each participant had one or more sample up to 3 samples) and 23 metabolites.

2.5. Statistical analysis

Differences in demographic and clinical characteristics, and in $HRSD_{17}$ scores, across the phenotypes were evaluated using Analysis of variance (ANOVA) (for continuous variables) and Pearson Chi-squared test (for categorical variables). All analyses were performed in a metabolite-wise (univariate analysis) manner. The presence of each pure phenotype (i.e., CD+, NVSM+, and ANX+, excluding subjects who met criteria for more than one phenotype) for each participant at baseline was stored as three binary variables. Baseline and week eight metabolite concentrations, and changes in metabolite concentrations after SSRI treatment, were tested. To examine the statistical significance of differences in metabolite levels between phenotypes, at baseline and at eight weeks, we fitted linear mixed effect models with participants as the random variable. Log2 metabolite levels were used as the dependent variable. Phenotype (8-level categorical variable: all possible combinations of [CD+|CD−][NVSM+|NVSM−][ANX+|ANX−]) and time of visit (2-level categorical variable: baseline; week eight) were used as independent variables while adjusting for age, sex, $HRSD_{17}$ scores at time of visit, and specific antidepressant (escitalopram or citalopram). The "emmeans" R package was employed to compute the least squared means of the contrasts of interest and their corresponding p-values at baseline and at eight weeks (i.e., 1: CD+ vs. NVSM+; 2: CD+ vs. ANX+; 3: NVSM+ vs. ANX+). To examine the statistical significance of the temporal changes in metabolite concentration over eight weeks across phenotypes, linear mixed effect models (participant as random) were fitted with log2-fold metabolite levels as the dependent variable and time of visit (2-level categorical variable: baseline; week eight) as the independent variable; the models were adjusted for age, sex, baseline $HRSD_{17}$ score,and specific antidepressant (escitalopram or citalopram).

3. Results

3.1. Participant characteristics

Plasma metabolite data were available from 240 MDD participants. At baseline, age, gender, and baseline $HRSD_{17}$ scores did not significantly differ across the three groups. The demographic and clinical characteristics of the study sample are detailed in Table 1.

Table 1
Baseline Demographics.

Feature	All Subjects (N = 92)	CD+* (N = 31)	NVSM+* (N = 17)	ANX+* (N = 44)	Test Statistic
AGE Mean (SD)	39.8 (13.6)	43.1 (13.9)	36.6 (14.8)	36.8 (12.3)	F = 1.7 df. = 3167 P = 0.17
Gender Male% (N)	33% (56)	32% (10)	12% (2)	34% (15)	Chi-square = 4 df. = 3 P = 0.26
Baseline $HRSD_{17}$* Mean (SD)	20.3 (3.7)	21.5 (3.9)	21.5 (3.10)	22.3 (3.36)	F = 16 df. = 3167 P < 0.001

Abbreviations: *$HRSD_{17}$: 17-item Hamilton Rating Scale for Depression; CD: Core Depression phenotype, ANX: Anxious Depression phenotype, NVSM: Neurovegetative Symptoms of Melancholia phenotype.

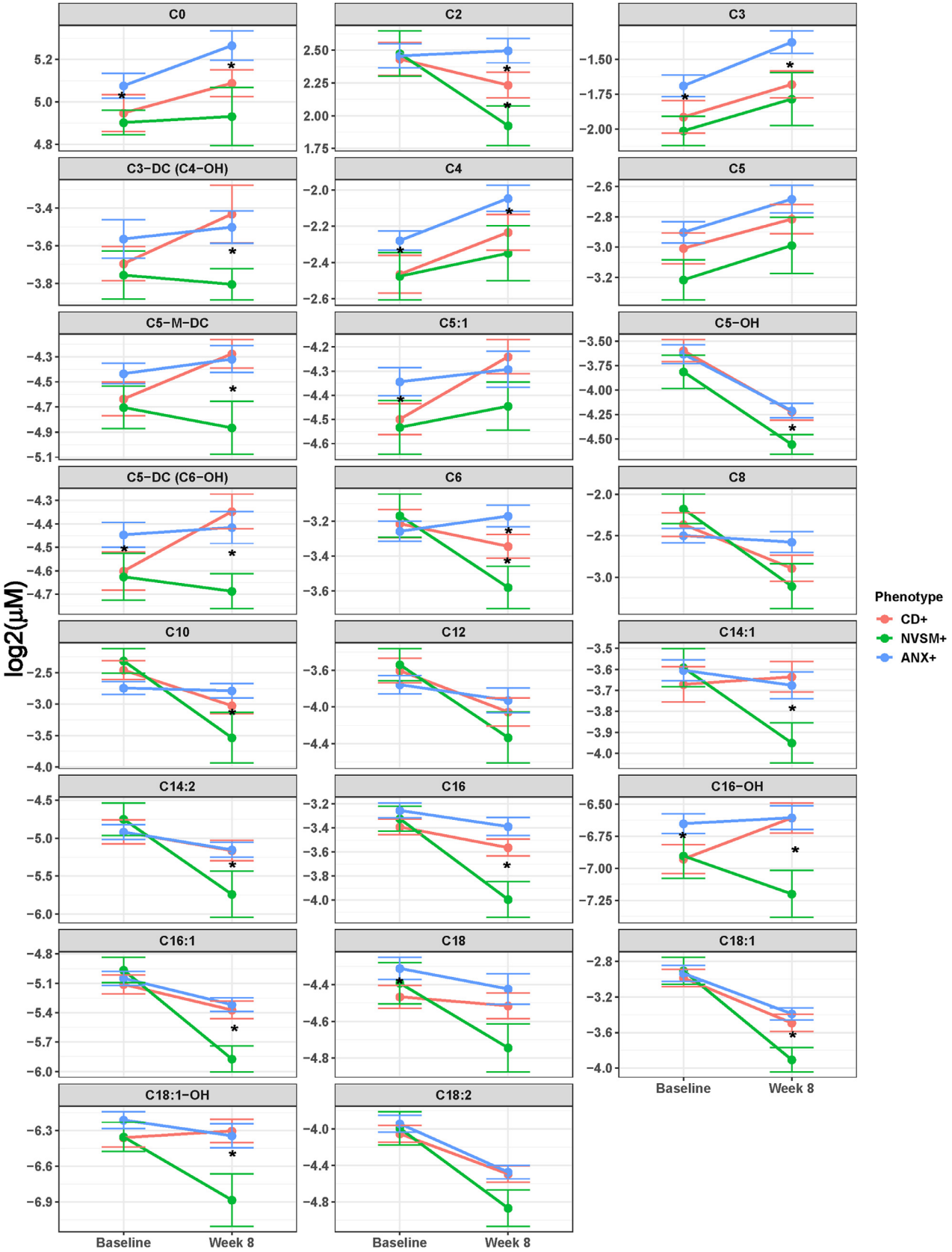

Fig. 2. Short-, Medium- & Long-Chain Acylcarnitines — Baseline and 8 Weeks Stratified by Phenotype (+).
These plots show the baseline and week-eight acylcarnitine metabolite concentrations for each of the three phenotypes (CD+: Core Depression, ANX+: Anxiety, NVSM+: Neurovegetative Symptom of Melancholia). Asterisks indicate statistical significance of mean differences between the two groups (unadjusted $p < 0.05$) at each visit. Error bars represent standard error of the means. P-values were obtained from linear mixed effect models corrected for age, sex, drug and 17-item Hamilton Rating Scale for Depression scores.

3.1.1. At baseline, acylcarnitine metabolomic patterns that differentiate each pure phenotype (CD+; NVSM+; ANX+) from the others

CD+ participants demonstrated significantly lower levels of C0, C3, C5:1, C5-DC/C6-OH, C16-OH, and C18 acylcarnitines compared to ANX + participants. Moreover, C5-DC/C6-OH and C16-OH acylcarnitines levels were significantly lower, and C10 significantly higher, in the NVSM+ participants compared to the ANX+ participants. Fig. 2 shows the baseline and week eight acylcarnitine metabolite concentrations for each of the three phenotypes. **Supplemental Table 3** details the baseline and week eight differences between each phenotype and significant p-values.

3.1.2. At eight weeks, acylcarnitine metabolomic patterns that differentiate each pure phenotype (CD+; NVSM+; ANX+) from the others

At eight weeks, CD+ participants had significantly higher levels of C5-DC/C6-OH, C5-M-DC, C6, C14:1, C16, C16-OH, C16:1, and C18:1-OH acylcarnitines compared to NVSM+ participants. CD+ participants had significantly lower levels of C2, C3 and C6 acylcarnitines compared to ANX+ participants. NVSM+ participants had significantly lower levels of C0, C2, C3, C5-DC/C6-OH, C5-M-DC, C6, C10, C14:1, C16, C16-OH, C16:1 and C18:1-OH acylcarnitines compared to ANX+ participants. **(**Fig. 2**, Supplemental Table 3).**

3.1.3. Baseline to week eight changes in acylcarnitine metabolomic patterns that differentiate each pure phenotype (CD+; NVSM+; ANX+) from the others

In all subjects, over the eight-week course of SSRI treatment, there were significant increases in the levels of several short-chain acylacrnitines and significant decreases in the levels of several medium- and long- chain acylacrnitines (Fig. 3). Different patterns in acylcarnitine changes were noted across the three phenotypes. Patients characterized by the greatest levels of sadness and anhedonis (CD+), demonstreated the significant increases in five short-chain acylcarnitines (C0, C3, C4, C5-M-DC and C5:1). The free (C0) and short-chain acylcarnitines C3, and C4 significantly increased in both CD+ and ANX+. The medium-chain acylcarnitines C8, C10, and C12 significantly decreased in CD+ and NVSM+. Finally, nearly all the long-chain acylcarnitines demonstrated highly significant decreases in the NVSM+ group, but not in the CD+ or ANX+. There were four acylcarnitines, C5-OH, C16:1, C18:1, and C18:2, that significantly decreased in all three phenotypes, perhaps reflecting the effects of exposure to SSRI. Significance of p-values ranged from <0.05 to <0.001. Fig. 3 **and Supplemental Table 4** show change of metabolite levels from baseline to eight weeks of SSRI treatment among the three phenotypes and significant p-values (rows represent the acylcarnitine and columns represent the phenotype).

4. Discussion

This study assessed whether three symptomatically defined phenotypes of MDD could be differentiated based on acylcarnitine profiles at baseline and after eight weeks of citalopram/escitalopram treatment. Both the cross-sectional levels and the changes in acylcarnitine levels distinguished the three pure phenotypes. In the temporal analyses assessing change from baseline to week 8 of SSRI treatment, significant increases in short-chain acylcarnitines were observed in the CD+ and ANX+ phenotypes but no much in the NVSM+ phenotype. The NVSM + phenotype was associated with significant decreases in medium- and long-chain acylcarnitines.

After eight weeks of SSRI treatment, concentrations of most of the 23 acylcarnitines were significantly lower in the NVSM+ than in ANX + participants. In addition, the NVSM+ group, which was defined by reduced appetite and insomnia) also showed the largest decrease in the levels of C2 after eight weeks of citalopram/escitalopram. Interestingly, although acylcarnitine concentrations are dependent upon dietary intake of carnitine, increased levels of C2 have been associated with the fasting state in healthy controls (Costa et al., 1999; Frohlich et al., 1978; Hoppel and Genuth, 1980). Recently, Davies and colleagues found increases in C2 after sleep deprivation (Davies et al., 2014) and concluded that changes in the acylcarnitines involved in fatty acid β-oxidation are implicated in sleep regulation (Davies et al., 2014). In our study, the C2 levels decreased significantly and consistently in the NVSM+ patients post drug exposure, from which we infer that the change is at least partially due to the effect ofthe drug treatment. Similarly, another study found a decrease in C2 concentrations after ketamine treatment (n = 29) compared to placebo (n = 29) (Moaddel et al., 2018). Other studies (Nasca et al.) have also reported perturbations in C2 levels in depression. Beyond C2, we also found that the NVSM+ and CD+ phenotypes were associated with decreased levels of medium and long-chain acylcarnitines, especially C8, C10 and C12, after eight weeks of treatment,.

At baseline, differences in acylcarnitines were most evident between the ANX+ and CD+ phenotypes. Most notable were the significantly lower levels of several short-chain acylcarnitines among the CD+ compared to the ANX+. After 8 weeks of SSRI treatment, the differences in acylcarnitines between these groups was reduced, though C2, C3, and C6 continued to be significantly lower in the CD+ group. With treatment, the C0, C3 and C4 levels significantly increased in both CD+ and ANX+, but C5 increased only in the ANX+ phenotype.

Previous work has found that C0 levels are inversely correlated with self-rated depressive symptoms (Fukami et al., 2014), and Tashiro and colleagues have suggested that C4 levels are associated with depressive symptoms (Tashiro et al., 2017). In our phenotyping approach, the CD + phenotype is defined by patients who are sad and anhedonic but not highly anxious, in contrast to the ANX+ phenotype, defined as having high anxiety but relatively low levels of sadness and anhedonia (i.e., below the threshold use to define CD+). Although these two groups had very similar total $HRSD_{17}$ scores (0.8 points difference in means),

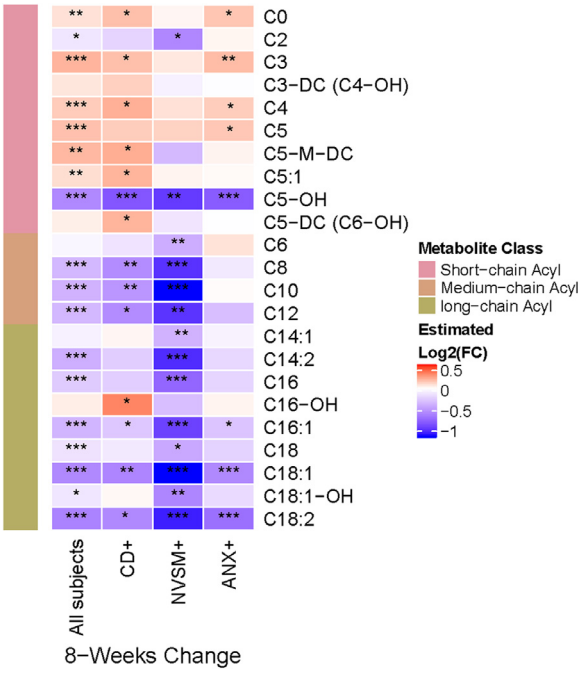

Fig. 3. Metabolic Signature of Eight Weeks of Exposure to SSRI.
The heatmap depicts the log2 fold change of metabolite levels from baseline to week eight of SSRI treatment. CD+: Core Depression, ANX+: Anxiety, NVSM +: Neurovegetative Symptom of Melancholia. P-values were obtained using linear mixed effect models controlling for age, sex and baseline 17-item Hamilton Rating Scale for Depression scores. Red indicates an increase and blue indicates a decrease in metabolite levels over eight weeks of treatment; *:p-value <0.05, **:p-value <0.01 and ***:p-value <0.001.

they differed in the items that drove the total score, reinforcing the importance of specific items for determining MDD phenotypes (Hieronymus et al., 2016). Taken together, these results are consistent with those of prior studies suggesting that more severe cores symptoms of depression are associated with greater reductions in short-chain acylcarnitines.

To summarize, the current data demonstrated that three clinically-defined depression phenotypes have distinct patterns of acylcarnitine levels at baseline and after eight weeks of antidepressant treatment. Also, these phenotypes were uniquely and distinctly related to changes in acylcarnitine levels induced by SSRI exposure. The most clinically relevant interpretation of our results is that:1) more severe forms of depression, defined by high levels of sadness and anhedonia, are associated with reductions in short-chain acylcarnitines, and 2) depression characterized by reduced sleep and appetite show substantial reductions in short-, medium-, and long-chain acylcarnitines after SSRI treatment. These findings, and the relationship of acylcarnitine levels with mitochondrial fatty acid β–oxidation and branched-chain amino acid catabolism, suggest that the pathobiologies of MDD may manifest in part through metabolomic dysfunction. Further, these findings may reflect changes in mitochondrial function or ATP production in patients with MDD. It is unfortunate that the $HRSD_{17}$ has only one item to assess a patient's energy level, with a narrow scoring range of 0–2. Application of broader subjective measures of energy, or objective measures of cognitive or physical fatiguability, may enable greater understanding between MDD symptoms and acylcarnitine disturbances. Another approach is to link acylcarnitine metabolomic data to genetic data, which has proven to be a powerful approach for highlighting variation in predicting antidepressant response among depressed individuals (Ji et al., 2011; Neavin et al., 2016). These approaches may help to develop a metabolomic profile or 'metabotype' or 'potential biomarkers' of MDD patients, as indexed by an electrochemistry metabolomics platform total output (digital map), with the aim of improving subtype classification of the MDD syndrome. However, these markers that did distinguish between phenotypes in this initial report, cannot be used as markers to distinguish among the phenotypes without replication in different samples. Moreover, the study did not produce thresholds that could be used to adequately distinguish between the phenotypes at the individual level, but the significant differences in acylcarnitines do provide guidance for further understanding the pathobiology of MDD.

4.1. Limitations

The study has several limitations. Analyses were performed on a subset of MDD participants, thereby limiting the generalizability of the findings. Some of the reported acylcarnitines, especially dicarboxylic and hydroxylated species (C3DC, C5DC, C5MDC, C16OH, C18OH)—are canonically elevated only in patients with rare inborn errors of metabolism (see Supplemental Table 2), and are of very low concentrations in most individuals. The flow-injection MS/MS method used in this study may lack molecular specificity to confirm exact structure for low-level species, even though the measures reported herein show excellent technical reproducibility. Importantly, we reported many acylcarnitines which are considered 'low abundance (i.e. <0.1 μM), and our average reported values for each analyte in the cohort are below the pathological clinical reference threshold reported by Mayo Clinic Laboratories (Mayo Clinic Laboratories, 2017). Confirmation of exact molecular speciation of the low-abundance acylcarnitines reported in this study may benefit from utilization of an assay with better molecular specificity, such as a research-grade LC-MS/MS assay reported previously(Minkler et al., 2015). The presence of outlier samples in our cohort might represent subtypes of MDD that will require larger sample sizes to characterize more fully, or may represent other metabolic disorders in those individuals. Our study lacked inclusion of a healthy control group with which to compare and contrast the acylcarnitine profiles of the MDD phenotypes, which could have determined whether or not these metabolite changes with SSRI treatment reflect changes toward normal concentrations.

5. Conclusions

Baseline and week eight acylcarnitine concentrations, evaluated both cross-sectionally and by temporal change, indicated unique acylcarnitine signatures that distinguish clinically-defined MDD phenotypes from each other. This study supports the possibility of using acylcarnitines as a tool for understanding personal variation in MDD drug response; this moght allow for sub-classifications of MDD to CD+, NVSM+ and ANX+ patients and the linking of acylcarnitine changes to mitochondrial fatty acid β–oxidation and branched-chain amino acid catabolism.

Authors statement

Dr. Kaddurah-Daouk serves as the overall PI and has developed the concepts of using metabolomics data for disease subclassification and for defining response to treatment. Drs. Ahmed, Frye, Rush, Dunlop, Craighead and Kaddurah-Daouk conceptualized the hypothesis, created the analysis plan, supervised the data analysis and the manuscript writing. Drs. Ahmed and MahmoudianDehkordi had access to all data and take responsibility for the accuracy of the data analysis and the writing of the manuscript. Drs. MahmoudianDehkordi, Bhattacharyya, and Ahmed performed the data analysis. All authors contributed to interpretation of findings and have approved the final manuscript.

Previous presentation

Presented in part at the Annual Meeting of the Society of Biological Psychiatry (SOBP) 2019 IL and in bioRxiv.

Declaration of Competing Interest

Richard Weinshilboum is a co-founder and stockholder in OneOme, LLC, a pharmacogenomic clinical decision support company. Dr. Kaddurah-Daouk is an inventor on patents related to metabolomics applications in the study of CNS diseases and holds equity in Metabolon, Inc., a biotechnology company that provides metabolic profiling capabilities. Dr. A. John Rush has received: consulting fees from Akili, Brain Resource Inc., Compass Inc., Curbstone Consultant LLC., Emmes Corp., Johnson and Johnson (Janssen), Liva-Nova, Mind Linc., Sunovion; speaking fees from Janssen and Liva-Nova; and royalties from Guilford Press and the University of Texas Southwestern Medical Center, Dallas, TX (for the Inventory of Depressive Symptoms and its derivatives). Dr. Rush is also named co-inventor on two patents: U.S. Patent No. 7795,033: Methods to Predict the Outcome of Treatment with Antidepressant Medication and U.S. Patent No. 7906,283: Methods to Identify Patients at Risk of Developing Adverse Events During Treatment with Antidepressant Medication. Drew Neavin's stipend has been supported in part by NIH T32 GM072474 and the Mayo Graduate School. Ahmed Ahmed's research was supported by National Institute of General Medical Sciences of the National Institutes of Health under award number T32 GM008685. Dr. Matthias Arnold was supported by National Institute on Aging [R01AG057452, RF1AG051550, R01AG046171], National Institute of Mental Health [R01MH108348], and Qatar National Research Fund [NPRP8-061–3–011]. The funders listed above had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. Dr. Dunlop has received research support from Acadia, Axsome, Janssen, Takeda and he serves as a consultant to Assurex Health and Aptinyx Inc. dr. Craighead is a board member of Hugarheill ehf, an Icelandic company dedicated to the prevention of depression, and he receives book

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2019.11.122.

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