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The metabolome as a biomarker of mortality risk in the common marmoset

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Abstract

Recently, the common marmoset has been proposed as a non-human primate model of aging. Their short lifespan coupled with pathologies that are similar to humans make them an ideal model to understand the genetic, metabolic, and environmental factors that influence aging and longevity. However, many of the underlying physiological changes that occur with age in the marmoset are unknown. Here, we attempt to determine if individual metabolites are predictive of future death and to recapitulate past metabolomic results after a change in environment (move across the country) was imposed on a colony of marmosets. We first determined that low levels of tryptophan metabolism metabolites were associated with risk of death in a two-year follow-up in the animals, suggesting these metabolites may be used as future biomarkers of mortality. We also discovered that betaine metabolism and methionine metabolism are associated with aging regardless of environment for the animals, or of metabolomic assay technique. These two metabolic pathways are therefore of particular interest to examine as future targets for health and lifespan extending interventions. Many of the pathways associated with age in our first study of marmoset metabolomics were not found to have significant age effects in our second study, suggesting more work is needed to understand the reproducibility of large scale metabolomic studies in mammalian models. Overall, we were able to show that while several metabolomics markers show promise in understanding health and lifespan relationships with aging, it is possible that choice of technique for assay and reproducibility in these types of studies are still issues that need to be examined further.

Authorship

JMH, DELP, and DPJ designed the experiment. CR and ST were in charge of marmoset husbandry and sample collection. VT and DPJ completed the metabolomic analyses. JMH analyzed the data, made the figures, and wrote the first draft of the manuscript. All authors commented on and edited the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Keywords

marmoset; aging; metabolomics; longevity; sex differences

Introduction

The number one risk factor for the majority of morbidities and mortalities in the developed world is age (Kaeberlein, Rabinovitch, & Martin, 2015). World populations are aging rapidly, yet many of the underlying physiological pathways that affect aging and longevity are unknown. Animal models have been developed to determine conserved genetic and metabolic pathways that influence aging, and thus mortality in an individual. A major drawback of standard laboratory organism research is that model organism biology is often not representative of human biology, especially when models are distantly related phylogenetically; to this end, non-human primate (NHP) models of human aging are beneficial. However, most NHP species are quite long lived, almost as long as humans in many species, which is not ideal for understanding the biology of aging as studies take decades and are quite cost prohibitive.

Recently, the common marmoset, *Callithrix jacchus*, has been proposed as an ideal NHP model of aging (Fischer & Austad, 2011; Tardif, Mansfield, Ratnam, Ross, & Ziegler, 2011). Marmosets are short-lived (Ross et al., 2017), neotropical primates that share many age-related morbidities in common with humans. For these reasons, the marmoset has the potential to be an ideal animal model to understand the genetic and environmental factors that influence aging and longevity.

The shorter lifespan of the common marmoset allows for the detection of potential predictive indicators of morbidity and mortality in a relatively short time frame. Over the past decade, researchers have become interested in studying the effects of individual metabolites and metabolic pathways as indicators of current and future health and longevity. Metabolomics, the systematic study of the intermediates and products of metabolism, has the potential to elucidate individual metabolites and metabolic pathways that are associated with different aging phenotypes. Within the biology of aging, metabolomics has been used extensively over the last decade to uncover potential biochemical changes that cause aging in model organisms (e.g. Fuchs et al., 2010; Hoffman et al., 2014; Houtkooper et al., 2011) and humans (Menni et al., 2013). There are two main approaches to metabolomics, both of which have been applied in a biology of aging context: global and targeted metabolomics. Global metabolomics involves the analysis of thousands of different small molecules, the majority of which cannot be annotated to a known metabolite. Targeted metabolomics, the focus of this paper, typically includes 100–200 well-defined metabolites. The two methods provide different information on the metabolome, but they should show similar corresponding results, as the same metabolic pathways will be changing in both samples, and pathways that reflect biological relevance should be detectable regardless of assay technique.

We have previously shown that global metabolomic profiles change with age in the common marmoset in a sex specific manner (Hoffman et al., 2016). A subset of the animals analyzed

in the initial project were moved from Southborough, MA to San Antonio, TX in 2015 due to the closure of the New England National Primate Research Center. As the metabolome is highly sensitive to shifts in both the physical and biological environment, the move of an entire colony of previously characterized animals allows for the exploration of hypotheses regarding metabolomic shifts associated with the likely stressors experienced during the move and acclimation to a completely new laboratory environment, with diet kept consistent between locations. We hypothesized that even with the large environmental shift, metabolic pathways associated with aging should remain consistent regardless of their housing environment. While associated changes may be comparable across studies, no one has yet demonstrated that physiological associations with age change are stable regardless of environmental change.

Here, we present a targeted metabolomic analysis on the effects of age and sex on the metabolome of a cohort of marmosets that were previously examined (Hoffman et al., 2016). This second analysis occurs after the original cohort was removed from its original environment (the New England Primate Research Center), with animals sent to one of two other centers, the Wisconsin National Primate Center, or the Southwest National Primate Center (SNPRC). Here we focus on those animals sent to SNPRC, looking in particular for metabolic predictors of future death of individuals. We then determined if the effects of age on metabolites were still observed after this significant shift in their environment, using a different metabolomics method, and we determine if the entire metabolome is associated with age and sex in the animals.

Methods

Marmosets and sample collection

Adult marmosets (ages 2–17) were transferred from the New England Primate Center, outside Boston, MA, to the SNPRC, Texas Biomedical Research Institute, San Antonio, TX, during the summer of 2015. The incoming animals were housed and maintained separately from the SNPRC marmoset colony. General husbandry protocols followed (Layne & Power, 2003). Room temperature ranged between 76 and 84° F (set point of 80° F), with a 12h light-dark cycle with lights off at 19:00. Rather than transitioning the marmosets to the standard marmoset diet at SNPRC, the New England marmosets stayed on their familiar diet consisting of Teklad 8794 extruded pellets and Zupreem. They also received three enrichment items daily consisting of one protein item (egg, cottage cheese, nuts, beans etc.), one fruit (fresh or dried), and one vegetable or other item (enrichment jumble, raisins, cranberries, etc).

Of the original 230 marmosets that were assessed in the 2012–2013 sampling (Hoffman et al, 2016), 96 marmosets were available at SNPRC for sampling immediately following the move to SNPRC. Distribution of ages for animals used in this analysis are shown in Figure S1. Fasted femoral vein plasma samples were collected from non-anesthetized marmosets by placing them into a restraint device to which they were habituated. Samples were placed in the –80°C freezer until shipment. Blood samples were collected in June/July 2015 and then again in December 2015/January 2016. Animal deaths were evaluated for this cohort of marmosets through Summer 2017 for comparison with metabolomic analysis from 2015.

This research was approved by the Institutional Animal Care and Use Committee at Texas Biomedical Research Institute, abided by the guidelines set forth by the American Society of Primatologists and all applicable U.S. Federal laws governing research with NHPs.

Metabolomics

Metabolomics samples were run at the Emory University Metabolomics Core, and the protocols used for sample preparation, mass spectrometry, and data quality control have been described previously (Go et al., 2014). Briefly, 100 μ l of acetonitrile was added to 50 μ l of blood plasma and 2.5 μ l of internal standard. Samples were maintained on ice for 30 min and then spun at 14,000g for 10 minutes at 4°C to remove protein content. 10 μ l of supernatant was analyzed by liquid chromatography (LC) coupled to ultra-high-resolution mass spectrometry; each sample was analyzed with three technical replicates on each of two columns, an anion exchange (AE) and a reverse-phase (C18) column. Two columns were used to extract a broader representation of metabolites. AE columns measure negatively charged metabolites while C18 columns measure hydrophobic metabolites. The two columns tend to have about a 30% overlap in the metabolites detected (Go et al., 2014). Metabolite detection was obtained using positive electrospray ionization on an LTQ-Velos Orbitrap mass spectrometer (ThermoFisher, Inc).

Statistical analyses

All statistical analyses were completed in the program R unless otherwise stated (R Core Team, 2016). First, counts from targeted metabolomics data from both columns, AE and C18, were log transformed. Triplicate runs of technical replicates were averaged for each metabolite within each sample. Metabolites with more than 30% missing values were removed from each dataset. Data were then centered and scaled to a mean of zero and standard deviation of one. Missing values were imputed using the metabolite mean in the Hmisc package in R (Harrell, 2018).

We first sought to determine if individual metabolites were indicative of future death in the animals. Animals were followed for two years and any naturally occurring deaths were recorded. We used those blood samples drawn in June/July 2015 (therefore, each animal was only measured once), and then ran a Cox proportional hazard model to determine if individual metabolite concentrations were associated with death in follow-up. Animals that were alive at the end of the study were censored. Correction for multiple comparisons was done using a False Discovery Rate (FDR) of $\alpha < 0.1$ (Benjamini & Hochberg, 1995). Metabolites found to be associated with death were plotted using stripcharts and boxplots.

We have previously shown that global metabolomic profiles are associated with age (Hoffman et al., 2016). Therefore, we were interested in opportunistically evaluating whether individual targeted metabolites showed similar relationships with age following a move to a new facility. As metabolomic profiles are significantly influenced by an individual's environment, we might expect to find that those metabolites that change with age are different across different environments. To this end, we ran a random effects linear model using the nlme package in R (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2012). We determined the associations of age and sex with metabolite concentration, controlling for the

random variation of each individual. To correct for multiple comparisons, we again considered variables to be associated with metabolite concentrations at an FDR of $\alpha < 0.1$.

After finding individual metabolites associated with age and sex, we ran pathway enrichment analysis using the program MetaboAnalyst 3.0 (Xia & Wishart, 2016). Using the known human metabolic pathways as a reference, due to this being the closest related species to marmosets with well-defined metabolic pathways, we asked what metabolic pathways were overrepresented for individual metabolites associated with age and/or sex. This allowed us to determine if the metabolic pathways associated with aging in our previous study could be recapitulated in the same animals living in a significantly different environment.

We were then interested in determining if individual metabolites changed from immediately post-move (June/July 2015) and 6 months later (December 2015/January 2016). This analysis allowed us to evaluate whether metabolomics shifts might return to pre-move levels after 6 months. To this end, we ran a linear model of each metabolite on age, using only those individuals for which we had data collected from both time points. We then took the residuals from the model and ran a paired t-test on the early and late time points. This allowed us to determine those metabolites that changed within the 6-month period, controlling for the effect of age, as the animals were 6 months older. We again used an FDR<0.1 for determining those metabolites that were significantly different from each other.

For our final analysis, we used a multivariate approach to study the extent to which the entire metabolome combined was associated with our three factors of interest: age, sex, and death during follow-up. We used only those samples that were collected in June/July 2015 to remove any effects due to replication of the same individuals, and this gave us a greater samples size as more animals were sampled at the first time point than the second. We ran unsupervised principal components analysis (PCA) to determine if any principal component axes were associated with our three factors of interest: age, sex, and death in follow-up.

Results

Our final dataset consisted of information from 93 AE column metabolites and 100 C18 column metabolites taken from 96 marmosets (54 males, 42 females). 49 marmosets had blood drawn at both time points (June/July 2015 and December 2015/ January 2016). During the two-year follow-up period, 21 (22%) of the animals died due to non-experimental causes. Animals that died were almost exclusively from the older age classes (average age at death = 10.45 years; range = 2.8–17.4 years; 86% of deaths were in animals 8 years of age or older). The majority of pathology findings in those deceased animals were in agreement with age-related morbidities (Table S1).

We were first interested in discovering if individual metabolites were associated with death over two years after an individual's first blood draw at the SNPRC. Across the two columns, we found only three metabolites that passed our FDR correction. 3-indoeacrylic acid, 3-indolepropionic acid, and tryptophan were all discovered in the C18 column, and all three metabolites were lower in those animals that died in the two-year follow-up controlling for

the effects of age and sex (Figure 1). Indoleacrylic acid can be a biologic metabolite of tryptophan but also can occur as an insource fragment of tryptophan produced during ionization; these cannot be distinguished in the current analysis. Indolepropionic acid is also a derivative of tryptophan. While not passing our FDR cutoff, two adduct forms of 3-indoleacrylic acid and tryptophan were detected and were also lower in animals that died in the follow-up period.

We then determined individual metabolites that were associated with age and sex across all animals. We found 13 and 17 metabolites associated with sex (Figure 2 and Table 1) in our C18 and AE column respectively, and 25 and 23 metabolites associated with age in our AE and C18 column respectively (Figure 3 and Table 2). We then ran enrichment analyses on these groups of metabolites and found that in both columns, betaine metabolism and glycine and serine metabolism significantly declined with age (Table 3). In addition, methionine metabolism, located downstream of betaine metabolism, was changed with age in the AE column. Many of these results differed from our previous study on age related metabolic pathways (Table 4). Our analysis of sex differences in the metabolome suggested that, as would be expected, steroidogenesis metabolism was significantly different between males and females (Table 3). This was driven mostly by significantly higher levels of progesterone in female as compared to male marmosets (Table 1).

We then looked to see if metabolite levels returned to a “normal” level 6 months after the move to determine whether some of the detected metabolic changes may have been an acute reaction to the move. We found 13 and 5 metabolites that changed between the two points in the AE and C18 column respectively (Table 5). Of those metabolites that did change after acclimation to the new environment, there was some overlap with our metabolites that changed with age, including betaine levels. In addition, allantoin and lysine were found to change in between the two timepoints in both columns.

Finally, we determined the extent to which the entire metabolome was associated with sex, age, and death. We first ran unsupervised principal components analysis on the early timepoint dataset, as it had a larger sample size. Principal Component 1 (PC1) and PC2 were significantly associated with age and sex, respectively, in the C18 column (Figures 4 and 5, $P < 0.0001$ for both). Similarly, PC1 and PC3 were correlated with age and sex, respectively, in the AE column (Figures 4 and 5, $P < 0.001$ for both). Death in follow-up was associated with PC4 in the C18 column, which explains a little more than 5% of the total metabolome variance ($P = 0.0006$). Of the top 10 principal components, PC2 in the AE column had the highest correlation with death in follow-up, but it did not reach statistical significance ($P = 0.080$).

Discussion

The overall goals of this study were to 1) reveal if any individual metabolites or metabolic pathways were associated with future death over a two-year follow-up period 2) discover if metabolomic profiles are associated with age and 3) determine whether the signature of changes in the metabolome with age are detectable regardless of environment or environmental change. First, we were able to find three metabolites associated with future

death in the marmosets over a two year follow up period from the first blood draw after controlling for the effects of age. This is one of the first studies to discover metabolites whose levels predict future mortality over a several year time span. Interestingly, the three metabolites that were predictive of future death, 3-indoleacrylic acid, 3-indolepropionic acid, and tryptophan, are all important metabolites in the tryptophan metabolism pathway. It must be noted, however, that the mass spectral identification of 3-indoleacrylic acid as an endogenous metabolite is equivocal because it could have arisen as an in-source fragment of tryptophan. All metabolites are nodes in the tryptophan metabolism network, however, meaning they have many different known metabolic connections and are both upstream and downstream targets of many metabolic pathways. With either interpretation, detection of both and concurrence of responses provides greater confidence in the conclusion that tryptophan metabolism is associated with death as an outcome. Any other physiological effects were potentially blunted because only a small percentage (22%) of the animals in the study died during the two years. Interestingly, tryptophan was not found to be correlated with age. While the majority of the animals that died were older, a large number of older individuals did not die within the two year follow up. This suggests tryptophan might be an ideal marker of future mortality in the animals because the levels appear to be driven not by the age of the animal specifically but by the overall health. This also might point to tryptophan changes being associated with resiliency and frailty. As the animals were moved and encountered a strong stressor, those animals that were able to survive after the major stressor, even in older age, showed a high level of resiliency. Thus tryptophan, might not just predict future mortality but also resiliency in animal populations. Future studies are needed to investigate this claim further, and to directly test if tryptophan levels can influence longevity in mammals.

Our previous marmoset metabolomics analysis implicated tryptophan metabolism in the aging process (Hoffman et al., 2016), and while our metabolic enrichment analysis with age did not recapitulate these results, it is interesting to note that the only predictors of death in follow-up were in the tryptophan metabolism pathway. In addition, our previous analysis did not measure tryptophan directly, as this metabolite was not annotated during our global analysis, most likely due to filtering of the data and removing low quality samples. This suggests that tryptophan metabolism is likely associated with aging and death in the marmoset. Tryptophan metabolism has previously been shown to be a strong indicator of health in other organisms, with lower metabolic levels associated with poorer health outcomes (Yao et al., 2011), and studies in worms (van der Goot et al., 2012), fruit flies (Hoffman et al., 2014; Oxenkrug, Navrotskaya, Voroboyva, & Summergrad, 2011) and mice (Miura, Ozaki, Shirokawa, & Isobe, 2008) have implicated tryptophan metabolism in the aging process. Previous research suggests that tryptophan levels decline with age and higher levels lead to longer lifespan in invertebrate models (Oxenkrug et al., 2011; van der Goot et al., 2012). Our results agree with this hypothesis, as tryptophan levels are significantly lower in marmosets who died than those who remained alive at the end of the study. Our results, coupled with those from the previous studies cited above, suggest that tryptophan metabolism may play a strong role in aging and longevity, and future research is needed to discern the extent to which this metabolic pathway may be used as a biomarker of future frailty, resilience, and longevity.

Interestingly, we found little overlap in our metabolic pathway enrichment analysis for metabolites associated with age in this study and our original study. Previously, our longitudinal analysis with age showed a strong association of purine metabolism with age in the animals. However, in this later targeted analysis, we fail to find individual metabolites in the purine metabolism pathway that are associated with age. In addition, our enrichment analysis failed to find a significant association of purine metabolisms with age. Our new targeted analysis suggests that betaine metabolism and serine and glycine metabolism both are significantly affected by the aging process. However, these two pathways were not as strongly associated in our previous longitudinal study completed two years prior to this study.

While we had predicted that some of the metabolic pathways associated with age in the first longitudinal study may be affected by environmental conditions, we were somewhat surprised by the broad differences between the two studies. There are several potential explanations for our results that we discuss in turn. First, the approach used here—targeted metabolomics—is considerably different from the global metabolomic approach we used in our first study (Hoffman et al., 2016). For example, with the selected targeted approach, many of the metabolites in the purine metabolism pathway were not assessed, so they were only measured in the global analysis. The lack of purine metabolites in the targeted analysis could have caused the disconnect in the measured metabolites associated with age in the two studies. Unfortunately, it was not possible to run targeted analyses on the original study samples to determine whether similar results would have been detected. Along the same lines, lack of annotation of metabolites using the global approach resulted in the ability to only annotate approximately 15% of the metabolites analyzed in the original study (Hoffman et al., 2016). Potentially, metabolites associated with betaine metabolism were affected by age in the original study, but we failed to accurately identify them. And then they were not included in our enrichment analysis. This result would not be unexpected as previous research on amino acids indicated that many metabolites did not overlap when comparing a targeted and global approach run on the *same* samples longitudinally (Klepacki et al., 2016). Thirdly, our two analyses were separated by two years, and all of the animals had aged. The first study included juveniles and individuals under a year of age, while in the second study the youngest were still young adults, but now were at least 2 years of age. This difference could potentially lead to shifted metabolomic profiles that allowed us to detect different metabolites that are associated with age. Lastly, not all the animals in the original assessment were moved to the SNPRC, so we are only analyzing a subset of the original population which might result in unintentional selection bias. However, these effects are probably fairly minor as we still had a reasonable sample size of the original population, and it would be unexpected for our cohort to be significantly different from the cohort not moved to the SNPRC.

A potentially more interesting reason for our lack of reproducibility between studies than those described above, is that metabolomic studies have an age-by-environment interaction. This would imply that metabolic pathways that are affected by age change based on an individual's environment. Our animals were moved from the New England Primate Research Center to the SNPRC in summer 2015. The SNPRC is a different environment with different handlers, husbandry practices, water, and air (though the animals were kept on

the same diet); along the same lines, the animals were most likely exposed to different microbiota in one facility as compared to the next. As upwards of 10–15% of the circulating metabolome in an organism is defined by the microbiome (Wikoff et al., 2009), this could be an important contributing factor to the age-by-environment interaction observed. Overall, the lack of major reproducibility suggests we still have a long way to go to optimize large metabolomic studies for studies with large environmental variation. This also suggests that the translational power of the marmoset to human studies will become stronger as our ability to reproduce large scale results improves.

To determine those metabolites that might have been affected acutely by the stress of the move, we looked at which metabolites changed in the 6-month post-move time period after controlling for the effects of age. This allowed us to see if any metabolites returned to a more “normal” controlled stage after the animals acclimated to the new environment. Interestingly, we found several of the metabolites that were associated with age, also changed between the two time points including: betaine, 2-aminobutyrate, N,N - dimethyllysine, valine, lysine, and tauroithocholic acid. These metabolites were found to change with age and still be different between the two time points after controlling for age. This suggests that these individual metabolites are affected not just by age but by acute stressors, the move in this instance. These metabolites might be potential targets to investigate responses of animals to various stressors, as well as those metabolites that are affected by age even in the face of a major stressor. It is interesting that the betaine metabolic pathway which was associated with the largest effect of aging, also expressed this timepoint effect. This suggests that the betaine pathway may be modified not just by age but by strong environmental stressors making it a strong novel marker of age and environment interactions.

In the current study, we found a strong association of betaine metabolism, as well as some evidence for changes in methionine metabolism with aging. These two pathways are closely intertwined with betaine being the methyl donor that is used to convert homo-cysteine to methionine (Lehninger, 2005). As the downstream methyl receiver from betaine, methionine metabolism has been of great interest to aging researchers, and studies in multiple organisms suggest that reducing levels of methionine may lead to healthier aging and lifespan extension (reviewed in Lee, Kaya, & Gladyshev, 2016; Sanchez-Roman & Barja, 2013). Our previous marmoset metabolomic study found betaine significantly declined with age in both males and females; however, we failed to find significant enrichment for betaine metabolism due to lack of annotation of metabolites in the betaine metabolism pathway. In addition, we discovered a significant change in methionine and cysteine metabolism with age in the animals with some metabolites in the pathway increasing while others appear to decrease; however, the effect was not as strong or as consistent as in our current study. In addition to donating methyl groups to produce methionine, betaine plays a large role in carnitine synthesis, which is required for fatty acid oxidation, and previous research has found declines in physiological function of the carnitine shuttle pathway as organisms age (Calabrese, Giuffrida Stella, Calvani, & Butterfield, 2006; Hoffman et al., 2014). While there was no significant pathway enrichment for carnitines, we did find a significant decline of acetylcarnitine in our marmosets (Table 2). While many studies have implicated methionine metabolism and carnitine metabolism in aging, there appears to be a dearth of

research on the upstream metabolite for both, betaine, and its effects on aging and longevity. Our results suggest that, at least in the marmoset, betaine metabolism and its downstream targets may be significantly changing as the organisms age, and this effect was at least partially conserved both across both large environmental changes and a different metabolomic assay technique. Future research is needed to determine if this change is causally associated with an aging phenotype in these animals.

This study took advantage of the unique opportunity to investigate metabolites associated with sex, as our original analysis analyzed the sexes separately. As a proof of concept and quality of our data, progesterone is the most significantly different metabolite between males and females in both the AE and C18 datasets with levels, not surprisingly, higher in females than males, and steroidogenesis was the most significantly different metabolic pathways between the two sexes. However, steroidogenesis and tryptophan metabolism were the only metabolic pathways found to be overrepresented with sex differences.

One potentially interesting sex difference in metabolomic profile that will need further investigation in the future was that cortisol and cortisone levels were significantly higher in female marmosets as compared to male marmosets. While it is possible that this heightened activation of the cortisol pathway was associated with sex-specific reactions to the stress of the move, it is much more likely to reflect sex-specific metabolic differences in a catabolic hormone. Female marmosets have been found to have significantly higher baseline salivary cortisol concentrations than males (Ash, Smith, Knight, & Buchanan-Smith, 2018), and they are known to significantly differ in their response to acute stressors such as the presence of intruders (Ross & French, 2011), and reactions to social challenge (de Menezes Galvao, Ferreira, de Sousa, & Galvao-Coelho, 2016). In addition, previous work on other marmoset colonies has suggested that males are longer lived than females both in median and maximum lifespan (Nishijima et al., 2012). Therefore, the sex differences we discovered between the animal might help partially explain some of the physiological changes that lead to sex differences in longevity in the species. Overall, while we find some interesting trends in sex differences in metabolic pathways in the marmoset, we discovered more metabolites associated with age than sex in the animals, suggesting that sex differences in metabolic pathway regulation may play a secondary role to the aging process. This hypothesis was supported by the principal components analysis which suggests that more variation in the metabolome is associated with age than with sex (Figures 4 and 5).

While the marmoset has been highly touted as an ideal translational NHP model due to its short lifespan and similar morbidities and mortalities as humans (Fischer & Austad, 2011; Tardif et al., 2011), our results suggest we still have a long way to go to really understand the translational biology of marmosets. However, the problems encountered in this study are similar to issues that would occur in human populations. Environments change which can have large shifts in metabolomic profiles impacting our interpretation of links of the metabolomic profile and aging. Overall, our results suggest we still have much research to accomplish to understand the molecular changes that occur in primates and humans as they age.

Conclusions

Here, we have provided a brief analysis of the potential effects of an environment-by-age interaction on metabolome profile as well as the extent to which the metabolome is a predictor of future longevity in the common marmoset. Overall, we are able to determine that declines in tryptophan metabolism may be a biomarker of future death. Future studies are needed to test the effects of tryptophan metabolism manipulation on health and longevity in animal models. Our current study coupled with our previous results suggest betaine metabolism coupled with methionine metabolism are potentially strongly involved in the aging process in the marmoset as they are conserved regardless of environment and metabolomics assay technique. Similar to our tryptophan biomarkers, these metabolites show potential as targets of future interventions to improve health and longevity in the marmoset. Our results show that there are still significant gaps in our knowledge with regards to reproducibility and understanding the true metabolic and physiological changes that occur with age in complex organisms, especially primates. We are hopeful that future research will enable us to discover those individual metabolites and metabolic pathways that are significantly influencing the aging process, and we will continue to develop the marmoset as a translational model of human aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Three metabolites are correlated with death in a two-year follow-up period.

After metabolomic analyses, animals were followed for two years and deaths recorded. Only three metabolites were associated with death in follow-up, controlling for the effects of sex in a Cox proportional hazard model. These metabolites are all potential biomarkers of aging and longevity in the marmoset. All three are involved in tryptophan metabolism. $P < 0.0001$ for each metabolite.

Figure 2. Three representative metabolites that are significantly different between the sexes. Each metabolite is associated with sex longitudinally across animals, controlling for the effects of age. $P < 0.0001$ for each metabolite.

Figure 3. Three representative metabolites that significantly change with age.
Each metabolite is associated with age longitudinally across animals, controlling for the effects of sex. $P < 0.0001$ for each of the three metabolites.

Figure 4. PCA effects of sex for the AE (A.) and C18 (B.) column.

PCA plots allow us to determine if the entire metabolome is associated with factors of interest. Females are colored in red, males are in blue. For both columns PC1 explains 10% of the variation in the metabolome. Note that the two sexes are differentiated on both PC1 and PC2 suggesting significant differences in metabolomic profiles between females and males.

Figure 5. PCA effects of age for the AE (A.) and C18 (B.) column.

Each number within the PCA indicates the age of the animals in that circle. Note that for both columns you can see a distinct shift in metabolomic profiles from young to old animals.

Table 1.

Metabolites associated with sex

Column	Metabolite	Sex with higher values	P-value
AE			
	Cortisone	females	5.53E-08
	Cortisol	females	2.25E-06
	17-Hydroxyprogesterone	females	2.62E-06
	Kynurenine	males	6.99E-06
	Uridine	males	9.54E-06
	Phenylpyruvate	males	1.23E-05
	Benzoate	males	4.02E-05
	5-Hydroxy-L-tryptophan	males	0.00014307
	Tryptophan	males	0.000954322
	Cystine	females	0.001689339
	Uric acid	males	0.002597271
C18			
	17-Hydroxyprogesterone	females	3.28E-15
	Cortisone	females	1.74E-06
	Tryptophan	males	3.19E-06
	Cortisol	females	3.58E-06
	3-Indoleacrylic acid	males	8.56E-06
	Phenylpyruvate	males	3.47E-05
	Uridine	males	0.000143934
	Thiamine	males	0.00041762
	Uric acid	males	0.00090037
	Sphingosine	males	0.00305602
	Palmitic acid	males	0.00409416
	Pirimiticarb	males	0.004224619
	Creatinine	males	0.004624424
	Niacin	males	0.005611201
	Pantothenic acid	males	0.008077247

Table 2.

Metabolites associated with age

Column	Metabolite	Age effect	P-value
AE	Acetylcarnitine	decrease	2.77E-09
	Asparagine	decrease	8.24E-09
	Betaine	decrease	3.59E-08
	Valine	decrease	3.59E-08
	Lysine	decrease	9.70E-07
	Cystine	increase	3.84E-06
	Threonine	decrease	3.69E-05
	Hypoxanthine	decrease	8.00E-05
	Benserazide	decrease	0.000135271
	Pyro-L-glutaminy-L-glutamine	decrease	0.000135271
	Glycero-3-Phosphocholine	decrease	0.000135271
	Sphingosine	decrease	0.000589739
	Citrate	decrease	0.005840078
	Acetylcarnitine	decrease	0.005965223
	Riboflavin	increase	0.006026805
	Methionine	decrease	0.008370098
	Taurocholic.acid	increase	0.009229463
	N,N -Dimethyllycine	increase	0.01132116
	2-Aminobutyrate	increase	0.01132116
	Aspartate	decrease	0.011547102
C18	Cystine	increase	0.011582895
	Methylinicotinic acid	decrease	0.020550319
	Kynurenine	increase	0.021467829
	Pyridoxamine	increase	0.021901727
	Methionine	decrease	1.12E-05
	Betaine	decrease	1.34E-05
	Valine	decrease	1.34E-05
	Threonine	decrease	3.76E-05
	Sphinganine	decrease	5.57E-05
	Hypoanthine	decrease	7.77E-05
	3-indolepropionic acid	increase	9.21E-05
	Lysine	decrease	0.000225906
	Sphingosine	decrease	0.000423997
	Methyl 3 phenylpropanoate	increase	0.003843728
	Dibutyl phthalate	increase	0.005998035
	Riboflavin	increase	0.006228517
	Indole-3-pyruvic acid	increase	0.006365443
	Anthine	decrease	0.00680882

Column	Metabolite	Age effect	P-value
	Methyl-3-phenylpropanoate	increase	0.007614055
	Indolelactic acid	increase	0.010818593
	Cinnamoylglycine	increase	0.010818593
	Cystine	increase	0.016565938
	2-Aminobutyrate	increase	0.024276321
	N,N -Dimethyllysine	increase	0.024276321
	Caffeine	increase	0.024785932
	Citrulline	increase	0.024845609

Table 3.
Metabolic pathways significantly associated with either age or sex.

Metabolic pathways that had a significant association with age or sex based on our linear model when run through enrichment analyses. Only those with $p < 0.05$ are shown.

Factor	Column	Metabolic pathway	p-value
Sex	AE	Steroidogenesis	0.00105
		Tryptophan Metabolism	0.0288
	C18	Steroidogenesis	0.0182
Age	AE	Betaine Metabolism	0.00678
		Glycine and Serine Metabolism	0.0241
		Methionine Metabolism	0.0478
	C18	Betaine Metabolism	0.0078
		Glycine and Serine Metabolism	0.0285

Table 4.
Comparison of metabolic pathways associated with age in current and previous study.

Our previous study found more pathways associated with age, but these were the pathways that appeared in at least 3 of the previous analyses.

Current	Previous
Betaine Metabolism	Tryptophan metabolism
Glycine and Serine Metabolism	Androgen and estrogen metabolism
Methionine Metabolism	Purine metabolism
	TCA cycle metabolites
	Porphyryn metabolism
	Cholesterol biosynthesis
	Xenobiotic metabolism
	Dynorphin metabolism
	Tyrosine metabolism

Table 5.

Metabolites that changed significantly between the first (June/July 2015) timepoint and 6 months later (December 2015/January 2016) after controlling for the effects of age.

AE metabolites	C18 metabolites
2-aminobutyrate	5-Ooproline
N,N - dimethyllycine	Pyroglutamic acid
Betaine	Lysine
Valine	Allantoin
Lysine	Taurolithocholate
Histidine	
Allantoin	
1-methyl l-histidine	
Daidzein	
17-hydroxyprogesterone	
Palmitoylcarnitine	
Taurolithocholate	
Taurocholic acid	