Short Report

Plasma Amino Acid Profiles in Healthy East Asian Subpopulations Living in Japan

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Objectives: Profiles of plasma free amino acids (PFAAs) have been utilized as biomarkers to detect various diseases. However, few studies have investigated whether ethnicity or specific subpopulations within East Asia influence PFAA concentrations.

Methods: A total of 95 healthy volunteers living in Japan, including 31 Japanese individuals, 36 Korean individuals and 28 Chinese individuals, were enrolled. Participants’ PFAA levels were measured by high-performance liquid chromatography mass spectrometry, and the effects of factors such as sex, age, body mass index (BMI) and subpopulation on PFAA profiles were analyzed.

Results: With the exception of glutamine and α-aminobutyric acid, there were no significant differences among the three examined subpopulations with respect to either the means or the distributions of PFAA concentrations. A multiple regression analysis revealed that most of the PFAA concentrations were significantly related to sex. Ornithine concentrations, glutamate concentrations, and glutamine and α-aminobutyric acid concentrations were significantly associated with age, BMI, and Chinese subpopulation, respectively.

Conclusion: The study results indicate that the contributions of subpopulation within East Asia to PFAA profiles are small, particularly relative to the contributions provided by sex. Am. J. Hum. Biol. 28:236–239, 2016. © 2015 The Authors American Journal of Human Biology Published by Wiley Periodicals, Inc.

Amino acids (AAs) play important roles as both basic metabolites and physiological regulators. Although AA metabolism is strictly regulated in healthy individuals, reports have indicated that certain diseases influence plasma free amino acid (PFAA) profiles (Holm et al., 1999; Hong et al., 1998; Miyagi et al., 2011; Felig et al., 1970). Based on these PFAA characteristics, “AminoIndex Technology,” which can evaluate specific health conditions and disease possibilities by analyzing PFAA status, has recently been described (Miyagi et al., 2011; Okamoto 2011).

PFAA homeostasis is primarily maintained by the balance among the endogenous synthesis of nonessential AAs, the degradation of AAs, and the synthesis and breakdown of proteins. Because these reactions are performed by various enzymes, PFAA profiles may be influenced by genetic variations in enzymes involved in AA metabolism. In fact, a prior genome-wide association study (GWAS) of the human metabolome indicated that certain AAs are associated with genetic loci encoding either AA transporters or enzymes involved in serine (Ser) biosynthesis (Rhee et al., 2013).

It has been reported that the frequency of single nucleotide polymorphisms in certain enzymes involved in drug metabolism is associated with ethnicity (Man et al., 2010). Genetic variations among three major East Asian subpopulations (Japanese, Korean and Chinese) have also been reported with respect to enzymes, including the CYP19*3 variant (Man et al., 2010), although variations are much smaller than between Europeans and Africans. However, there have been no reports regarding the genetic heterogeneity of enzymes involved in AA metabolism, and few studies have described the relationship between PFAA profiles and either ethnicity or subpopulations.

In this study, we compared the PFAA concentrations of healthy Japanese, Korean, and Chinese individuals living in Japan and investigated how subpopulation, as well as age, sex, and body mass index (BMI), affected PFAA profiles.

MATERIALS AND METHODS

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki, and experimental protocols were approved by the ethics committee of Ajinomoto Co., Inc. All subjects provided written informed consent for their inclusion before participating in the study. All data were analyzed anonymously throughout the study.

Enrollment

A total of 95 healthy volunteers, including 31 Japanese individuals, 36 Korean individuals and 28 Chinese individuals (86 percent of whom were Han Chinese) were recruited for this study. Each participant’s subpopulation was determined using a questionnaire that verified the Japanese, Korean or Chinese backgrounds of both of the participant’s parents. All subjects had been living in Japan for at least one year. Information about diet was

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**TABLE 1. Plasma AA concentrations in three major East Asian subpopulations**

| Amino Acid | Japanese | Korean | Chinese | P-value by ANOVA | P-value by Levene's test |
|------------|----------|--------|---------|-----------------|-------------------------|
| **Val**    | 213.6 ± 59.5 | 207.4 ± 40.4 | 224.7 ± 38.5 | 0.3419 | 0.0711 |
| **Ile**    | 61.1 ± 19.4 | 61.7 ± 14.9 | 63.6 ± 15.2 | 0.8355 | 0.3469 |
| **Leu**    | 118.6 ± 34.6 | 116.9 ± 27.8 | 127.2 ± 24.6 | 0.3510 | 0.4097 |
| **His**    | 85.8 ± 10.6 | 83.2 ± 9.2 | 82.4 ± 9.2 | 0.3669 | 0.8350 |
| **Phe**    | 57.5 ± 10.1 | 55.4 ± 9.6 | 59.0 ± 9.2 | 0.3237 | 0.6421 |
| **Trp**    | 54.9 ± 11.8 | 56.8 ± 9.8 | 55.3 ± 9.6 | 0.7390 | 0.4979 |
| **Met**    | 26.8 ± 5.8 | 27.7 ± 5.9 | 25.0 ± 6.5 | 0.1977 | 0.8616 |
| **Thr**    | 133.7 ± 33.1 | 138.0 ± 29.4 | 119.2 ± 32.4 | 0.0558 | 0.5712 |
| **Lys**    | 173.1 ± 31.4 | 181.9 ± 28.4 | 181.2 ± 38.1 | 0.4916 | 0.6991 |

**Nonessential amino acids (μmol/L)**

| Amino Acid | Japanese | Korean | Chinese | P-value by ANOVA | P-value by Levene's test |
|------------|----------|--------|---------|-----------------|-------------------------|
| **Ser**    | 113.3 ± 23.8 | 124.3 ± 20.8 | 116.2 ± 22.8 | 0.1198 | 0.6604 |
| **Gly**    | 246.4 ± 58.9 | 240.1 ± 53.4 | 229.0 ± 40.4 | 0.4311 | 0.3460 |
| **Ala**    | 384.6 ± 97.1 | 370.7 ± 94.0 | 372.1 ± 108.9 | 0.8292 | 0.7391 |
| **Tyr**    | 59.8 ± 16.6 | 59.4 ± 13.6 | 62.5 ± 13.6 | 0.6642 | 0.7540 |
| **Glut**   | 26.3 ± 12.0 | 24.1 ± 11.9 | 32.7 ± 20.1 | 0.0699 | 0.0351 |
| **Pro**    | 146.2 ± 48.3 | 159.9 ± 49.7 | 150.5 ± 60.1 | 0.5573 | 0.6801 |
| **Gln**    | 617.9 ± 65.1 | 578.2 ± 62.96 | 554.0 ± 70.7 | 0.0013* | 0.6794 |
| **Cit**    | 31.1 ± 6.2 | 29.6 ± 7.0 | 30.2 ± 7.3 | 0.6699 | 0.5085 |
| **Arg**    | 96.1 ± 20.6 | 101.0 ± 16.8 | 101.9 ± 25.9 | 0.5068 | 0.1013 |
| **Orn**    | 47.9 ± 15.2 | 48.6 ± 10.6 | 50.0 ± 14.9 | 0.8256 | 0.1480 |
| **Asn**    | 48.8 ± 5.6 | 48.2 ± 7.8 | 48.6 ± 9.7 | 0.9534 | 0.0591 |
| **α-ABA**  | 14.5 ± 3.6 | 17.6 ± 5.43 | 20.4 ± 6.5 | 0.0003* | 0.0355 |

Data are presented as means ± SD (range). The chi-squared test was used to test for differences in sex distributions. Levene's test was performed to assess the homogeneity of variances. Significant differences among the three groups were determined by one-way ANOVA followed by Tukey's test and are presented in bold. Different characters indicate significant differences (P<0.002). *A significance level of P < 0.002 was established (a Bonferroni-corrected threshold).

Based on a 3-month recall interview. The frequency of food intake from their home countries (e.g., Korean food for Korean and Chinese food for Chinese) was investigated by choosing from “daily,” “4–6 times a week,” “1–3 times a week,” “2–3 times month,” once a month or less often,” and “never.” Fifty-eight percent of Korean and 50% of Chinese consume their local food more than 4–6 times a week. The exclusion criteria of taking medication, pregnancy, mental disorders and cancer were applied at the beginning of the study.

**Quantification of plasma AA concentrations**

Blood samples (5 ml) were collected from forearm veins in the morning following overnight fasting into tubes containing ethylenediaminetetraacetic acid. These samples were immediately placed on ice. Plasma was prepared via centrifugation at 2,010 g and 4°C for 15 min and stored at −80°C until needed for analysis. Subsequently, plasma samples were deproteinized with acetonitrile at a final concentration of 80%. The samples were then subjected to precolumn derivatization followed by high-performance liquid chromatography (HPLC)-electrospray ionization (ESI)-mass spectrometry (MS) for AA quantification, which was performed as previously described (Shimbo et al., 2009a; Shimbo et al., 2009b; Yoshida et al., 2015). The following 21 AAs were quantified: alanine (Ala), alpha-aminobutyric acid (α-ABA), arginine (Arg), asparagine (Asn), citrulline (Cit), glutamate (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val).

**Statistical analysis**

Study data are presented as means ± standard deviation (SD). Statistical and multivariate analyses were
performed using the JMP 9.0.0 program (SAS Institute Inc., NC). To assess differences among the three subpopulations, Tukey’s test was performed following a one-way analysis of variance (ANOVA). Levene’s test was performed to assess the homogeneity of variances. Multiple linear regression (MLR) was used to evaluate the contributions of background factors (sex, age, BMI or subpopulation) to PFAA concentrations. P < 0.05 was established as the level of significance following Bonferroni multiple comparison tests.

RESULTS

Table 1 presents the characteristics of the Japanese, Korean, and Chinese subjects enrolled in this study. The sex ratios of subjects in these three subpopulations did not differ. The mean age of the Korean participants was significantly lower than the mean age of the Japanese and Chinese participants. The mean BMI of the Chinese subjects was significantly lower than the mean BMI of the Japanese and Korean subjects. There was no significant difference in sex or BMI between the Japanese and Korean subpopulations. Further investigations are needed to confirm whether PFAA profile changes associated with residence and dietary habits on PFAA profiles (e.g., Korean and Chinese subjects living in Japan for less than one year).

MLR analysis demonstrated that PFAA profiles, particularly with respect to essential AAs, were more strongly related to sex than to age, BMI or subpopulation. Our previous study also found that sex is a more influential factor for PFAA profiles than age or BMI (Yamamoto et al., 2015). In addition, reports have indicated that PFAA levels are strongly correlated with visceral fat area and insulin-related parameters but not with subcutaneous fat area (Nakamura et al., 2014; Yamakado et al., 2012). Furthermore, the ingestion of a low-protein diet leads to decreased PFAA levels (Fujita et al., 1978). These results suggest that PFAA levels may be more strongly influenced by sex, physiological conditions or dietary habits than by genetic factors.

In conclusion, we have demonstrated that there is little to no difference among the PFAA profiles of Japanese, Korean and Chinese individuals living in Japan, although this is a preliminary study and needs confirmation with larger numbers of subjects. These results suggest that it may be feasible to clinically utilize PFAA profiles for the detection of diseases such as cancer or metabolic syndrome. Further investigations are needed to confirm whether PFAA profile changes associated with these diseases and physiological conditions are similar among these subpopulations.
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