

Amino Acid Profiles in Human Tear Fluids Analyzed by High-Performance Liquid Chromatography and Electrospray Ionization Tandem Mass Spectrometry

MINA NAKATSUKASA, CHIE SOTOZONO, KAZUTAKA SHIMBO, NOBUKAZU ONO, HIROSHI MIYANO, AKIRA OKANO, JUNJI HAMURO, AND SHIGERU KINOSHITA

- **PURPOSE:** To identify the 23 amino acid profiles in human tear fluids, and to evaluate whether the ocular disease conditions reflect the amino acid profiles.
- **DESIGN:** Laboratory investigation.
- **METHODS:** We evaluated the concentrations and relative composition of 23 amino acids in tear fluids obtained from 31 healthy volunteers using reversed-phase high-performance liquid chromatography and electrospray ionization tandem mass spectrometry, and compared them with those in plasma and aqueous humor. We also evaluated the tear-fluid amino acid profiles from 33 affected subjects.
- **RESULTS:** The amino acid profiles of the basal tear and reflex tear were found to be similar, and 4 distinct groups of healthy volunteers (male, female, young, and elderly) showed similar profiles. Absolute concentrations of taurine (Tau) and L-glutamine were significantly dominant in these tear fluids. The relative compositions of Tau, L-glutamic acid, L-arginine (Arg), and citrulline in the tear fluid were significantly higher than those in the plasma and aqueous humor. Analysis of the hierarchical clustering of the amino acid profiles clearly distinguished severe ocular surface diseases from non-ocular surface diseases. The relative compositions of Tau, L-methionine, and Arg decreased in severe ocular surface disease subjects compared with non-ocular surface disease subjects.
- **CONCLUSIONS:** Tear-fluid amino acid profiles differ from those in plasma and aqueous humor. Steady-state tear-fluid amino acid profiles might reflect ocular-surface homeostasis and the observed changes of amino acids might have a close relation with the disease conditions on the ocular surface. (Am J Ophthalmol 2011;151:799–808. © 2011 by Elsevier Inc. All rights reserved.)

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From the Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan (M.N., C.S., J.H., S.H.); the Life Science Research Laboratories, AJINOMOTO Co, Inc, Kawasaki, Japan (K.S., H.M.); and the Pharmaceutical Research Laboratories, AJINOMOTO Co, Inc, Kawasaki, Japan (N.O., A.O.).

Inquiries to Shigeru Kinoshita, Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Hirokoji-agaru, Kawaramachi-dori, Kamigyo-ku, Kyoto 602-0841, Japan; e-mail: shigeruk@koto.kpu-m.ac.jp

METABOLISM CAN BE VIEWED AS A NETWORK that can adapt to various nutritional conditions and that may become disturbed during disease and physiologic insults. Specific variations in amino acid profiles in blood have been reported in the context of liver failure,¹ renal failure,² cancer,³ diabetes,⁴ and so on. Conventionally, amino acid has long been considered a source of protein synthesis in the nutritional term, and the existence of a free amino acid supply to the tissues plays a pivotal role in maintaining organ and body protein homeostasis.⁵ However, besides their role as substrates for protein synthesis, amino acids have multiple and critical functions, not only in maintaining baseline steady-state homeostasis, but also in the pathophysiology of diverse human disorders. It is now widely accepted that changes in amino acid availability have profound effects on many aspects of cellular functions, including the regulation of cell signaling, gene expression, and the transport of amino acids themselves.^{6–8} For example, the pathophysiologic relevance of L-glutamine (Gln, GluNH₂), L-arginine (Arg), and L-leucine (Leu) have been implicated in severely traumatized patients,⁹ in the inflammatory response,¹⁰ and in activating the mammalian target of rapamycin (mTOR).^{11,12}

Tear fluids provide oxygen and other nutrients, as well as chemical mediators including antimicrobial and immunologic mediators. Considering the relevance of amino acids and glucose in the homeostatic metabolism of tissues, the profound understanding of the function of amino acid in tear fluids is as crucial as that of chemical mediators. Previously, a few reports have presented contradicting results on the amount of a limited number of amino acids in human tears.^{13,14} The existence of a significantly higher concentration of L-valine (Val), L-isoleucine (Ile), and L-histidine (His) has been reported, and with the exception of L-aspartate (Asp), L-glutamic acid (Glu), and taurine (Tau), the quantities found in tears were at a comparable level with those found in plasma.^{13,14}

Today, amino acid profiles for biological specimens are commonly analyzed by ion-exchange chromatography, a method in which amino acids and related compounds can be measured.^{12,15} Recently, a new method for rapidly analyzing amino acids was developed that involves derivatization with a novel reagent, followed by reversed-phase

high-performance liquid chromatography and electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS).¹⁶ Now, more than 100 different analytes with amino groups can be measured within 10 minutes by the combination of the precolumn derivatization and HPLC/ESI-MS/MS techniques. This method represents an alternative to traditional amino acid analysis techniques. The aim of this study was to reveal and describe the amino acid profiles in human tear fluids since this new method now makes it possible to analyze samples in trace amounts (below 0.5 μ L) and with a superior reproducibility.¹⁶ In addition, the possible physiological and pharmacologic function of amino acids will be discussed.

METHODS

• **NORMAL VOLUNTEER SUBJECTS:** Enrolled in this study were normal volunteer subjects with no corneal-, conjunctival-, or lacrimal-system abnormalities as assessed by slit-lamp examination. Free amino acids were evaluated in blood samples obtained from 11 healthy young male (mean age: 22.9 ± 1.4 years), 6 healthy young female (mean age: 21.0 ± 0.7 years), 6 healthy elderly male (mean age: 69.5 ± 3.8 years), and 11 healthy elderly female (mean age: 71.5 ± 4.0 years) volunteer subjects. Basal tear samples were collected from the young and elderly subjects ($n = 31$; young male: 10; young female: 6; elderly male: 6; elderly female: 9) and reflex tear samples were also collected from the young subjects ($n = 16$; male: 10; female: 6). Basal and reflex tear fluid samples (0.5–1.0 μ L) were collected from the inferior tear meniscus of each subject using a microcapillary tube. Reflex-tear stimulation was initiated by inserting an applicator into the nose of each subject. Aqueous humor samples were obtained from the elderly subjects ($n = 16$; male: 6; female: 10) through the use of a 1-mL syringe with a 30-gauge needle prior to cataract surgery, and all of the obtained samples were free of contamination by blood fluids.

• **PREPARATION OF TEAR FLUID AND AQUEOUS HUMOR SAMPLES:** Each tear sample was transferred into a 0.5-mL sterile microfuge tube and then centrifuged. Supernatants were stored at -70°C until assaying for the amino acid levels. After thawing, 0.5 μ L of each tear sample was diluted with 4.5 μ L of sterile purified water, and then extracted by the addition of 20 μ L of acetonitrile and mixing with a vortex mixer. The samples were centrifuged at 10 000 rpm for 1 minute, and the supernatants were then analyzed. A quantity of 1.0 μ L of aqueous humor was used for each sample, with the same dilution as that of the tear fluid.

• **PATIENTS WITH OCULAR SURFACE DISEASE AND CORNEAL OR SCLERAL DISEASE:** Tear samples were collected from 33 affected subjects composed of 18 subjects

with severe ocular surface diseases and 15 subjects with corneal or scleral diseases. Severe ocular surface diseases included Stevens-Johnson syndrome (SJS) ($n = 10$), chemical injury ($n = 4$), thermal burn ($n = 2$), and stem cell deficiency from an unknown cause ($n = 2$). The corneal or scleral diseases of the other 15 subjects included granular dystrophy ($n = 2$), band-shaped keratopathy ($n = 2$), keratoconus ($n = 2$), bullous keratopathy ($n = 2$), lattice dystrophy ($n = 1$), corneal erosion ($n = 1$), postcorneal infection ($n = 2$), necrotizing keratitis ($n = 1$), and scleritis ($n = 2$). Those 15 subjects were all classified as non-ocular surface disease.

• **BIOCHEMICAL ASSAYS OF TEAR FLUID AMINO ACID AND AQUEOUS HUMOR AMINO ACID:** For measurement of the amino acid concentration in the tear fluid and aqueous humor samples, we adopted precolumn derivatization with AccQ-Tag (Waters Corporation, Milford, Massachusetts, USA) to increase the ionization efficiency of the adducts before being analyzed by the multiple-reaction monitoring mode of reversed-phase HPLC HP1100 series (Agilent Technologies, Inc, Palo Alto, California, USA) and triple quadrupole tandem mass spectrometry (API4000 LC/MS/MS system; Applied Biosystems, Inc, Foster City, California, USA).^{16–18} For derivatization with 20 μ L of AccQ-Tag reagent, 20 μ L of a deproteinized tear sample was added to 60 μ L of 0.2-M borate buffer (pH 8.8) and then heated at 55°C for 10 minutes. The reaction mixture was diluted with 100 μ L of 0.2% acetic acid and 5 μ L of the mixture was then injected onto the HPLC column (L-Column, 50 mm $\times$ 2.0 mm, 3- μ m particles; Chemicals Evaluation and Research Institute, Tokyo, Japan) prior to MS detection at a flow rate of 0.25 mL/min.

• **BIOCHEMICAL ASSAYS OF PLASMA AMINO ACID:** The plasma was separated and deproteinized in a final concentration of 3% sulfosalicylic acid. All samples were stored at -70°C until measurement using HPLC (SRL Inc, Tokyo, Japan). The basic amino acid and related molecules (23 compounds) were measured and used in the analysis. Those compounds are as follows: L-tyrosine (Tyr), Val, Leu, L-methionine (Met), Ile, Gln, L-serine (Ser), L-lysine (Lys), L-asparagine (Asn, AspNH₂), L-threonine (Thr), L-alanine (Ala), Glu, His, L-ornithine (Orn), L-cystine (Cys₂), L-proline (Pro), L-tryptophan (Trp), Arg, Tau, glycine (Gly), citrulline (Cit), Asp, and L-phenylalanine (Phe).

• **STATISTICAL ANALYSIS:** Statistical analysis was performed using SPSS statistical analysis software (SPSS Inc, Chicago, Illinois, USA). Hierarchical clustering analysis was performed using JMP7.0 software (SAS Institute, Inc, Cary, North Carolina, USA). The correlations among 23 amino acid concentrations as well as

TABLE 1. Concentration (Mean $\pm$ SEM, μ M) of Each Amino Acid in the Basal Tears, Reflex Tears, Aqueous Humor, and Plasma of Normal Subjects

	Basal Tear (n = 31)	Reflex Tear (n = 16)	Aqueous Humor (n = 16)	Plasma (n = 34)
Gly	10.7 $\pm$ 2.8	14.4 $\pm$ 7.1	0.4 $\pm$ 0.1	231.3 $\pm$ 11.1
Ala	23.8 $\pm$ 4.8	19.5 $\pm$ 8.5	14.4 $\pm$ 2.7	385.5 $\pm$ 14.5
Val	3.5 $\pm$ 0.9	3.8 $\pm$ 1.4	6.7 $\pm$ 1.1	244.7 $\pm$ 8.5
Leu	10.1 $\pm$ 1.9	6.4 $\pm$ 1.9	3.3 $\pm$ 0.6	125.4 $\pm$ 6.1
Ile	1.1 $\pm$ 0.3	1.1 $\pm$ 0.5	1.5 $\pm$ 0.3	68.7 $\pm$ 3.6
Ser	14.4 $\pm$ 4.2	26.4 $\pm$ 13.0	3.9 $\pm$ 0.8	113.6 $\pm$ 3.3
Thr	5.4 $\pm$ 1.2	8.4 $\pm$ 3.6	3.5 $\pm$ 0.7	127.6 $\pm$ 3.8
Met	2.4 $\pm$ 0.1	2.2 $\pm$ 0.1	0.9 $\pm$ 0.2	28.1 $\pm$ 1.3
AspNH ₂ (Asn)	1.4 $\pm$ 0.3	1.5 $\pm$ 0.7	1.0 $\pm$ 0.2	49.6 $\pm$ 1.5
GluNH ₂ (Gln)	42.1 $\pm$ 6.8	25.4 $\pm$ 6.3	28.7 $\pm$ 5.2	589.5 $\pm$ 11.0
Pro	8.4 $\pm$ 1.4	6.7 $\pm$ 1.5	0.5 $\pm$ 0.1	180.2 $\pm$ 8.5
Phe	10.7 $\pm$ 1.9	5.7 $\pm$ 1.5	2.8 $\pm$ 0.5	63.7 $\pm$ 2.0
Tyr	1.3 $\pm$ 0.5	1.1 $\pm$ 0.5	2.8 $\pm$ 0.6	73.5 $\pm$ 3.8
Trp	7.1 $\pm$ 0.7	6.6 $\pm$ 1.0	0.9 $\pm$ 0.1	52.9 $\pm$ 1.8
Asp	13.2 $\pm$ 4.5	18.2 $\pm$ 11.6	0.0 $\pm$ 0.0	1.8 $\pm$ 0.2
Glu	30.2 $\pm$ 4.8	20.9 $\pm$ 4.8	0.1 $\pm$ 0.0	42.3 $\pm$ 2.8
Lys	5.7 $\pm$ 1.2	3.5 $\pm$ 1.2	3.1 $\pm$ 0.6	195.7 $\pm$ 7.2
Arg	18.7 $\pm$ 1.6	14.4 $\pm$ 2.5	2.5 $\pm$ 0.5	82.7 $\pm$ 3.0
His	1.9 $\pm$ 0.7	3.2 $\pm$ 1.9	1.5 $\pm$ 0.3	83.7 $\pm$ 2.0
Cit	10.1 $\pm$ 0.8	8.2 $\pm$ 1.5	1.2 $\pm$ 0.2	35.1 $\pm$ 1.7
Orn	3.3 $\pm$ 1.1	7.1 $\pm$ 3.0	0.4 $\pm$ 0.1	79.3 $\pm$ 4.9
Cys ₂	1.0 $\pm$ 0.2	0.6 $\pm$ 0.2	0.3 $\pm$ 0.1	49.0 $\pm$ 2.4
Tau	195.1 $\pm$ 26.9	100.1 $\pm$ 18.7	1.2 $\pm$ 0.3	57.7 $\pm$ 2.0

Ala = L-alanine; Arg = L-arginine; Asp = L-aspartate; AspNH₂ (Asn) = L-asparagine; Cit = citrulline; Cys₂ = L-cystine; Glu = L-glutamic acid; GluNH₂ (Gln) = L-glutamine; Gly = glycine; His = L-histidine; Ile = L-isoleucine; Leu = L-leucine; Lys = L-lysine; Met = L-methionine; Orn = L-ornithine; Phe = L-phenylalanine; Pro = L-proline; Ser = L-serine; Tau = taurine; Thr = L-threonine; Trp = L-tryptophan; Tyr = L-tyrosine; Val = L-valine.

RESULTS

• **COMPARISON OF AMINO ACID PROFILES AMONG TEAR FLUID, AQUEOUS HUMOR, AND PLASMA SAMPLES OF NORMAL SUBJECTS:** Amino acid concentrations in a trace amount of tear fluids of 0.5 to 1.0 μ L were able to be measured reproducibly by HPLC/ESI-MS/MS. The reproducibility of the measurements was confirmed in separate experiments using normal tear fluids. The Table shows the concentrations of each amino acid in the basal tear, the reflex tear, the aqueous humor, and the plasma of normal subjects. A significant difference of amino acid profiles between the tear fluid, the aqueous humor, and the plasma samples was confirmed. The concentration of total amino acids in the tear fluid samples (means $\pm$ SEM: 382.1 $\pm$ 42.6 μ M) was lower than that in the plasma samples (2961.6 $\pm$ 63.1 μ M) but higher than that in the aqueous humor samples (81.6 $\pm$ 15.2 μ M). Ala, Val, and Gln were dominant components in the plasma samples (more than 240 μ M). Gly, Pro, and Lys were also major components in the plasma samples.

among plasma and/or ophthalmic fluid (basal tear, reflex tear, and aqueous humor) samples were clustered using the Ward method. For the red>gray>blue color system, the depth in the red color or blue color reflected the different degree of values above or below the mean (gray color), respectively. Graphic presentations of box plots were generated using R (R Foundation for Statistical Computing, Vienna, Austria). In each box plot, the top and bottom of the boxes were the first and third quartiles, respectively, and the length of the box represented the inter-quartile range within 50% of the values that were included. The horizontal line within each box represented the median, the vertical lines showed the largest/smallest observation that fell within a distance of 1.5 times the box size from the nearest quartile in the box, and the additional points were considered “extreme” values and are shown separately.

These presentations and analyses were performed based on each of the absolute amino acid concentrations (μ M) or relative amino acid composition (percentage), calculated by each amino acid concentration/total 23 amino acid concentrations.

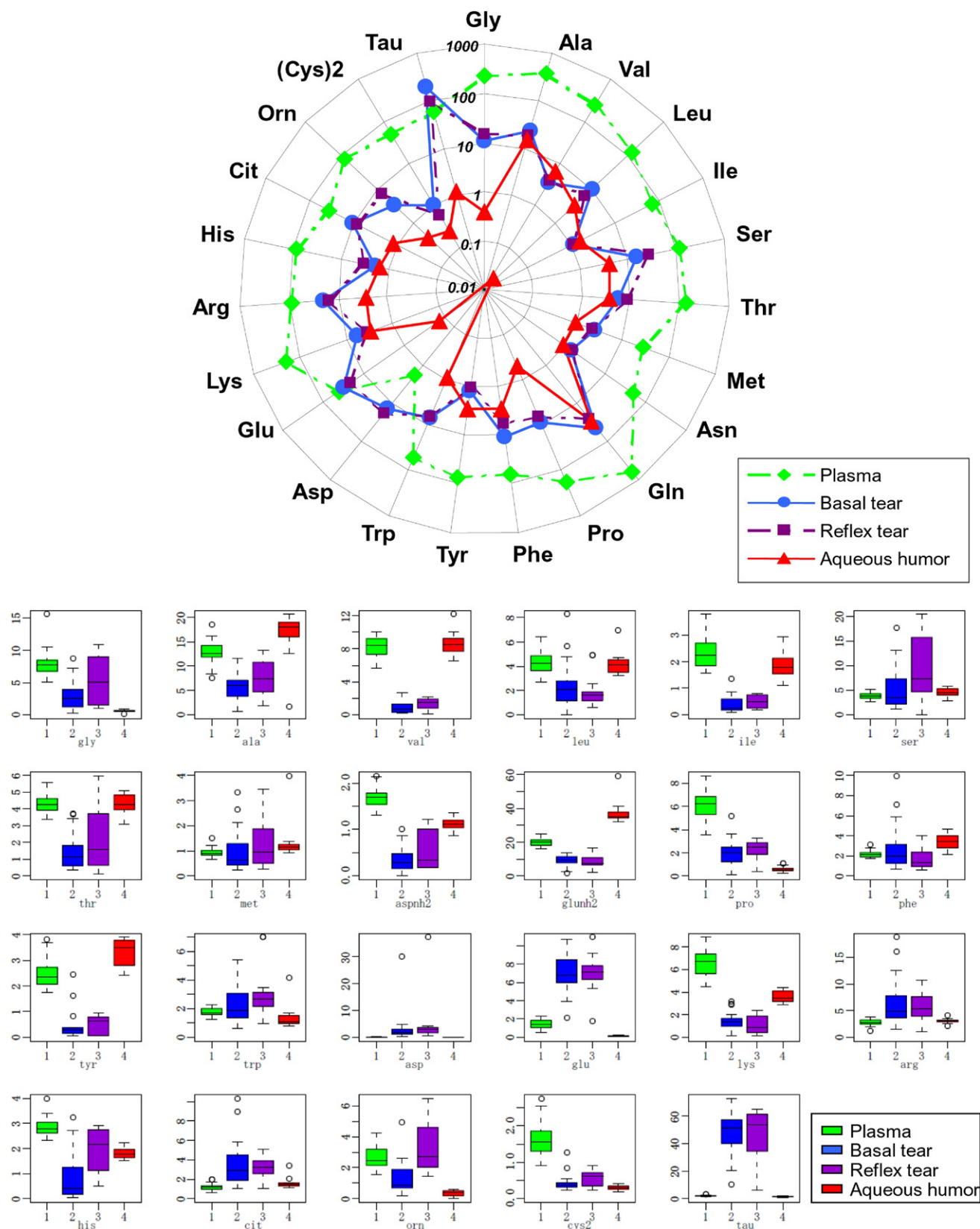


FIGURE 1. The differences of amino acid profiles of normal subjects between basal tear, reflex tear, and aqueous humor samples, compared with that of plasma samples. (Top) Mean values of 23 amino acid concentrations (μM) of basal tear ($n = 31$) (blue), reflex tear ($n = 16$) (purple), aqueous humor ($n = 16$) (red), and plasma ($n = 34$) (green) samples from normal subjects are shown in

In the tear fluids, Tau, Glu, and Gln were dominant components (more than 25 μM). Arg and Cit were of a higher concentration than other amino acid concentrations, whereas Val, Ile, Met, Asn, Tyr, His, and Orn were of a lower concentration (below 5 μM) (Table, and Figure 1, Top). Notably, the concentrations of Tau and Glu were significantly higher than (3.4 times higher) or comparable with (0.7 times higher) those in plasma samples. Asp also was of a higher value than that in plasma samples. The amino acid concentrations and profiles of the basal tear fluid and reflex tear fluid were found to be similar.

Since it has been shown that the metabolomic profiling of amino acid can be helpful in revealing specific physiological conditions or states, and that the ratios between some specific amino acids can be useful in diagnosing them,¹⁹ we performed some analyses based on not only amino acid concentrations but also relative amino acid composition (Figure 1, Bottom). The composition of amino acid in aqueous humors was rather analogous to that in plasmas, although the levels of Gly, Pro, and Orn were low. In the tear fluid samples, Tau and Glu were of a higher relative amino acid composition (25.0 times higher and 4.9 times higher, respectively; $P < .001$) (Tukey test) than those in the plasma samples, yet the aqueous humor samples were not (Tau: 0.73 times higher, Glu: 0.09 times higher). The relative compositions of Arg and Cit in the tear fluid samples were smaller but statistically significantly higher than those in the plasma and aqueous humor samples. There was no significant difference between the basal tear fluid and reflex tear fluid in 23 relative amino acid compositions (Tukey test).

In an aim to build up a simple visual presentation, the correlations among 23 amino acid concentrations and the relative amino acid composition of 31 basal tear fluid, 16 reflex tear fluid, 16 aqueous humor, and 34 plasma samples were clustered (Figure 2). Plasma samples were clearly clustered at the highest position, followed by tear fluid samples and then aqueous humor samples.

• **COMPARISON OF TEAR-FLUID AMINO ACID PROFILES AMONG YOUNG AND ELDERLY VOLUNTEERS:** The change of amino acid concentration attributable to aging is known to exist in other body fluids.²⁰ In this study, a preliminary analysis was carried out on the basal tear fluid and plasma samples of adults that differed by both age and sex. The mean values of 23 amino acids in relation to concentration and composition that were obtained from young male ($n = 10$), young female ($n = 6$), elderly male ($n = 6$), and elderly female ($n = 9$) normal subjects were examined. The Tukey test showed

that the differences were not statistically significant. Hierarchical clustering charts of both the concentration and composition did not exhibit any distinct clustering trends among these 4 groups (data not shown).

• **DISTINCTIVE CHANGE OF AMINO ACID PROFILES IN TEAR FLUIDS FROM DISEASED EYES:** The difference of amino acid concentrations and profiles among tear fluids from individuals of severe ocular surface disease and non-ocular surface disease was investigated. Means of the 23 amino acid concentrations (μM) from severe ocular surface disease ($n = 18$) and non-ocular surface disease ($n = 15$) were shown in a radar chart (Figure 3, Top). The concentrations of amino acid were highly elevated in tear fluids from severe ocular surface disease subjects compared with those from non-ocular surface disease subjects. Four amino acids (Val, Ile, Tyr, and Glu) showed a significant P value of less than .01, and 15 amino acids showed a P value of less than .05 (Student t test). Of important note is the fact that the changes were not restricted to the concentration, but also extended to the amino acid profile, namely composition.

Next, the distribution pattern of the amino acid composition in tear fluids from the severe ocular surface disease subjects was compared with those from the non-ocular surface disease subjects. Amino acid composition (%) of tear fluids from 15 non-ocular surface disease subjects (column 1, left) and 18 severe ocular surface disease subjects (column 2, right) are shown in the box plots (Figure 3, Bottom). The significant changes were the decrease in Arg, Met, and Tau and the increase in Orn, Lys, and Thr in the severe ocular surface disease subjects.

Analysis of the hierarchical clustering of the amino acid profiles of tear fluids from severe ocular surface disease subjects and non-ocular surface disease subjects indicated clear clustering of 12 of the 18 specimens from individuals with severe ocular surface disease when classified on the basis of amino acid composition (Figure 4). Interestingly, the composition-based clustering exhibited 5 distinctive clusters of amino acid, namely Tau/Met/Arg, Asn/Tyr/Val/Ile/Lys, Glu/Gln, Cit/Leu/Phe/Trp/Pro, and Asp/His/Thr/Orn/Ser/Gly/Ala. Decreased amino acid, Tau, Met, and Arg were included in 1 cluster, which suggests a correlation of the metabolism and/or transport of these amino acids in inflammatory eyes.

DISCUSSION

IN THIS STUDY, WE PERFORMED THE SYSTEMATIC QUANTIFICATION of free amino acids in human tear fluids by HPLC/ESI-MS/

the radar chart. (Bottom) Amino acid composition data (percentages) from plasma ($n = 34$) (green), basal tear ($n = 31$) (blue), reflex tear ($n = 16$) (purple), and aqueous humor ($n = 16$) (red) samples are shown in the box plots. The box represents the first and third quartiles, and the horizontal line within each box represents the median. The vertical lines show the largest/smallest observation, and the additional points are "extreme" values.

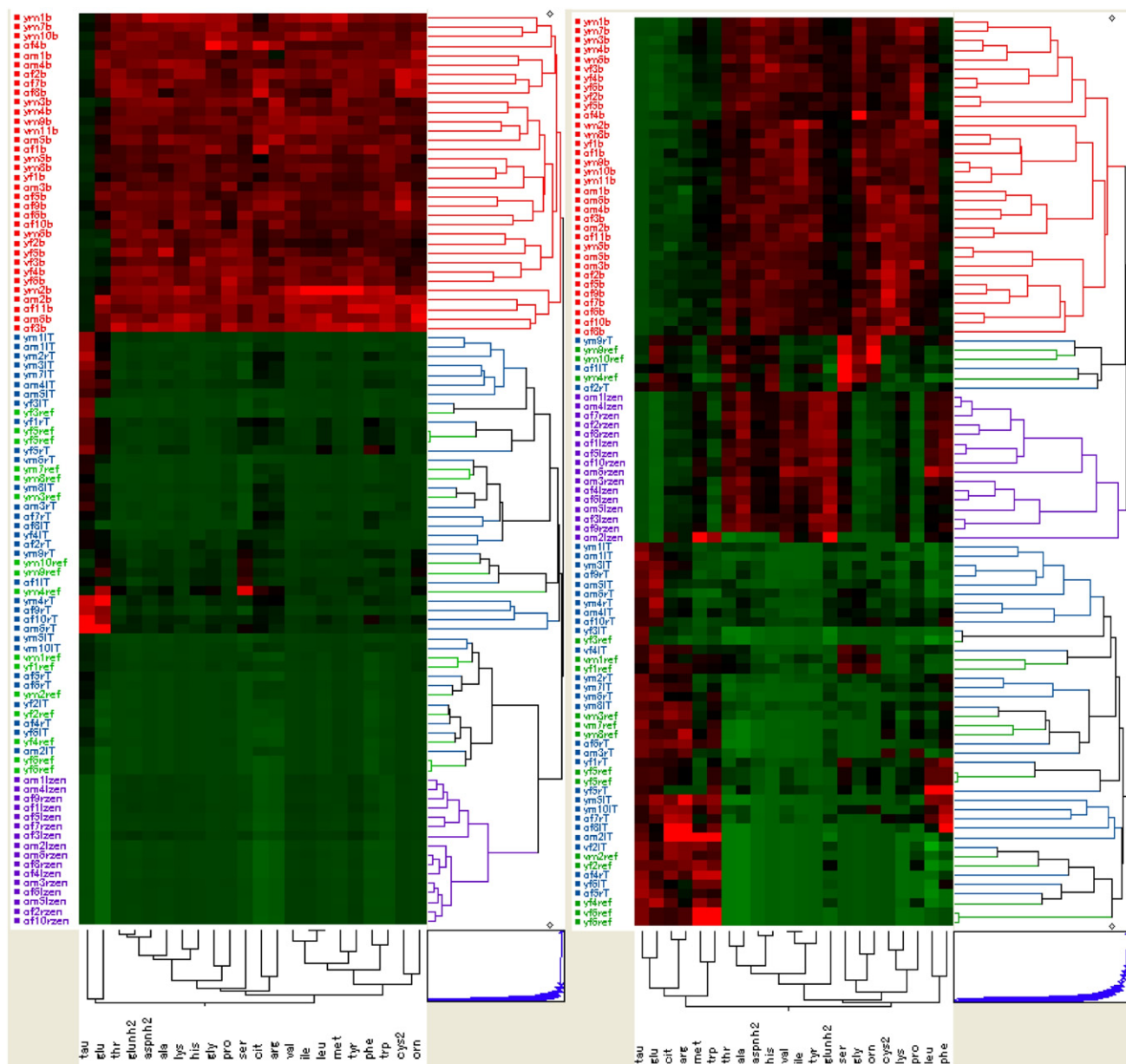


FIGURE 2. Hierarchical clustering of the amino acid profiles of basal tear, reflex tear, aqueous humor, and plasma samples of normal subjects. The correlations among 23 amino acid concentrations (Left) and relative amino acid compositions (Right) of basal tear (n = 31) (blue), reflex tear (n = 16) (green), aqueous humor (n = 16) (purple), and plasma (n = 34) (red) samples were clustered using the Ward method.

MS. The amino acid profiles showed the following characteristics: 1) the amino acid profiles in tear fluids differ from those in aqueous humors and plasmas, 2) the absolute concentrations of Tau, Glu, and Asp in tear fluids are significantly higher than, or comparable with, those in plasma samples, 3) the amino acid profile in the aqueous humor samples was rather analogous to that in the plasma samples, 4) the amino acid profiles of the tear fluid of distinct groups (male vs female, young vs elderly) are similar, and 5) amino acid profiles of tear fluids from severe ocular surface disease subjects differed from those of non-ocular surface disease subjects.

The combination of the precolumn derivatization and HPLC/ESI-MS/MS techniques enabled the analysis of 120 body fluid samples in a single day and may possibly provide an alternative to traditional techniques used for amino acid analysis. From this viewpoint, we theorize that our high-speed, reliable procedure for amino acid analysis will prove to be a useful technique for the diagnosis and management of inherited disorders of amino acid metabolism in the clinical setting. In this study, we discovered that almost all amino acids exist in tear fluid. The plasma concentration of amino acid is the sum of its rates of appearance in and disappearance from plasma, and amino acid appearance

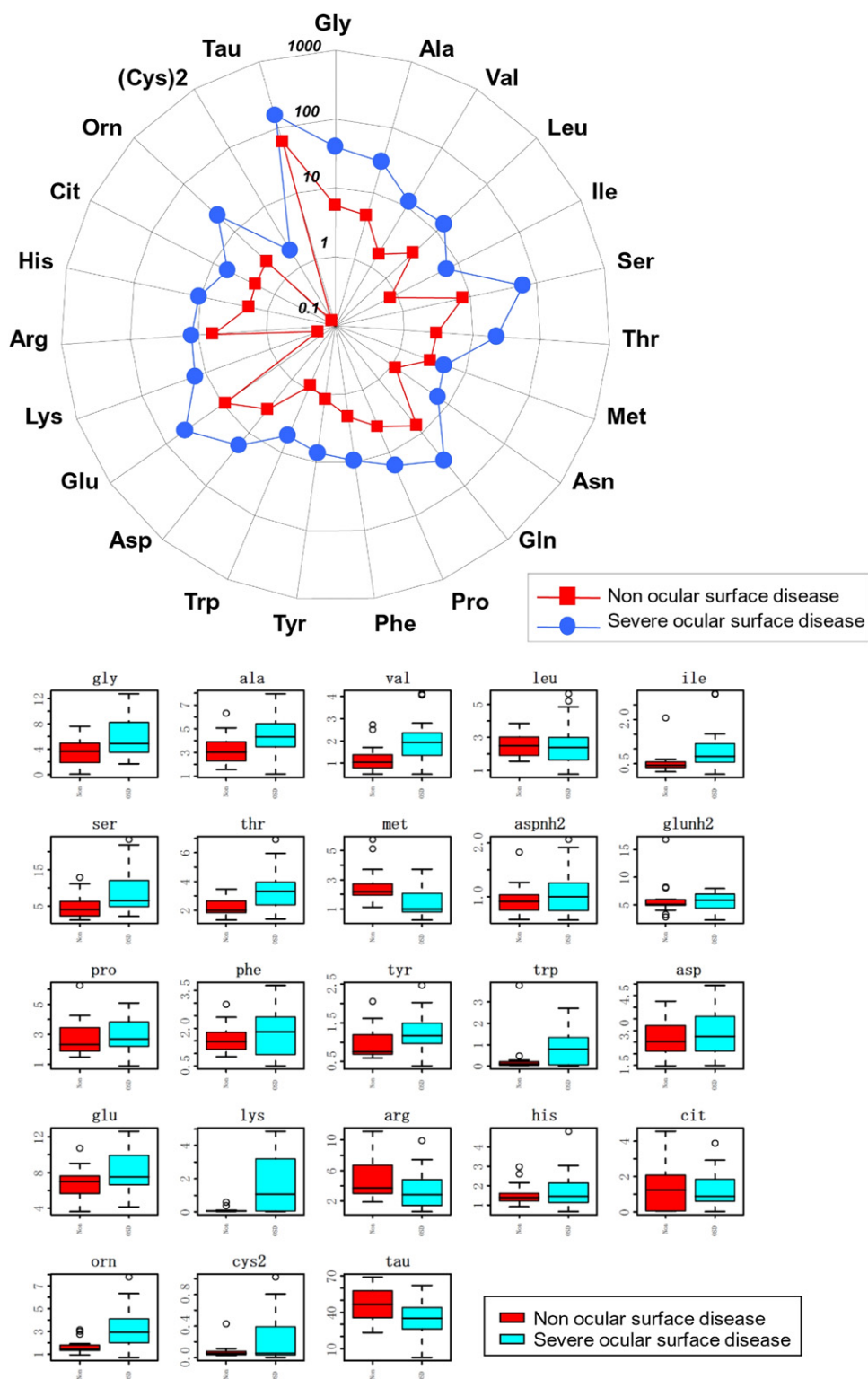


FIGURE 3. The differences of amino acid concentrations between tear fluids from severe ocular surface disease subjects and non-ocular surface disease subjects. (Top) Means values of 23 amino acid concentrations (μM) of tears from severe ocular surface disease subjects ($n = 18$) (blue) and non-ocular surface disease subjects ($n = 15$) (red) are shown in the radar chart. (Bottom) Amino acid composition data (percentages) of tears from non-ocular surface disease ($n = 15$) (red; left) and severe ocular surface disease ($n = 18$) (blue; right) subjects are shown in the box plots.

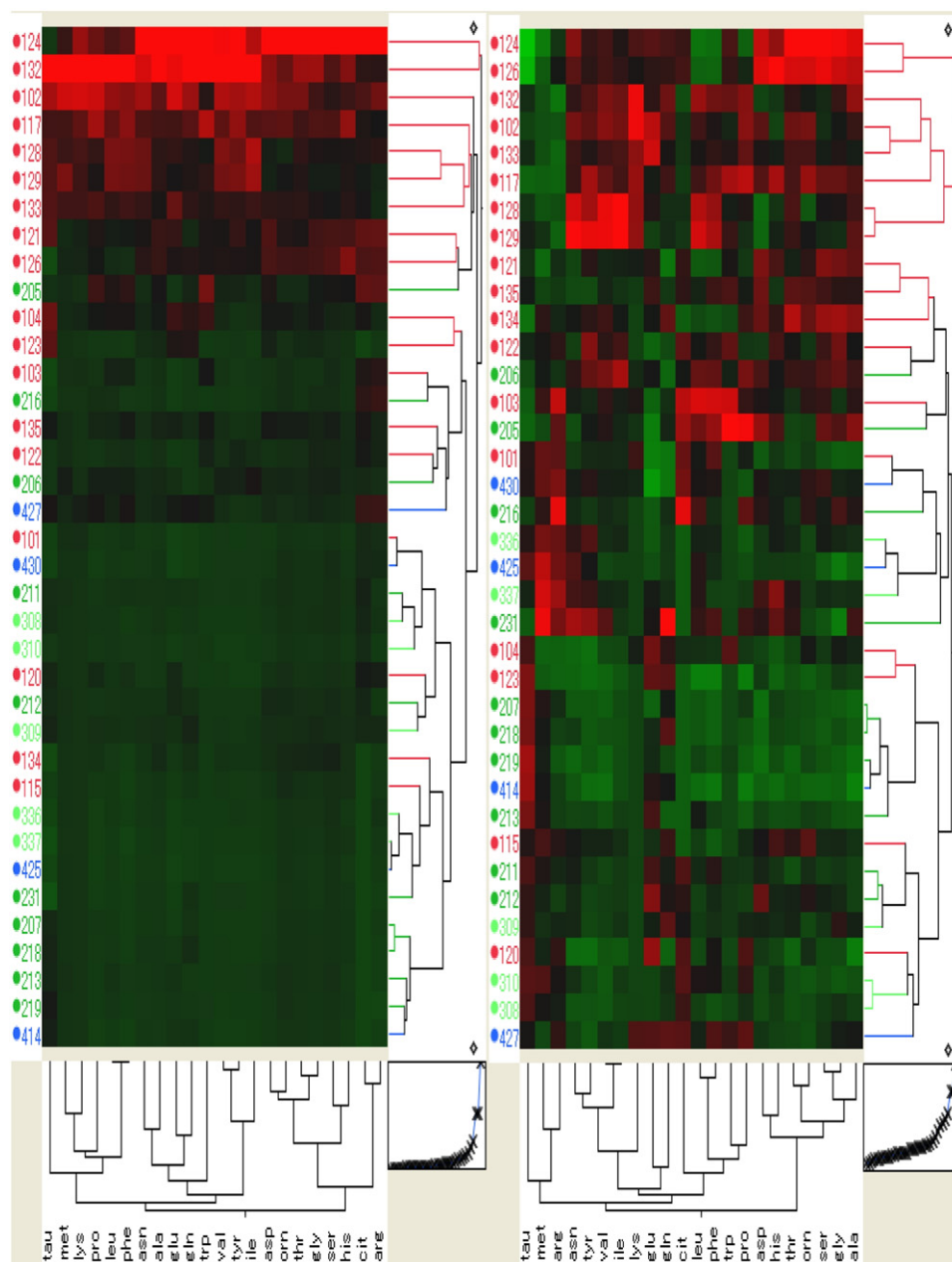


FIGURE 4. Hierarchical clustering of the amino acid profiles of tear fluids from severe ocular surface disease subjects and non-ocular surface disease subjects. The correlations among 23 amino acid concentrations (Left) and relative amino acid compositions (Right) of tear samples from severe ocular surface disease subjects ($n = 18$) (red) and non-ocular surface disease subjects ($n = 15$) (green) were clustered using the Ward method. The concentrations and relative composition of 23 amino acids in tear fluids differ from those in plasma and aqueous humor. Analysis of the hierarchical clustering of the amino acid profiles distinguished severe ocular surface diseases from non-ocular surface diseases. Steady-state tear-fluid amino acid profiles might reflect ocular-surface homeostasis and the observed changes of amino acids might have a close relation with the disease conditions on the ocular surface.

and disappearance are tightly regulated. In some circumstances, tissue uptake of amino acid and further metabolism depend on plasma amino acid concentrations. Mere determinations of plasma amino acid concentrations at the basal state (ie, postabsorptive) provide a rather limited amount of information.²¹

Previously, a few reports have presented contradicting results on the amount of limited kinds of free amino acids

in human tears.^{13,14} In those studies, the quantities found in tear fluids were at a comparable level with those found in plasma, except for the levels of Asp, Glu, and Tau. The high level for Tau in tear fluids was reported previously,¹⁴ and Tau has been identified as a major player in numerous biological functions^{22,23} and is involved in the regulation of the proinflammatory responses.²² Very

recently, it has been demonstrated that Tau plays a key role in regulating epithelial barrier function.²⁴ In this study, the concentration of Tau was confirmed to be very high in the tear fluid samples compared with that in the plasma samples, consistent with the previous reports. These findings signal the necessity to discriminate the subtle function of Tau under physiological and pathologic environments. Plasma Tau levels are usually high, although decreases are observed in response to surgical injury and numerous pathologic conditions, including cancer and sepsis.²⁵ The observed higher concentration of Glu (and probably that of Asp as well) may reflect the energy demand at the luminal side of corneal/conjunctival epithelial cells. Mucosal tissue exhibits a high rate of aerobic glycolysis, and in intestinal metabolism, luminal Gln, Glu, and Asp, but not glucose, has been found to contribute critically to the respiratory CO₂ produced in this tissue.²⁶ Although considered nonessential, Gln becomes conditionally essential during severe catabolic stress in which intracellular and plasma Gln levels decrease rapidly.^{27–29}

It is very difficult to discuss with clarity the underlying mechanism for the regulation of amino acid profiles in tear fluids, because to date, almost no information has been presented on the dynamic roles of ocular surface epithelial cells in regard to amino acid transportation. The presence of the Na⁺-dependent neutral amino acid transporter has been reported in a rabbit primary corneal epithelial cell culture and rabbit corneas.³⁰ Whether all or part of the amino acid component of tear fluids is attributable to secretion, filtration, local synthesis, or local degradation of proteins is unknown. However, it is speculated that the amino acids are transported from tear fluids into the corneal tissues by this transport system. It is important to discuss the characteristic variance of amino acid profiles in tear fluid between the individuals with and without ocular surface disease. Diseases that develop because of the loss of corneal epithelial stem cells are known as limbal stem cell deficiency. Among limbal stem cell deficiency, SJS, ocular cicatricial pemphigoid, and severe chemical or thermal burns are known as severe ocular surface disease, because these diseases are intractable, even with corneal epithelial transplantation, and visual prognosis is poor. In cases of severe ocular surface disease, corneal neovascularization, ingrowth of fibrous tissue, and stromal scarring occur and often progress with chronic inflammation. However, the mechanisms of chronic inflammation and pathophysiology are still unclear. In this study, both the concentrations and relative composition of

the amino acids differed between the severe ocular surface disease subjects and the non-ocular surface disease subjects. Hierarchical clustering on the amino acid profiles of tears clearly distinguished severe ocular surface disease subjects from non-ocular surface disease subjects. Surprisingly, hierarchical clustering also distinguished the eyes with SJS at the chronic stage as severe ocular surface disease, in which at least clinically, no inflammation and no scarring were detected. Amino acid profiles might be a sensitive marker of ocular surface inflammation, or they may be a new method to reflect ocular surface pathologic dynamics.

It is well known that supplemental Arg promotes wound healing following trauma shock. The major catabolic products of Arg are Orn and Cit. Because of the competition between nitric oxidase synthase and arginase for the same substrate Arg, their activities are regulated reciprocally. As for conditionally essential amino acids, Arg exhibited a significant decrease with a moderate decrease in Cit in the eyes with severe ocular surface disease, while Orn exhibited a prominent increase, indicating the possible polarization of tissue inflammation to the axis of arginase (oxidative tissue regeneration).^{31–34} In this context, the reason why the increased composition of Orn over Cit, reflecting the high arginase activity, exists only in the tear fluids of individuals with chronic ocular surface inflammation is a subject that requires further investigation.

We can now harness information buried in amino acid profiles for the generation of diagnostic and surrogate markers. The methods described in this study might be applicable to the clinical setting and prove useful in diagnosing various physiologic and disease states at the ocular surface. Our results using cluster analysis of amino acid profiles in tear fluids suggest that the analysis can help us understand the complex interrelations that make up the metabolism of the ocular surface. In the future, a far more stringent study with controlled background factors will need to be performed to solidify the present observations. Amino acid represents a convenient set of metabolites that can be easily measured. Once the association of abnormalities in individual amino acid concentrations and/or composition with specific ocular surface diseases or physiologic conditions has been addressed, amino acid profiles for the generation of diagnostic and surrogate markers may prove to help advance biomedical and nutritional science in the field of ocular physiology.

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Biosketch

Mina Nakatsukasa, MD, graduated and received her medical degree from Kyoto Prefectural University of Medicine, Kyoto, Japan in 2001, and completed her residency training at the Department of Ophthalmology at Kyoto Prefectural University Hospital, Kyoto, Japan. Dr Nakatsukasa currently specializes in clinical research and cornea-related diseases.