Metabolic Profiling of Impaired Cognitive Function in Patients Receiving Dialysis

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ABSTRACT

Retention of uremic metabolites is a proposed cause of cognitive impairment in patients with ESRD. We used metabolic profiling to identify and validate uremic metabolites associated with impairment in executive function in two cohorts of patients receiving maintenance dialysis. We performed metabolic profiling using liquid chromatography/mass spectrometry applied to predialysis plasma samples from a discovery cohort of 141 patients and an independent replication cohort of 180 patients participating in a trial of frequent hemodialysis. We assessed executive function with the Trail Making Test Part B and the Digit Symbol Substitution test. Impaired executive function was defined as a score $\geq$2 SDs below normative values. Four metabolites—4-hydroxyphenylacetate, phenylacetylglutamine, hippurate, and prolyl-hydroxyproline—were associated with impaired executive function at the false-detection rate significance threshold. After adjustment for demographic and clinical characteristics, the associations remained statistically significant: relative risk 1.16 (95% confidence interval [95% CI], 1.03 to 1.32), 1.39 (95% CI, 1.13 to 1.71), 1.24 (95% CI, 1.03 to 1.50), and 1.20 (95% CI, 1.05 to 1.38) for each SD increase in 4-hydroxyphenylacetate, phenylacetylglutamine, hippurate, and prolyl-hydroxyproline, respectively. The association between 4-hydroxyphenylacetate and impaired executive function was replicated in the second cohort (relative risk 1.12; 95% CI, 1.02 to 1.23), whereas the associations for phenylacetylglutamine, hippurate, and prolyl-hydroxyproline did not reach statistical significance in this cohort. In summary, four metabolites related to phenylalanine, benzoate, and glutamate metabolism may be markers of cognitive impairment in patients receiving maintenance dialysis.

Uremia is the clinical syndrome accompanying kidney failure that is primarily attributed to retention of metabolic waste products in plasma.[1] Neurologic symptoms, including cognitive impairment, were among the earliest described clinical features of uremia. Cognitive impairment accompanying uremia has been reported to improve with maintenance dialysis or kidney transplantation.[2–4] Contemporary studies indicate that cognitive impairment is common among patients receiving maintenance dialysis.[5] However, the extent to which incomplete removal of uremic metabolites by dialysis contributes to cognitive impairment remains unclear for several reasons. First, the number of known metabolites retained in uremia exceeds 200; presumably, many more remain uncharacterized.[6,7] To date, few metabolites with neurotoxicity in animal models have been

identified and human studies are even more limited.[8] Second, more intensive hemodialysis does not appear to yield improvement in cognitive function.[9] In contrast, nonrandomized studies suggest kidney transplantation is associated with improved cognitive function.[4] Whether failure of higher-dose hemodialysis reflects ineffective clearance of putative uremic metabolites and/or adverse effects

of the hemodialysis procedure, such as cerebral stunning, is unknown.[10] Alternatively, cognitive impairment may not be primarily related to retention of uremic metabolites, but may reflect health conditions which would not be expected to improve with renal replacement therapies, such as vascular dementia.[11,12]

Metabolic profiling refers to high-throughput analysis of plasma metabolites using mass spectrometry. This technique has been utilized to characterize novel uremic metabolites and identify uremic metabolites associated with cardiovascular risk.[13–15] We used metabolic profiling to identify and validate uremic metabolites associated with cognitive impairment using two cohorts of patients receiving maintenance dialysis. The discovery cohort consisted of patients receiving peritoneal dialysis or hemodialysis in Northern California who participated in a prospective study of cognitive function. The replication cohort consisted of subjects enrolled in the Frequent Hemodialysis Network (FHN) Daily Trial, a randomized clinical trial of six times per week versus three times per week hemodialysis.

RESULTS

Patient Characteristics in the Discovery and Replication Cohorts

Subjects in the discovery cohort had a mean age of 56.6±14.6 years, 64.5% were male, and 42.6% were white (Table 1). The median dialysis vintage was 25 (interquartile range [IQR], 48) months. There were 81 subjects (57.5%) in the discovery cohort with impaired executive function. Compared with subjects without impaired executive function, those with impaired function were similar in most characteristics except that they had a higher dialysis vintage and were less likely to be treated with peritoneal dialysis versus hemodialysis (Supplemental Table 1).

At baseline, before the FHN intervention, subjects in the replication cohort had a mean age of 50.6±13.8 years, 61.9% were male, and 26.0% were white (Table 1). The median dialysis vintage was 42 (IQR, 60) months. There were 117 subjects (64.6%) in the replication cohort with impaired executive function. Compared with subjects in the cohort without impaired executive function, those with impaired executive function were older and had fewer years of education (Supplemental Table 1).

Metabolites Associated with Impaired Executive Function in Discovery Cohort

Mean metabolite values for subjects with and without impaired executive function are provided in Supplemental Table 2. Of the 95 uremic metabolites included on the platform, four metabolites were significantly associated with impaired executive function at the false-detection rate (FDR) threshold P value ($P<0.05$) (Figure 1). Two were related to phenylalanine metabolism (4-hydroxyphenylacetate and phenylacetylglutamine), one was related to benzoate metabolism (hippurate), and one was a dipeptide related to glutamate metabolism (prolyl-hydroxyproline). When we repeated the analyses using all metabolites and the ratio of all metabolite pairs, three metabolite ratios met the FDR threshold P value—all with phenylacetylglutamine in the denominator (Supplemental Table 3). The p-gain statistic indicated that these associations were not different from the association of the individual metabolites with executive function impairment.

The correlation coefficient between phenylacetylglutamine and hippurate was 0.62; all other correlation coefficients among the four metabolites were <0.30. None of the four metabolites were correlated with urea or the urea reduction ratio. Mean phenylacetylglutamine concentration was 3.0±3.1 mg/dl and mean hippurate concentration was 4.1±5.5 mg/dl, as measured by quantitative tandem mass spectrometry (methods for direct quantification of 4-hydroxyphenlacetate and prolyl-hydroxyproline are not yet available). There was a strong correlation between metabolite levels obtained from the Metabolon Inc. platform and direct quantification of phenylacetylglutamine and hippurate (Supplemental Figure 1).

Table 1. Characteristics of dialysis patients in the discovery cohort and replication cohort

Patient Characteristics	Discovery Cohort n=141	Replication Cohort n=180
Demographic and Clinical Characteristics		
Age, yr	56.6±14.6	50.6±13.8
Male, %	64.5	61.7
White versus nonwhite, %	42.6	26.1
Months receiving dialysis[a]	25 (12, 60)	42 (20, 80)
Education, %		
< High school	12.8	16.7
High school graduate	27.0	24.4
Post–high school	60.2	58.9
English- versus Spanish-speaking, %	95.0	80.6
Diabetes, %	46.1	41.7
Stroke, %	12.1	8.9
Peritoneal dialysis, %	7.1	0
Systolic BP, mmHg	141.3±26.1	147.1±18.2
Diastolic BP, mmHg	77.0±14.9	80.1±12.0
Hemoglobin, g/dl	11.9±1.0	12.1±1.2
Urea reduction ratio, %	67.8±8.6	73.0±5.7
BUN, mg/dl	67.2±19.0	58.0±17.6
Cognitive Function		
Trail Making Test Part B	130.8±69.8	162.4±97.2
Digit Symbol Substitution test	49.9±17.5	49.1±19.0[b]
Impaired executive function, %	57.5	64.4

Higher scores on the Trail Making Test Part B indicate poorer cognitive function. Lower scores on the Digit Symbol Substitution test indicate poorer cognitive function.
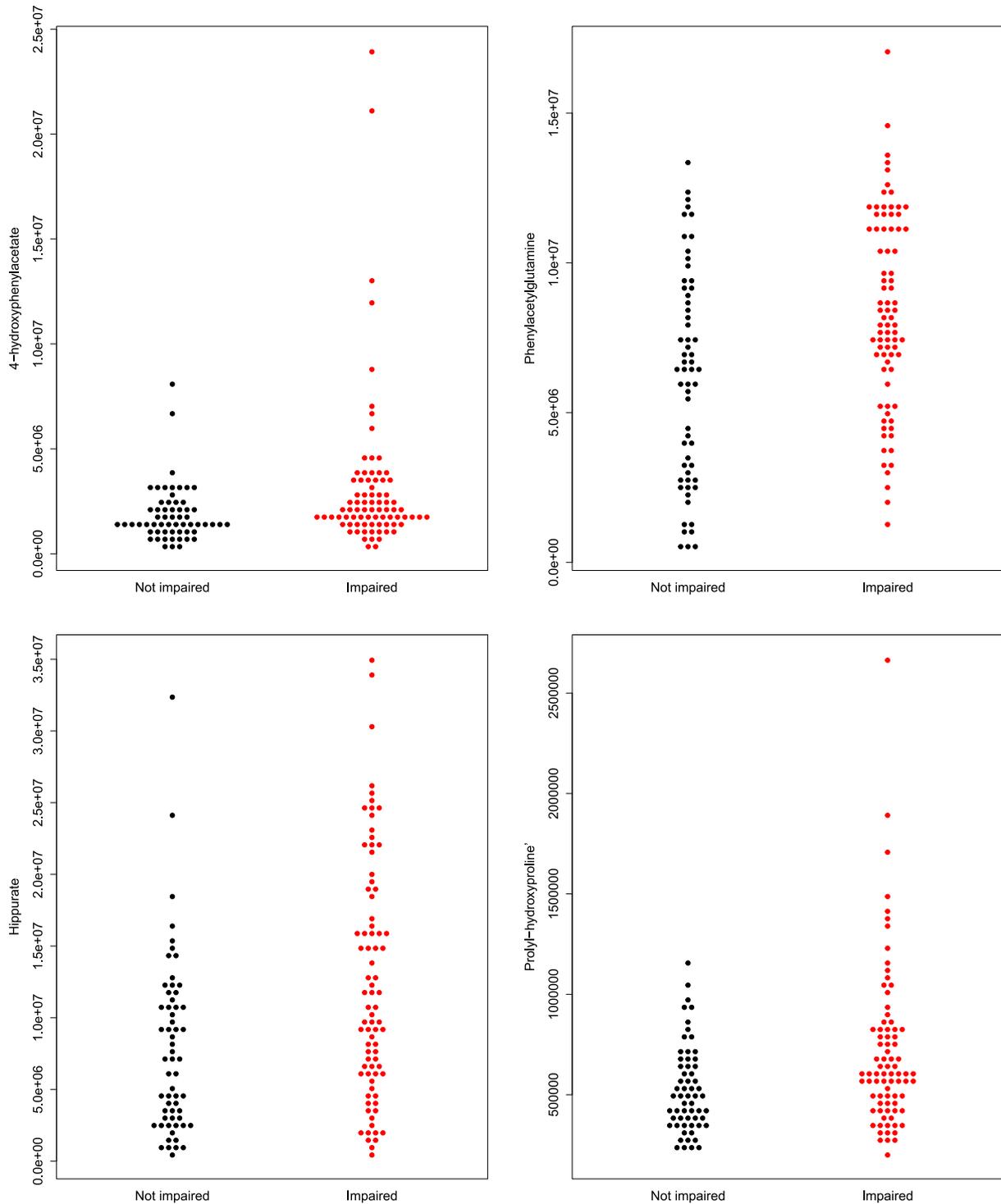[a]Median (25th, 75th percentile).
[b](n=59).

Figure 1. Distribution of (A) 4-hydroxyphenylacetate, (B) phenylacetylglutamine, (C) hippurate, and (D) prolyl-hydroxyproline among subjects with (red) and without (black) impaired executive function in the discovery cohort. The beeswarm plots illustrate significantly higher metabolite levels among subjects with versus without impaired executive function.

In models adjusted for vintage and dialysis modality, and then additionally adjusted for age, education, language, and stroke, there remained a significant association between each metabolite and impaired executive function ($P<0.05$; Supplemental Table 4, Table 2). Results were similar when we

excluded subjects receiving peritoneal dialysis and when we adjusted for the urea reduction ratio. The results were also similar when we substituted the concentrations of phenylacetylglutamine (relative risk [RR] per SD increase 1.12; 95% confidence interval [95% CI], 1.00 to 1.27) and hippurate

Table 2. Adjusted association between selected metabolites and RR of impaired executive function in discovery cohort (*n*=141)

Metabolite	Adjusted RR (95% CI)	
	Model One	Model Two
4-hydroxyphenylacetate per SD increase in log	1.19 (1.06 to 1.33)	1.16 (1.03 to 1.32)
Phenylacetylglutamine per SD increase in log	1.38 (1.12 to 1.68)	1.39 (1.13 to 1.71)
Hippurate per SD increase in log	1.23 (1.02 to 1.49)	1.24 (1.03 to 1.50)
Prolyl-hydroxyproline per SD increase in log	1.20 (1.05 to 1.36)	1.20 (1.05 to 1.38)

Model 1 is adjusted for vintage and peritoneal dialysis (versus hemodialysis).
Model 2 is adjusted for age, education, language, stroke, vintage, and peritoneal dialysis.

(RR per SD increase 1.13; 95% CI, 1.01 to 1.25) from direct quantification.

In adjusted linear regression models, higher levels of phenylacetylglutamine were associated with poorer scores on tests of executive function, as well as psychomotor speed and memory (Pegboard and Rey Auditory Verbal Learning tests, respectively [Table 3]). Higher levels of hippurate were associated with poorer scores on tests of executive function and memory. There was no significant linear association between the other two metabolites (4-hydroxyphenylacetate and prolyl-hydroxyproline) with either test of executive function or with tests of other cognitive domains.

There were 48 subjects (34%) with no metabolites elevated, 35 (25%) with one elevated metabolite, 27 (19%) with two elevated metabolites, and 31 (22%) with three or more elevated metabolites. After adjustment for vintage, modality, age, education, language, and stroke, there was an increased risk of impaired executive function among subjects with two elevated metabolites (RR 1.91; 95% CI, 1.25 to 2.92), and three or more elevated metabolites (RR 1.93; 95% CI, 1.21 to 3.08), compared with subjects with no elevated metabolites (Figure 2).

Associations among Selected Metabolites and Impaired Executive Function in the Replication Cohort

After adjustment for age, education, language, stroke, vintage, and the day of the week that blood was sampled, there was a significant association between higher levels of 4-hydroxyphenylacetate and impaired executive function in the replication cohort (Supplemental Table 4, Table 4). There were borderline significant associations between higher levels of phenylacetylglutamine (*P*=0.1) and prolyl-hydroxyproline (*P*=0.05) and impaired executive function. There was no significant association between hippurate and impaired executive function in the replication cohort. The results were similar when we substituted the concentrations of phenylacetylglutamine and hippurate from direct quantification.

DISCUSSION

In a cohort of patients receiving maintenance dialysis, we identified four uremic metabolites independently associated with impaired executive function. Compared with subjects with no elevated metabolite levels, there was a higher risk of impairment among subjects with two or more elevated metabolites. Furthermore, the association for one of these metabolites, 4-hydroxyphenylacetate, was replicated in an independent sample of patients, whereas two other metabolites, phenylacetylglutamine and prolyl-hydroxyproline, had borderline, nonsignificant associations with impaired executive function in the replication cohort.
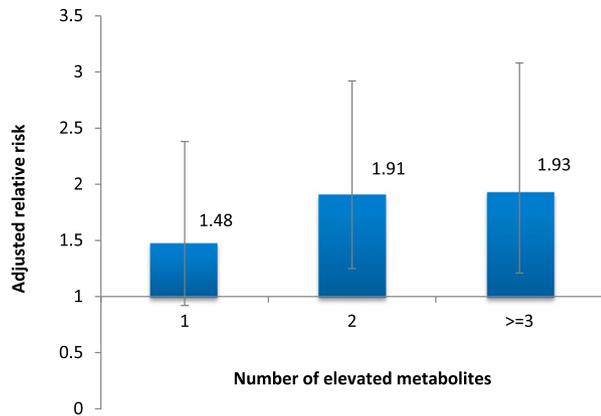
Table 3. Adjusted association of selected metabolites with cognitive test scores in discovery cohort

Cognitive Test	4-hydroxyphenylacetate per SD Increase in Log		Phenylacetylglutamine per SD Increase in Log		Hippurate per SD Increase in Log		Prolyl-hydroxyproline per SD Increase in Log	
	$\beta \pm$ SEM	P Value	$\beta \pm$ SEM	P Value	$\beta \pm$ SEM	P Value	$\beta \pm$ SEM	P Value
Executive function								
Trail Making Test Part B	2.6±5.6	0.65	14.4±5.4	0.01[a]	9.4±5.8	0.11	3.0±5.5	0.58
Digit Symbol Substitution	−2.2±1.4	0.13	-4.9±1.4	<0.001[a]	-3.5±1.5	0.02[a]	0.7±1.4	0.65
Attention and psychomotor speed								
Trail Making Test Part A	2.3±2.1	0.29	3.4±2.1	0.10	2.1±2.2	0.35	1.2±2.1	0.57
Pegboard, dominant hand	6.1±4.9	0.21	8.6±4.4	0.05[a]	2.8±4.8	0.56	5.3±4.7	0.26
Pegboard, nondominant hand	7.8±5.6	0.17	10.3±4.6	0.03[a]	2.0±4.9	0.69	3.1±4.8	0.52
Language and memory								
Controlled Oral Word Association	−0.01±1.2	0.99	−1.3±1.2	0.30	−1.4±1.2	0.24	1.2±1.8	0.31
Rey Auditory Verbal Learning, delayed recall	−0.2±0.3	0.45	-0.6±0.3	0.03[a]	-0.6±0.3	0.05[a]	−0.1±0.3	0.74

Models are adjusted for age, vintage, education, language, modality, and stroke. The Trail Making Test Part B, Trail Making Test Part A, and the Pegboard test are timed tests measured in seconds. A positive parameter estimate indicates poorer cognitive function. The Digit Symbol Substitution, Controlled Oral Word Association, and Rey Auditory Verbal Learning tests are scored as the number of correct responses. A negative parameter estimate indicates poorer cognitive function.
[a]Uncorrected *P* value <0.05.

Figure 2. Adjusted RR of impaired executive function for subjects with one, two, and three or more elevated metabolites, compared with subjects with no elevated metabolites. The figure illustrates a significantly higher risk for impaired executive function among subjects with two or more elevated metabolites. An elevated metabolite is defined as the highest tertile for each metabolite: 4-hydroxyphenylacetate, phenylacetylglutamine, hippurate, and prolyl-hydroxyproline.

The metabolites 4-hydroxyphenylacetate and phenylacetylglutamine are derived from metabolism of phenylalanine and tyrosine by colonic microbes.[16,17] Although 4-hydroxyphenylacetate has not been extensively studied, dialytic clearance of phenylacetylglutamine is less than half that of native kidney clearance.[18] While the bound fraction of phenylacetylglutamine is below 20%, it is secreted by the kidneys—a function not replicated by hemodialysis; therefore, levels are increased more than 100-fold in patients receiving hemodialysis compared with persons with normal kidney function.[18] In patients with inborn errors of urea synthesis, phenylacetylglutamine is an alternative vehicle for nitrogen disposal.[19] Accumulation of phenylacetate and phenylacetylglutamine after infusion of high-dose phenylacetate results in confusion, lethargy, and nausea.[20,21] The serum phenylacetylglutamine concentration associated with toxicity varies in reports between 1.6- and 6-fold higher than the average concentration observed in patients receiving hemodialysis.[20,21] Administration of phenylacetate together with sodium benzoate, a precursor of hippurate, increases renal excretion of glutamine-associated nitrogen, and is Food and Drug Administration-approved for the treatment of hyperammonemia in patients with inborn errors of urea synthesis.[22,23] These agents, as well as a similar agent, ornithine phenylacetate, are currently being tested as treatments for hepatic encephalopathy.[24] Elevated phenylacetylglutamine levels have also been identified in the cerebrospinal fluid of patients with HIV-associated cognitive impairment.[25]

Hippurate is a product of the conjugation of benzoate with glycine. In addition to being a food preservative, benzoate is produced by microbial metabolism of polyphenols, purines, aromatic organic acids, and amino acids.[26] Like phenylacetylglutamine, hippurate is secreted by the kidneys, so dialytic clearance of it is low (<30%) relative to native kidney clearance, and serum concentrations are increased more than 100-fold in patients receiving hemodialysis compared with persons with normal kidney function.[18] It is speculated that hippurate inhibits organic anion transporters, which mediate efflux of uremic metabolites across the blood–brain barrier; however, there is limited evidence of toxicity in humans.[27–29]

Prolyl-hydroxyproline is a dipeptide produced from collagen breakdown. Metabolism of prolyl-hydroxyproline occurs in the kidney, resulting in release of glutamine, which is a precursor for several neurotransmitters.[30] To our knowledge, there is no known association of prolyl-hydroxyproline with central nervous system function.

Like urea, the dialytic reduction ratio for phenylacetylglutamine and hippurate is high (75–80%).[18] Therefore, increasing clearance parameters or session length of conventional thrice-weekly hemodialysis does not lower the postdialysis concentration much more. Lowering the pretreatment plasma concentration of these metabolites might theoretically be achieved by altering dietary intake and/or the colonic microbiome, or by increasing the frequency of hemodialysis. It is plausible that even if one or more of the metabolites identified in this study are causally related to cognitive impairment, other factors, such as vascular disease, could play a larger role.

This study has several limitations. First, the discovery and replication cohorts were small, and may not be representative of the larger population of patients receiving dialysis. Most patients in this study were receiving hemodialysis, thus these findings may not be generalizable to patients receiving peritoneal dialysis. Second, blood sampling was not performed on a uniform day of the dialysis cycle in the replication cohort; this would be expected to bias the results toward the null. Furthermore, adjustment for day of the week did not appreciably change the results. Third, executive function was assessed with a single test in a subset of participants in the replication cohort, which may have led to misclassification of impairment status. Finally, the concentration of uremic metabolites in cerebrospinal fluid is likely to be more important than serum concentrations with respect to cognitive function; however, this was not assessed in this study.

In summary, higher levels of 4-phenylacetylglutamine were associated with impaired executive function in independent samples of patients receiving dialysis, whereas three other metabolites, phenylacetylglutamine,

Table 4. Adjusted association between selected metabolites and RR of impaired executive function in replication cohort (n=180)

Metabolite	Adjusted RR (95% CI)	
	Model One	Model Two
4-hydroxyphenylacetate per SD Increase in Log	1.12 (1.02 to 1.22)	1.12 (1.02 to 1.23)
Phenylacetylglutamine per SD Increase in Log	1.10 (0.98 to 1.25)	1.11 (0.98 to 1.26)
Hippurate per SD Increase in Log	0.96 (0.83 to 1.06)	0.96 (0.86 to 1.08)
Prolyl-hydroxyproline per SD Increase in Log	1.11 (0.99 to 1.23)	1.11 (1.00 to 1.24)

Model 1 is adjusted for age, education, and language. Model 2 is adjusted for age, education, language, stroke, vintage, and day of blood sampling.

hippurate, and prolyl-hydroxyproline, were associated with impaired executive function in one, but not both cohorts. Further research is needed to determine whether these markers of phenylalanine, benzoate, and glutamate metabolism represent modifiable risk factors for uremic cognitive impairment.

CONCISE METHODS

Subjects

The discovery cohort was comprised of subjects recruited from March 2009 through October 2010 from five outpatient dialysis clinics in Northern California for a study of cognitive function.[31] Eligible participants were at least 21 years of age and receiving dialysis for at least 90 days. Participants were excluded if they were not fluent in English or Spanish, had an active psychiatric disorder, or had significant visual or hearing impairment. We contacted 346 eligible individuals; 148 (43%) were enrolled. Of the 148 enrolled subjects, 141 (95%) had blood samples available for metabolic profiling.

The replication cohort was comprised of subjects enrolled in the FHN Daily Trial. The design and main outcomes of the FHN trials have been previously reported.[9,32,33] From January 2006 to March 2009, patients receiving maintenance hemodialysis were recruited from clinics in the United States and Canada. Major exclusion criteria included age <13 years, inability to achieve a mean equilibrated Kt/V_{urea} ≥1.0, life expectancy <6 months, medical need for hemodialysis >3 times per week, residual urea clearance >3 ml/min per 35L, poor adherence to hemodialysis, inability to communicate in English or Spanish, and anticipated kidney transplantation or relocation within the next 14 months. Of the 387 enrolled subjects, 330 completed cognitive testing at baseline; of these, 180 (55%) subjects had blood samples available. Both studies were reviewed by institutional review boards at each clinical center and all subjects gave informed consent.

Cognitive Function Assessment

The primary outcome for these analyses was impairment in executive function. In the discovery cohort, executive function was assessed with the Trail Making Test Part B (Trails B) and the Digit Symbol Substitution test. In the replication cohort, we designated *a priori* the Trails B as the primary performance metric within the cognitive function domain. A subset (*n*=59) of FHN subjects also received the Digit Symbol Substitution test in a cognitive ancillary study. In both cohorts, cognitive testing was administered before a midweek dialysis session. We defined impairment in executive function as a score on the Trails B or Digit Symbol Substitution test at least two SDs below normative values, accounting for age and grade level attainment.[5,34] Additional cognitive tests assessing attention, psychomotor speed, language, and memory were administered to subjects in the discovery cohort, as previously described.[31] These were evaluated as secondary outcomes because they were not available for most subjects in the replication cohort.

Metabolite Profiling

In the discovery cohort, blood samples were drawn one week after cognitive testing, before a Monday or Tuesday hemodialysis session

(*i.e.*, after a 67-hour dialysis interval). In the replication cohort, blood samples were drawn before hemodialysis according to each center's monthly lab schedule. Accordingly, 29% of baseline blood samples were drawn on Monday/Tuesday, 61% on Wednesday/Thursday, and 17% on Friday/Saturday.

Metabolon Inc. performed metabolomic profiling.[35,36] Briefly, select compounds were added to each plasma sample before processing for quality control. Individual samples were deproteinized and aliquoted for analysis by gas chromatography-mass spectrometry and by tandem mass spectrometry in positive and negative modes. The platform identifies compounds using software which compares the chromatographic and mass spectral patterns of potential compounds observed in samples to an in-house library consisting of purified chemical standards. The peak area for each compound is automatically recorded when the quality of identification is considered high and hand-checked when the quality of identification is considered intermediate. No value is recorded for samples in which identification does not meet a threshold value.

The Metabolon analysis detected a total of 562 compounds in at least one plasma sample from the discovery and replication cohorts. Of these, 96 have been previously identified as uremic metabolites on the basis of finding of higher levels in patients receiving dialysis compared with healthy subjects in a recent study using the same platform.[37] One metabolite was measured in <90% of subjects and was excluded. The list of 95 metabolites included in these analyses is provided in Supplemental Table 2. To confirm the results, we performed quantitative analysis of selected metabolites by tandem mass spectrometry with isotopic dilution as previously described.[38] For this analysis, plasma samples were deproteinized with methanol in 1:4 vol/vol ratio and diluted 40 times before mass spectrometric analysis. In supplementary analyses, we repeated the analysis using all metabolites detected in at least 90% of samples (363 metabolites) and all pairs of metabolite ratios.

Statistical Analyses

We expressed continuous variables as a mean (±SD) or median (IQR) and compared these using the *t* test or Kruskall–Wallis test. We expressed categorical variables as proportions and compared these using the chi-squared test. In the discovery cohort, we compared the raw area counts of 95 uremic metabolites among subjects with impairment in executive function versus subjects without impairment, accounting for multiple comparisons using the FDR.[39] For the supplementary analyses of all metabolites and the ratios of metabolite pairs, we evaluated the association using the FDR *P* value (accounting for a larger number of tests) and the p-gain statistic. The p-gain statistic indicates whether the association between the ratio of two metabolites and the outcome of interest is different than the association of the individual metabolites.[40,41] We log transformed metabolite values for analysis. To summarize the results, we plotted the distribution of untransformed metabolites in subjects with versus those without impairment in executive function.

Next, we assessed whether metabolites meeting the FDR threshold were independently associated with impairment in executive function after accounting for potential confounders. For these analyses, we used Poisson regression to estimate the RR of impairment in executive

function. We used Poisson regression rather than logistic regression because the odds ratio does not approximate the RR when the outcome is common.[42] We analyzed metabolites as log-transformed continuous variables divided by their SD. We constructed two adjusted models. The first model adjusted for clinical characteristics that differed among subjects with impairment versus those without impairment in executive function in each cohort. The second model adjusted for clinical characteristics that differed among subjects with impairment versus those without impairment in executive function in either cohort. In complementary analyses, we used linear regression to assess the relationships among metabolites meeting the FDR threshold with scores on the Trails B and Digit Symbol Substitution tests, as well as additional tests of attention, psychomotor speed, language, and memory. To determine whether the metabolites had additive effects, we determined the proportion of subjects with elevated levels of one or more of the metabolites meeting the FDR threshold, defined as the highest tertile for each metabolite. We then estimated the RR of impairment for subjects with zero, one, two, and three or more elevated metabolites. To validate the results, we repeated the analysis using the replication cohort. To account for the fact that blood samples were drawn on different days of the dialysis cycle in the validation cohort, we included day of the week in adjusted models in addition to other covariates.

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DISCLOSURES

B.S. is chief medical officer of Satellite Healthcare (San Jose, CA). A.R.N. is chief medical officer of DaVita Inc. (El Segundo, CA).

REFERENCES

1. Meyer TW, Hostetter TH: Uremia. *N Engl J Med* 357: 1316–1325, 2007
2. Teschan PE, Ginn HE, Bourne JR, Ward JW, Hamel B, Nunnally JC, Musso M, Vaughn WK: Quantitative indices of clinical uremia. *Kidney Int* 15: 676–697, 1979
3. Rasbury WC, Fennell RS 3rd, Morris MK: Cognitive functioning of children with end-stage renal disease before and after successful transplantation. *J Pediatr* 102: 589–592, 1983
4. Harciarek M, Biedunkiewicz B, Lichodziejewska-Niemierko M, Dębska-Ślizień A, Rutkowski B: Continuous cognitive improvement 1 year following successful kidney transplant. *Kidney Int* 79: 1353–1360, 2011
5. Murray AM, Tupper DE, Knopman DS, Gilbertson DT, Pederson SL, Li S, Smith GE, Hochhalter AK, Collins AJ, Kane RL: Cognitive impairment in hemodialysis patients is common. *Neurology* 67: 216–223, 2006
6. Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jörres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W; European Uremic Toxin Work Group (EUTox): Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney Int* 63: 1934–1943, 2003
7. Meyer TW, Hostetter TH: Approaches to uremia. *J Am Soc Nephrol* 25: 2151–2158, 2014
8. De Deyn PP, D'Hooge R, Van Bogaert PP, Marescau B: Endogenous guanidino compounds as uremic neurotoxins. *Kidney Int Suppl* 78: S77–S83, 2001
9. Kurella Tamura M, Unruh ML, Nissenson AR, Larive B, Eggers PW, Gassman J, Mehta RL, Kliger AS, Stokes JB; Frequent Hemodialysis Network (FHN) Trial Group: Effect of more frequent hemodialysis on cognitive function in the frequent hemodialysis network trials. *Am J Kidney Dis* 61: 228–237, 2013
10. Eldehni MT, Odudu A, McIntyre CW: Randomized clinical trial of dialysate cooling and effects on brain white matter. *J Am Soc Nephrol* 26: 957–965, 2015
11. Martinez-Vea A, Salvadó E, Bardají A, Gutierrez C, Ramos A, García C, Compte T, Peralta C, Broch M, Pastor R, Angelet P, Marcas L, Saurí A, Oliver JA: Silent cerebral white matter lesions and their relationship with vascular risk factors in middle-aged predialysis patients with CKD. *Am J Kidney Dis* 47: 241–250, 2006
12. Drew DA, Bhadelia R, Tighiouart H, Novak V, Scott TM, Lou KV, Shaffi K, Weiner DE, Sarnak MJ: Anatomic brain disease in hemodialysis patients: a cross-sectional study. *Am J Kidney Dis* 61: 271–278, 2013
13. Rhee EP, Souza A, Farrell L, Pollak MR, Lewis GD, Steele DJ, Thadhani R, Clish CB, Greka A, Gerszten RE: Metabolite profiling identifies markers of uremia. *J Am Soc Nephrol* 21: 1041–1051, 2010
14. Kalim S, Clish CB, Wenger J, Elmariah S, Yeh RW, Deferio JJ, Pierce K, Deik A, Gerszten RE, Thadhani R, Rhee EP: A plasma long-chain acylcarnitine predicts cardiovascular mortality in incident dialysis patients. *J Am Heart Assoc* 2: e000542, 2013
15. Toyohara T, Akiyama Y, Suzuki T, Takeuchi Y, Mishima E, Tanemoto M, Momose A, Toki N, Sato H, Nakayama M, Hozawa A, Tsuji I, Ito S, Soga T, Abe T: Metabolomic profiling of uremic solutes in CKD patients. *Hypertens Res* 33: 944–952, 2010
16. Aronov PA, Luo FJ, Plummer NS, Quan Z, Holmes S, Hostetter TH, Meyer TW: Colonic contribution to uremic solutes. *J Am Soc Nephrol* 22: 1769–1776, 2011
17. Ross AB, Pere-Trépat E, Montoliu I, Martin FP, Collino S, Moco S, Godin JP, Cléroux M, Guy PA, Breton I, Bibiloni R, Thorimbert A, Tavazzi I, Tornier L, Bebuis A, Bruce SJ, Beaumont M, Fay LB, Kochhar S: A whole-grain-rich diet reduces urinary excretion of markers of protein catabolism and gut microbiota metabolism in healthy men after one week. *J Nutr* 143: 766–773, 2013
18. Sirich TL, Funk BA, Plummer NS, Hostetter TH, Meyer TW: Prominent accumulation in hemodialysis patients of solutes normally cleared by tubular secretion. *J Am Soc Nephrol* 25: 615–622, 2014
19. Posada-Ayala M, Zubiri I, Martin-Lorenzo M, Sanz-Maroto A, Molero D, Gonzalez-Calero L, Fernandez-Fernandez B, de la Cuesta F, Laborde CM, Barderas MG, Ortiz A, Vivanco F, Alvarez-Llamas G: Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease. *Kidney Int* 85: 103–111, 2014
20. MacArthur RB, Altincatal A, Tuchman M: Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers. *Mol Genet Metab* 81[Suppl 1]: S67–S73, 2004
21. Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor AO, Tompkins AC, Weinberger MS, Headlee DJ, McCall NA, Samid D, Myers, CE: A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. *Cancer Res* 54: 1690–1694, 1994
22. Smith I: The treatment of inborn errors of the urea cycle. *Nature* 291: 378–380, 1981
23. Misel ML, Gish RG, Patton H, Mendler M: Sodium benzoate for treatment of hepatic encephalopathy. *Gastroenterol Hepatol (N Y)* 9: 219–227, 2013

24. Kristiansen RG, Rose CF, Fuskevåg OM, Mæhre H, Revhaug A, Jalan R, Ytrebø LM: L-Ornithine phenylacetate reduces ammonia in pigs with acute liver failure through phenylacetylglycine formation: a novel ammonia-lowering pathway. *Am J Physiol Gastrointest Liver Physiol* 307: G1024–G1031, 2014

25. Cassol E, Misra V, Dutta A, Morgello S, Gabuzda D: Cerebrospinal fluid metabolomics reveals altered waste clearance and accelerated aging in HIV patients with neurocognitive impairment. *AIDS* 28: 1579–1591, 2014

26. Williams HR, Cox IJ, Walker DG, Cobbold JF, Taylor-Robinson SD, Marshall SE, Orchard TR: Differences in gut microbial metabolism are responsible for reduced hippurate synthesis in Crohn's disease. *BMC Gastroenterol* 10: 108, 2010

27. Cathcart-Rake W, Porter R, Whittier F, Stein P, Carey M, Grantham J: Effect of diet on serum accumulation and renal excretion of aryl acids and secretory activity in normal and uremic man. *Am J Clin Nutr* 28: 1110–1115, 1975

28. Deguchi T, Isozaki K, Yousuke K, Terasaki T, Otagiri M: Involvement of organic anion transporters in the efflux of uremic toxins across the blood-brain barrier. *J Neurochem* 96: 1051–1059, 2006

29. Kaluzna-Czaplinska J, Jozwik J, Zurawicz E: Analytical methods used in autism spectrum disorders. *Trends Analyt Chem* 62: 20–27, 2014

30. Lowry M, Hall DE, Brosnan JT: Metabolism of glycine- and hydroxyproline-containing peptides by the isolated perfused rat kidney. *Biochem J* 229: 545–549, 1985

31. Kurella Tamura M, Meyer JB, Saxena AB, Huh JW, Wadley VG, Schiller B: Prevalence and significance of stroke symptoms among patients receiving maintenance dialysis. *Neurology* 79: 981–987, 2012

32. Suri RS, Garg AX, Chertow GM, Levin NW, Rocco MV, Greene T, Beck GJ, Gassman JJ, Eggers PW, Star RA, Ornt DB, Kliger AS; Frequent Hemodialysis Network Trial Group: Frequent Hemodialysis Network (FHN) randomized trials: study design. *Kidney Int* 71: 349–359, 2007

33. Chertow GM, Levin NW, Beck GJ, Depner TA, Eggers PW, Gassman JJ, Gorodetskaya I, Greene T, James S, Larive B, Lindsay RM, Mehta RL, Miller B, Ornt DB, Rajagopalan S, Rastogi A, Rocco MV, Schiller B, Sergeyeva O, Schulman G, Ting GO, Unruh ML, Star RA, Kliger AS; FHN Trial Group: In-center hemodialysis six times per week versus three times per week. *N Engl J Med* 363: 2287–2300, 2010

34. Yaffe K, Ackerson L, Kurella Tamura M, Le Blanc P, Kusek JW, Sehgal AR, Cohen D, Anderson C, Appel L, Desalvo K, Ojo A, Seliger S, Robinson N, Makos G, Go AS; Chronic Renal Insufficiency Cohort Investigators: Chronic kidney disease and cognitive function in older adults: findings from the chronic renal insufficiency cohort cognitive study. *J Am Geriatr Soc* 58: 338–345, 2010

35. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E: Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. *Anal Chem* 81: 6656–6667, 2009

36. Dehaven CD, Evans AM, Dai H, Lawton KA: Organization of GC/MS and LC/MS metabolomics data into chemical libraries. *J Cheminform* 2: 9, 2010

37. Tanaka H, Sirich TL, Plummer NS, Weaver DS, Meyer TW: An Enlarged Profile of Uremic Solutes. *PLoS One* 10: e0135657, 2015

38. Sirich TL, Aronov PA, Plummer NS, Hostetter TH, Meyer TW: Numerous protein-bound solutes are cleared by the kidney with high efficiency. *Kidney Int* 84: 585–590, 2013

39. Benjamini Y, Hochberg Y: Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J R Stat Soc Series B Stat Methodol* 57: 289–300, 1995

40. Petersen AK, Krumsiek J, Wägele B, Theis FJ, Wichmann HE, Gieger C, Suhre K: On the hypothesis-free testing of metabolite ratios in genome-wide and metabolome-wide association studies. *BMC Bioinformatics* 13: 120, 2012

41. Suhre K, Schwartz JE, Sharma VK, Chen Q, Lee JR, Muthukumar T, Dadhania DM, Ding R, Ikle DN, Bridges ND, Williams NM, Kastenmüller G, Karoly ED, Mohney RP, Abecassis M, Friedewald J, Knechtle SJ, Becker YT, Samstein B, Shaked A, Gross SS, Suthanthiran M: Urine Metabolite Profiles Predictive of Human Kidney Allograft Status. *J Am Soc Nephrol* 27: 626–636, 2016

42. Spiegelman D, Hertzmark E: Easy SAS calculations for risk or prevalence ratios and differences. *Am J Epidemiol* 162: 199–200, 2005