Association of Transcobalamin II (*TCN2*) and Transcobalamin II-Receptor (*TCblR*) Genetic Variations With Cobalamin Deficiency Parameters in Elderly Women

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Abstract

Cobalamin (vitamin B12) deficiency is a subtle progressive clinical disorder, affecting nearly 1 in 5 individuals > 60 years old. This deficiency is produced by age-related decreases in nutrient absorption, medications that interfere with vitamin B12 absorption, and other comorbidities. Clinical heterogeneity confounds symptom detection for elderly adults, as deficiency sequelae range from mild fatigue and weakness to debilitating megaloblastic anemia and permanent neuropathic injury. A better understanding of genetic factors that contribute to cobalamin deficiency in the elderly would allow for targeted nursing care and preventive interventions. We tested for associations of common variants in genes involved in cobalamin transport and homeostasis with metabolic indicators of cobalamin deficiency (homocysteine and methylmalonic acid) as well as hematologic, neurologic, and functional performance features of cobalamin deficiency in 789 participants of the Women's Health and Aging Studies. Although not significant when corrected for multiple testing, eight single nucleotide polymorphisms (SNPs) in two genes, transcobalamin II (*TCN2*) and the transcobalamin II-receptor (*TCblR*), were found to influence several clinical traits of cobalamin deficiency. The three most significant findings were the identified associations involving missense coding SNPs, namely, *TCblR* G220R (rs2336573) with serum cobalamin, *TCN2* S348F (rs9621049) with homocysteine, and *TCN2* P259R (rs1801198) with red blood cell mean corpuscular volume. These SNPs may modify the phenotype in older adults who are more likely to develop symptoms of vitamin B12 malabsorption.

Keywords

cobalamin, vitamin B12 deficiency, transcobalamin, genetic association, pernicious anemia, older adults

To maintain health, humans must have an adequate intake of cobalamin (vitamin B12) and retain the ability to absorb and assimilate the vitamin. Absorption of cobalamin is dependent upon the coordinated actions of binding proteins that attach to ingested dietary protein in the stomach and intestine and a family of receptors that facilitate entry into the plasma and tissues (Herrmann, Obeid, Schorr, & Geisel, 2003; Rosenblatt & Fenton, 2001). After an ileal enterocyte absorbs dietary cobalamin bound to intrinsic factor from the intestinal lumen, it is paired with the transport protein transcobalamin II (TCN2), released into the portal circulation, and carried to cells in target tissues. Under normal physiologic conditions, TCN2 is 20–30% saturated and represents 10–20% of the total cobalamin pool in plasma (Refsum, Johnston, Guttormsen, & Nexo, 2006). TCN2 bound to cobalamin, also called holotranscobalamin (holoTC), is the

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biologically active form of cobalamin in the body and has a half-life of approximately 2 hr (Lindgren, Kilander, Bagge, & Nexo, 1999). The biologically active cobalamin/TCN2 complex binds to the transcobalamin II-receptor (TCblR) found in the plasma membrane of most cell types. The receptor/TCN2/cobalamin complex is endocytosed and transported to several subcellular compartments (Christensen & Birn, 2002). Approximately 80% of the circulating cobalamin is bound to transcobalamin I and III (haptocorrin). This complex remains in the circulation with a 10-day half-life (Carmel, 1985). In addition to acting as a storage pool for cobalamin, haptocorrin removes potentially toxic cobalamin analogs from the bloodstream. Haptocorrin binds to the liver-specific asialoglycoprotein receptor. As these receptors are not expressed in other tissues, the role of haptocorrin in transporting the active form of cobalamin is unclear (Burger, Schneider, Mehlman, & Allen, 1975; Hardlei & Nexo, 2009).

Biochemically, cobalamin is a cofactor for two essential enzymatic reactions. In the first, methylcobalamin serves as a cofactor for the cytoplasmic enzyme methionine synthase. This enzyme transfers methyl groups from 5-methyl-tetrahydrofolate to homocysteine, producing tetrahydrofolate for nucleic acid synthesis and methionine for methylation reactions and protein synthesis. The second cobalamin-dependent reaction is catalyzed by the mitochondrial enzyme L-methylmalonyl coenzyme A mutase to produce succinyl coenzyme-A, which enters the Krebs cycle (Stabler, 2013). In the event of decreased cobalamin availability, homocysteine and methylmalonic acid (MMA, a by-product of methyl-malonyl coenzyme-A) accumulate in blood. Homocysteine is also elevated in folate deficiency. For this reason, homocysteine and MMA are commonly used as deficiency indicators in addition to serum cobalamin levels, as normal serum cobalamin concentrations can occur in the presence of clinical symptoms (Stabler, 2013).

The pathophysiology of cobalamin deficiency manifests hematologic and neurologic sequelae. When cobalamin availability is low, nucleic acid synthesis is compromised (reviewed in Shane & Stokstad, 1985). In rapidly dividing hematopoietic precursor cells, abnormal DNA synthesis halts cell division, which affects up to 90% of a patient's red blood cell precursors (Aster, 2005). Clinical testing shows decreased hemoglobin concentration and elevated mean corpuscular volume (MCV), the joint presence of which is a classic hallmark indicator of megaloblastic anemia (Andres et al., 2006). Because cobalamin metabolic pathways overlap with folate metabolism in the cytoplasm, megaloblastic work-ups must always include evaluation of a cobalamin metabolite profile in addition to serum folate. Well-meaning health care providers aiming to treat megaloblastic anemia with folic acid without fully reviewing metabolic cobalamin parameters can improve the patient's hematologic status while leaving neurologic pathology untreated and likely to progress (Pfeiffer, Caudill, Gunter, Osterloh, & Sampson, 2005).

Cobalamin deficiency has long been associated with age-related neuropathology, but the molecular mechanisms remain unknown (reviewed in Reynolds, 2006). In older adults, cobalamin deficiency can present with a heterogeneous preclinical spectrum impacting multiple facets of health. For example, older adults with preclinical cobalamin deficiency experience nonspecific symptoms erroneously attributed to aging, including malaise, vertigo, fatigue, mood alterations such as depression, and sleep and gait disturbances (Carmel, 2000). In a number of studies, supplementation with a combination of vitamin B12, vitamin B6, and folic acid has slowed the rate of brain atrophy and cognitive decline, suggesting a potential role for vitamin B12 in these age-related neurological changes (de Jager, Oulhaj, Jacoby, Refsum, & Smith, 2012; Douaud et al., 2013; Smith et al., 2010). Factors such as medications that impair absorption, age-related decreases in gastric function, and comorbidities that drive increased needs result in a high prevalence of cobalamin deficiency for older adults, estimated at 6% to more than 40% (Allen, 2009; Baik & Russell, 1999; Johnson et al., 2003; Lindenbaum, Rosenberg, Wilson, Stabler, & Allen, 1994; Pennypacker et al., 1992). Unrecognized cobalamin deficiency in older adults may result in an exaggerated disability trajectory involving higher frequency of hospital admissions, lengthier and more severe hospitalizations, or greater degrees of chronic disablement significantly affecting mobility and quality of life (Bartali et al., 2006). Recently, the geriatric syndrome of frailty, a chronic and progressive disablement stemming from age-related declines in energy metabolism and the inflammatory, endocrine, skeletal muscle, and neurologic systems, has been linked to the cobalamin metabolite MMA (Matteini et al., 2008).

Disablement and frailty in elderly persons is a significant public health concern because of increased vulnerability to morbidity, mortality, and adverse health outcomes (Fried et al., 2001). Preventing or delaying the disablement and frailty process in older adults is a key area of research, and improved understanding of the genetic factors that contribute to its development may allow the identification of susceptible individuals and suggest personalized strategies for intervention and prevention (Walston et al., 2006). For example, nurses caring for elderly patients experiencing vague, nonspecific symptoms could query their genetic makeup and combine this with laboratory tests to determine if cobalamin could be used to prevent disability or frailty.

Genetic variants affecting proteins that transport cobalamin in blood and deliver it into cells may influence the amount of cobalamin available to cells. The TCN2 carrier molecule and its receptor (TCblR) are an important ligand and receptor in which structural variations can affect vitamin B12 binding. Altered TCN2 structure results in decreased cellular availability for essential metabolic reactions and influences susceptibility to deficiency (Miller, Ramos, Garrod, Flynn, & Green, 2002). To explore these factors, we tested for associations between common genetic variants in the *TCN2* and *TCblR* genes and cobalamin-related biochemical and clinical

outcomes in 795 Women's Health and Aging Studies (WHAS) participants.

Materials and Methods

Study Design and Population

We conducted a candidate gene association study using genetic material from participants in the WHAS. Because allele frequencies and physiologic and metabolic traits can differ between Caucasians and African Americans, we included genetic variants unique to each population and used race and genotype as independent variables in our study design (1000 Genomes Project Consortium et al., 2012; Cheng, Chan, Cembrowski, & van Assendelft, 2004; Stabler et al., 1999).

Previous authors have described the WHAS in detail (Fried, Bandeen-Roche, Chaves, & Johnson, 2000; Guralnik, Fried, Simonsick, Bandeen-Roche, & Kasper, 1995). In brief, WHAS 1 (1992–1995) and WHAS 2 (1994–1996) were prospective, observational research initiatives examining physical-disability trajectories in community-dwelling elderly women in Baltimore, MD. WHAS 1 sampled the one-third most disabled women and WHAS 2 sampled the two-thirds least disabled women; WHAS subject data can be pooled to represent the community-dwelling elderly (Bandeen-Roche et al., 2006; Chaves, Ashar, Guralnik, & Fried, 2002; Chaves et al., 2005; Leng, Xue, Tian, Walston, & Fried, 2007; Semba, Garrett, Johnson, Guralnik, & Fried, 2000; Semba et al., 2005; Walston et al., 2005). The WHAS protocols were approved by the Johns Hopkins Medical Institutions Institutional Review Board. The genotyping portion of this research was approved by the Office of Human Subjects Research at the National Institutes of Health and the Institutional Review Board of the University of Utah.

Outcome Variable Selection

The overarching hypothesis of this study was that genetic variation contributes to the clinical heterogeneity observed for cobalamin deficiency in older adults. The use of a population with well-characterized and measured phenotypes is instrumental in exploring genetic associations. For this reason, we generated genetic data on blood samples from WHAS to take advantage of the extensive and sophisticated clinical trait information available on these study participants.

We selected nine outcome variables from the broader WHAS data set to reflect a cobalamin deficiency phenotype in the older adult; these include biochemical, hematologic, neurologic, and functional performance parameters. For biochemical outcomes, we chose measurements commonly used in the diagnosis and management of cobalamin deficiency, namely, serum cobalamin, homocysteine, and MMA. For hematologic outcomes, we chose hemoglobin and MCV, as low cobalamin produces hallmark changes of megaloblastic anemia. For neurologic outcomes, we chose the following clinical measures known to be associated with age-related cobalamin deficiency: depression, as measured by the Geriatric Depression Scale (GDS) and peripheral neuropathy, as measured by vibration perception testing (VPT). For functional performance outcomes, we used measures of decreased muscular strength and capacity that indicate altered mitochondrial energy/oxidation states, that is, hand grip strength and 4-meter walking speed.

Blood Analyte Measurements

Measurements of serum cobalamin, folate, creatinine, homocysteine, MMA (Penninx et al., 2000; Stabler et al., 1999), hemoglobin, and MCV (Chaves et al., 2006; Williamson et al., 1995) were obtained as previously reported.

Previous studies of elderly individuals have demonstrated that modest elevations in homocysteine and MMA can occur as a result of renal insufficiency (Lindenbaum et al., 1994; Pennypacker et al., 1992; Stabler, Lindenbaum, & Allen, 1996). However, in older adults, singular use of serum creatinine as a kidney function assessment is recognized to significantly underascertain presence of impaired renal status (Giannelli et al., 2007). In this study, we used serum creatinine to estimate creatinine clearance for WHAS 1 and 2 subjects through the Cockcroft–Gault formula: creatinine clearance $(ml/s) = $ weight $(kg) \times [140 - $ age $(years)]/[72 \times$ serum creatinine $(mg/dl) \times 0.85]$ (Cockcroft & Gault, 1976).

Neurologic Clinical Measures

Depression. Depressive symptoms were evaluated with the GDS, a psychometric depression assessment for elderly adults (Feher, Larrabee, & Crook, 1992; Yesavage et al., 1982). The GDS is a validated 30-item survey comprising dichotomous (yes/no) questions and requiring approximately 8 min for administration.

Peripheral neuropathy. VPT was conducted to assess for peripheral neuropathy. As WHAS 1 protocols used a vibrometer and WHAS 2 protocols used a tuning fork, WHAS 2 subject data were excluded to ensure reliability. In a test modeled after a diabetic neuropathy protocol, subjects placed their right toe on the Vibratron II's (Physitemp Instrument, Inc., Clifton, NJ) platform and reported a vibratory stimulus using a yes/no forced choice procedure (Ferrucci, Kittner, Corti, & Guralnik, 1995; Maser et al., 1989). Measured vibration units were converted to microns and a mean vibratory threshold was identified (Resnick, Vinik, Heimovitz, Brancati, & Guralnik, 2001; Volpato, Leveille, Blaum, Fried, & Guralnik, 2005).

Functional Performance Measures

Hand grip strength. A JAMAR hand dynamometer was used to measure hand grip strength (Model #BK-7498; Fred Sammons Inc., Burr Ridge, IL). Testing was performed with subjects in a seated position and elbow flexure at a 90° angle. Subjects were asked to grasp the dynamometer and squeeze as hard as possible 3 times on each hand; the best measure in the stronger hand was recorded.

4-meter walking speed. Subjects walked a 4-meter course at their usual speed two times and once more as fast as possible. Using the faster of the two usual-pace walks, average walking speed was calculated by dividing the length of the walk (in meters) by the time in seconds required to complete it. Subjects were permitted to use a cane, walker, or walking aid but not assistance from another person.

Genetic Variant Measurement

Single nucleotide polymorphism (SNP) selection. SNPs were selected to cover common variation in *TCN2* and *TCblR* using public databases (HapMap [Phases 1 and 2 full data set], dbSNP [Build 125], and NCBI [Build 35]). For both Caucasian and African American ancestral groups, Haploview (4.0) Tagger (Broad Institute) was used to select tag SNPs in the candidate genes, including 10 kilobases (kb) flanks on both ends of the genes. Because both candidate genes were relatively small (19 kb and 6 kb) and there was high likelihood of dropped assays due to working with whole-genome amplified (WGA) material, we built redundancy into our assays. An r^2 threshold was set at 0.9 and minor allele frequency at 0.05. Additional candidate SNPs included variants with probability of exerting a functional effect, such as those located in or near exons or promoters. A total of 51 SNPs were selected, that is, 29 SNPs in *TCN2* and 22 SNPs in *TCblR*.

SNP genotyping. Genotyping of the WHAS WGA samples was performed using the Sequenom MassArray iPLEX platform (San Diego, CA). Allele-specific primer extension products were resolved using matrix-assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectroscopy. Primer sequences (Supplementary Table 1) were designed and used as described in the published protocol (Gabriel, Ziaugra, & Tabbaa, 2009).

To ensure quality of genotypes obtained with WGA DNA, all samples were genotyped twice and unresolved discordant genotypes dropped. For each SNP, an average of 93.8% (range: 90.6–97.1%) of all samples were successfully typed in duplicate. The average concordance of duplicate genotypes was 99.3% (range: 97.8–100%). The average call rate was 95.2% (range: 92.2–98.4%). SNPs with large departures from Hardy–Weinberg equilibrium (HWE, $p < .001$) in either racial subset were excluded. The only exception was the retention of *TCblR* rs2336573 ($p = 4.9 \times 10^{-13}$) because previous work indicates that genotyping error is an unlikely explanation for failure to adhere to HWE for this SNP (Pangilinan et al., 2010).

Statistical Analysis

Descriptive and inferential statistics were analyzed using the SPSS program (v12.0 and v20.0). For the study's sociodemographic and health characteristics and outcome variables, summary statistics, frequency distributions, and independent *t*-tests were evaluated. Assessment of the outcome and covariate variables for linearity, homogeneity of variance, homogeneity of regression slopes, and measurement reliability indicated no

Table 1. Selected Sociodemographic and Health Characteristics for WHAS Subjects.

Characteristic	African American Mean (SD) $n = 198$	Caucasian Mean (SD) $n = 591$
Age (years)	75.1 (6.4)*	76.7 (6.8)
Education (years completed)	9.1 (3.5)*	11.3 (3.8)
No. chronic diseases[a]	1.3 (1.5)	1.0 (1.3)
BMI (kg/m^2)[b]	31.0 (14.3)*	27.2 (5.7)

Note. BMI = body mass index; WHAS = Women's Health and Aging Study.
[a]Self-reported diseases include angina, myocardial infarction, coronary artery disease, congestive heart failure, peripheral artery disease, stroke, diabetes mellitus, and cancer.
[b]For BMI measures, $n = 181$ for African Americans and 562 for Caucasians.
*Significant differences from independent *t*-tests between African American and Caucasian subjects ($p < .01$).

violations of statistical assumptions. Severe outliers (>3 standard deviations away from the mean) were removed from analysis.

Two-way analysis of variance (ANOVA) and covariance (ANCOVA) tests were conducted to evaluate associations between the biochemical, hematological, neurologic, and physical-performance outcome parameters and the two independent variables, SNP genotype and race. WHAS subjects were placed into one of the three genetic categories (AA, AB, and BB) according to their genotypes and stratified by race. Several outcomes in the two-way ANCOVA models were adjusted for appropriate covariates. Significant ($p < .05$) SNP main effect *F* tests were explored further using the Tukey least significant difference procedure. The Bonferroni adjustment was used to correct for α inflation arising from multiple testing.

Results

Demographic and Clinical Characteristics

Demographic and other baseline health characteristics of the 789 WHAS study participants are shown in Table 1. The 591 Caucasian participants represented 75% of the study sample and had more years of total education and lower mean body mass index. Biochemical and clinical outcome summary statistics are shown in Table 2. There were significant differences between Caucasians and African Americans, making race an important independent variable. Because there are several hundred publications devoted to investigation of WHAS subjects, we do not provide a detailed discussion of sample characteristics here (Chaves, Garrett, & Fried, 2000; Ferrucci, Guralnik, et al., 1995; Fried et al., 2000; Kasper & Simonsick, 1995; Penninx et al., 2000; Stabler et al., 1999; Williamson et al., 1995).

Genetic Data

Of the total 51 SNPs selected for genotyping in *TCN2* and *TCblR*, 38 SNPs were successfully genotyped. Of these, 6 SNPs demonstrated significant, unexplained HWE departures and were removed from analysis. Genotype and allele frequencies

Table 2. Outcome Variable Summary Statistics by Race.

| | African American | | Caucasian | |
| | (n = 198) | | (n = 591) | |
Outcome Variable	n	Mean (SD)	n	Mean (SD)
Cobalamin (pmol/L)	192	415.4 (194.8)*	580	340.1 (163.8)
Homocysteine (μmol/L)	188	11.0 (4.1)	570	10.6 (3.8)
Methylmalonic acid (nmol/L)	189	209 (133)*	565	253 (141)
Hemoglobin (gm/dl)	185	12.4 (1.5)*	580	13.3 (1.2)
Mean corpuscular volume (fl)	184	90.3 (6.4)*	560	94.2 (5.2)
Geriatric Depression Scale score	198	6.6 (5.0)	591	6.7 (5.6)
Peripheral neuropathy[a] (μ)	144	8.4 (4.6)	354	8.3 (4.4)
Hand grip strength (kg)	172	23.2 (6.3)*	546	21.7 (5.7)
4-meter walking speed (m/s)	188	0.6 (0.3)*	579	0.8 (0.4)

[a]Vibratron II peripheral neuropathy measurements for Women's Health and Aging Study 1 participants only.
*Significant differences in means from independent t-tests between African American and Caucasian subjects (p < .01).

for the remaining genotyped SNPs are presented in Table 3. Of these, 11 SNPs had minor allele frequencies <0.05 in both Caucasian and African American populations and were excluded. A final total of 21 SNPs were entered into the ANOVA and ANCOVA models: 14 SNPs from the *TCN2* gene and 7 from the *TCblR* gene. Bonferroni adjustment for multiple testing of the 21 SNPs across the nine study outcomes yielded a corrected α significance threshold of 0.0003 (0.05/189).

ANOVA and ANCOVA Findings

Table 4 provides a summary of the significant genetic associations we obtained. Due to well-characterized differences between Caucasians and African Americans in the WHAS sample for most study outcomes, we highlight only significant genetic findings.

No genetic main effect reached the stringent Bonferroni-adjusted threshold level of significance of 0.0003. However, eight SNPs were significant at the $p < .01$ or $p < .05$ level. For these eight SNPs, Table 5 summarizes significant post hoc comparison tests at the $p < .05$ and $p < .01$ level observed for genotype group comparisons in the overall WHAS sample and within stratified race groups.

Cobalamin. We identified two significant SNP associations for cobalamin level, *TCN2* rs4820888 and *TCblR* rs2336573. *TCN2* rs4820888 A>G is located in intron 7 and showed a statistically significant main genetic effect for cobalamin, $F(2, 692) = 3.17$, $p = .04$. *TCblR* rs2336573 G>A is a missense polymorphism located in exon 4, resulting in a glycine-to-arginine amino acid change at codon position 220 (G220R). There was a significant association between rs2336573 and serum cobalamin levels, $F(2, 684) = 3.25$, $p = .04$.

Table 3. Genotype and Allele Frequencies for *TCN2* and *TCblR* Single Nucleotide Polymorphisms.

| | African American | | | | | Caucasian | | | | |
SNP	AA	AB	BB	A	B	AA	AB	BB	A	B
TCN2										
rs16988828	5	39	141	0.13	0.87	10	87	454	0.10	0.90
rs7289549	19	67	103	0.28	0.72	11	92	458	0.10	0.90
rs7286107	12	60	117	0.22	0.78	3	1	561	0.01	0.99
rs16988838[a]	1	13	178	0.04	0.96	0	0	575	0.00	1.00
rs740234	4	29	157	0.10	0.90	35	159	371	0.20	0.80
rs11557600[a]	0	0	190	0.00	1.00	0	3	559	0.00	1.00
rs12159600[a]	0	1	190	0.00	1.00	0	0	569	0.00	1.00
rs35838082	9	54	126	0.19	0.81	0	7	562	0.01	0.99
rs17849434[a]	0	0	187	0.00	1.00	0	0	555	0.00	1.00
rs11912238[a]	0	0	186	0.00	1.00	0	0	563	0.00	1.00
rs1801198	16	65	108	0.26	0.74	133	251	183	0.46	0.54
rs4820021	0	6	187	0.02	0.98	14	92	455	0.11	0.89
rs9621049	7	51	134	0.17	0.83	14	101	448	0.11	0.89
rs4820886	4	50	135	0.15	0.85	12	102	450	0.11	0.89
rs4820887	4	27	157	0.09	0.91	9	85	465	0.09	0.91
rs4820888	38	90	62	0.44	0.56	127	235	199	0.44	0.56
rs2301958	11	59	121	0.21	0.79	32	172	368	0.21	0.79
rs1131603[a]	0	1	195	0.00	1.00	2	49	531	0.04	0.96
rs4820889	5	31	154	0.11	0.89	2	23	547	0.02	0.98
rs2072194	4	44	136	0.14	0.86	115	238	200	0.42	0.58
TCblR										
rs173665	7	33	148	0.13	0.88	10	79	466	0.09	0.91
rs250510	3	22	157	0.08	0.92	1	6	539	0.01	0.99
rs2232788[a]	1	13	169	0.04	0.96	0	0	544	0.00	1.00
rs2232787[a]	1	3	186	0.01	0.99	1	0	547	0.00	1.00
rs2232786[a]	2	5	182	0.02	0.98	0	0	558	0.00	1.00
rs2227288	13	50	125	0.20	0.80	12	98	427	0.11	0.89
rs2336573	21	72	94	0.30	0.70	9	35	512	0.05	0.95
rs2232779	2	18	174	0.06	0.94	5	2	573	0.01	0.99
rs2927707	16	51	118	0.22	0.78	57	202	288	0.29	0.71
rs2232768[a]	0	0	186	0.00	1.00	0	0	545	0.00	1.00
rs3760680	29	75	78	0.37	0.63	81	221	242	0.35	0.65
rs36263[a]	1	7	181	0.02	0.98	0	0	545	0.00	1.00

Note. SNP = single nucleotide polymorphism; *TCN2* = transcobalamin II; *TCblR* = transcobalamin II-receptor. SNP data in genomic order as occurring on the chromosome. *A* denotes the minor allele and *B* denotes the major allele.
[a]SNPs with minor allele frequency < .05 in both races; excluded from analyses.

Homocysteine. We identified two significant SNP associations for homocysteine level, *TCN2* rs9621049 and *TCN2* rs4820887. *TCN2* rs9621049 is a C>T missense polymorphism in exon 7 that results in an amino acid change of a serine to phenylalanine at codon position 348 (S348F). This SNP demonstrated the strongest level of statistical significance for a genetic effect, $F(2, 673) = 4.97$, $p = .007$, among all of the SNPs. The second strongest association in this study was between mean homocysteine levels and *TCN2* rs4820887, a G>A SNP in intron 7, $F(2, 666) = 4.73$, $p = .009$.

Hematologic parameters. We identified two significant SNP associations with hematologic parameters, *TCN2* rs2072194 with mean hemoglobin concentration and *TCN2* rs1801198 with MCV. *TCN2* rs2072194 is an A>G

Table 4. *TCN2* and *TCblR* Two-Way Analysis of Covariance (ANCOVA) and Variance (ANOVA) Significant SNP Association Results.

	Interaction	Main SNP Effect	Main Race Effect
Study Outcome	F (p)	F (p)	F (p)
Cobalamin[a]			
TCN2 rs4820888	1.59 (0.21)	3.17 (0.04)*	22.33 (0.00)
TCblR rs2336573	0.27 (0.76)	3.25 (0.04)*	6.78 (0.01)
Homocysteine[b]			
TCN2 rs9621049	2.66 (0.07)	4.97 (0.007)**	6.91 (0.01)
TCN2 rs4820887	4.58 (0.01)	4.73 (0.009)**	7.94 (0.01)
Hemoglobin[c]			
TCN2 rs2072194	5.57 (0.04)	4.10 (0.02)*	50.29 (0.00)
MCV[c]			
TCN2 rs1801198	5.37 (0.01)	3.73 (0.03)*	66.29 (0.00)
Peripheral neuropathy			
TCN2 rs4820886	1.29 (0.28)	3.67 (0.03)*	1.10 (0.30)
4-meter walking speed[d]			
TCN2 rs7286107	1.52 (0.22)	4.18 (0.02)*	0.54 (0.47)

Note. MCV = mean corpuscular volume; SNP = single nucleotide polymorphism. Complete F tests are shown for only the SNPs with significant genetic effects. Results do not approach the Bonferroni corrected threshold of 0.0003.
[a]Covariate: creatinine clearance. [b]Covariates: creatinine clearance, serum folate, and serum cobalamin. [c]Covariate: serum folate. [d]Covariate: standing height.
*Uncorrected significant main SNP effect results at $p < .05$
**Uncorrected significant main SNP effect results at $p < .01$.

polymorphism in intron 8 of the *TCN2* gene and was significantly associated with mean hemoglobin concentrations of WHAS subjects, $F(2, 700) = 4.10$, $p = .02$. *TCN2* rs1801198 is a missense C>G polymorphism in exon 6 that results in an amino acid change of a proline to an arginine at codon position 259 (P259R). It demonstrated a significant association with MCV, $F(2, 709) = 3.73$, $p = .03$. Important to interpreting this result is the difference between the Caucasian subjects' mean MCV (94.2 fl) and the African American subjects' mean MCV (90.3 fl). We observed a bimodal peak in the outcome variable's distribution curve, and Levene's test for equality of variances was violated (data not shown). SNPs for this outcome not meeting the more stringent Bonferroni-corrected α significance levels should be cautiously interpreted (Tabachnik & Fidell, 2007). For this study, it may be reasonable to view the positive association for rs1801198 with additional caution.

Neurologic parameters. We identified one significant SNP association with a neurologic parameter, *TCN2* rs4820886 with peripheral neuropathy mean micron measurements. *TCN2* rs4820886 is a G>T polymorphism in intron 7 and demonstrated a significant main genetic effect, $F(2, 470) = 3.67$, $p = .03$.

Functional performance parameters. One SNP in *TCN2*, rs7286107, was associated with 4-meter walking speed. *TCN2* rs7286107 is a C>T polymorphism in intron 1 and is close to an area known to impact gene regulation. *TCN2* rs7286107

Table 5. Post Hoc Results, Women's Health and Aging Study (WHAS) Participant Means and Sample Sizes by Race for Significant Single Nucleotide Polymorphism (SNP) F-tests.

	Overall	African American	Caucasian
Trait and SNP	Mean (n)	Mean (n)	Mean (n)
Cobalamin (pmol/L)			
TCN2 rs4820888			
GG	346.7 (151)	398.1 (35)	331.1 (116)
AG	349.9 (302)	385.5 (79)	337.3 (223)
AA	373.8 (239)	459.4 (53)	349.5 (186)
	AG|AA $p = .02$*		
TCblR rs2336573			
AA	424.9 (30)	455.0 (21)	354.7 (9)
AG	422.5 (97)	444.2 (65)	378.2 (32)
GG	343.7 (557)	384.1 (78)	337.2 (479)
	AG|GG $p = .02$*	AG|GG $p = .04$*	
Homocysteine (μmol/L)			
TCN2 rs9621049			
TT	12.5 (21)	13.8 (7)	11.8 (14)
TC	10.6 (130)	10.1 (40)	10.8 (90)
CC	10.5 (522)	11.0 (114)	10.4 (408)
	TT|TC $p < .01$**	TT|TC $p < .01$**	
	TT|CC $p < .01$**	TT|CC $p < .01$**	
TCN2 rs4820887			
AA	12.9 (12)	17.0 (3)	11.5 (9)
GA	10.6 (96)	9.1 (20)	11.0 (76)
GG	10.6 (558)	11.1 (134)	10.4 (424)
	AA|GA $p < .01$**	AA|GA $p < .01$**	
	AA|GG $p < .01$**	AA|GG $p < .01$**	
Hemoglobin (gm/dl)			
TCN2 rs2072194			
GG	13.3 (114)	10.3 (4)	13.4 (110)
GA	13.1 (267)	12.4 (43)	13.3 (224)
AA	12.9 (316)	12.3 (123)	13.2 (193)
	GG|GA $p < .01$**	GG|GA $p < .01$**	
	GG|AA $p < .01$**	GG|AA $p < .01$**	
MCV (fl)			
TCN2 rs1801198			
GG	93.5 (143)	86.2 (14)	94.3 (129)
CG	93.4 (296)	91.4 (63)	94.0 (233)
CC	92.8 (270)	89.7 (97)	94.5 (173)
	GG|CG $p < .01$**		
	GG|CC $p = .03$*		
Peripheral neuropathy (μ)			
TCN2 rs4820886			
GG	8.8 (10)	6.5 (3)	9.8 (7)
GT	7.4 (88)	6.9 (34)	7.6 (54)
TT	8.6 (372)	9.0 (99)	8.5 (273)
	GT|TT $p = .04$*		
4-meter walking speed (m/s)			
TCN2 rs7286107			
CC	0.55 (13)	0.55 (10)	0.56 (3)
CT	0.54 (58)	0.54 (57)	0.33 (1)
TT	0.78 (637)	0.67 (105)	0.35 (532)
	CT|TT $p = .02$*	CT|TT $p = .05$*	CT|TT $p = .04$*

Note. MCV = mean corpuscular volume. Significant post hoc comparison test results shown.
*$p < .05$. **$p < .01$.

was significantly associated with mean walking speeds, $F(2, 708) = 4.18$, $p = .02$.

Discussion

We examined the associations of genetic variants with selected metabolic and clinical outcome parameters in WHAS participants, including serum cobalamin, its two primary metabolites (homocysteine and MMA), and six cobalamin-deficiency clinical phenotype characteristics. Although no result reached the stringent Bonferroni threshold of $p = .0003$, eight variants were significant at the $p = .007$ to $p = .04$ level. Of these, five variants (*TCN2* rs9621049, *TCN2* rs4820887, *TCN2* rs2072194, *TCN2* rs4820886, and *TCN2* rs7286107) had decreased cell sizes (<10), where genotype group differences in the overall WHAS sample could affect strength of the reported association. Stratifying the sample clinical characteristics produces some groups with small numbers, making adequate comparisons difficult for some SNPs in this study. Population genotype distributions in this study are similar to those reported in Hap-Map data.

Three associations involved coding SNPs (*TCblR* rs2336573, *TCN2* rs9621049, and *TCN2* rs1801198). The first is an association between the *TCblR* G220R missense coding SNP (rs2336573) and the mean serum cobalamin concentrations. Regardless of race and accounting for estimated creatinine clearance, individuals with a GG (220GG) genotype had significantly lower mean serum cobalamin, demonstrating a difference of 81.2 pmol/L from subjects with an AA (220RR) genotype. This SNP is strongly linked to a neighboring SNP (*TCblR* rs150384171, $D' = 1$, $r^2 = 0.39$) on the same chromosome, a phenomenon called linkage disequilibrium (LD). The *TCblR* rs150384171 SNP is a three base-pair deletion in exon 2 that results in a glutamic acid deletion. We previously reported the stronger influence of *TCblR* rs150384171 on serum cobalamin compared to an SNP in perfect LD with *TCblR* rs2336573 (rs9426, $D' = 1$, $r^2 = 1$; Stone et al., 2011). Although direct testing of rs150384171 was not possible in our WHAS sample due to genotyping platform incompatibility and low minor allele frequency (<0.01), it remains the best candidate for being the underlying causal variant driving the detected association between serum cobalamin and *TCblR* rs2336573.

A second key finding was the association between the *TCN2* S348F missense SNP (rs9621049) and the serum homocysteine levels. After adjusting for several covariates, TT (348FF) homozygotes have ~ 2.0 μmol/L higher mean homocysteine concentrations than CC (348SS) homozygotes or CT (348SF) heterozygotes ($p = .003$). Although the strength of this association is not definitive, we obtained very similar results for *TCN2* rs4820887, in intron 7. *TCN2* rs9621049 and rs4820887 share significant LD ($D' = 1$, $r^2 = 0.678$), indicating that they are not independent. As a missense SNP, rs9621049 (S348F) is an attractive candidate for being functional and directly influencing homocysteine levels.

The association between *TCN2* S348F (rs9621049) and homocysteine we found in this study is not consistent with findings of a previous study. Lievers et al. (2002) failed to find an association between homocysteine and *TCN2* S348F (rs9621049) but did find an association between the *TCN2* P259R missense SNP (rs1801198) and homocysteine that was dependent upon total serum cobalamin level. These differences may be due to dissimilarity in patient sampling and demographics, as Lievers et al. (2002) studied an ethnically Dutch population of 190 middle-aged individuals with cardiovascular disease and 601 normal healthy control subjects.

Although *TCN2* rs1801198 was not associated with homocysteine in our study, it was associated with MCV—our third key finding. After accounting for serum folate, individuals with a GG (259RR) genotype had significantly higher MCV, demonstrating a 0.7 fl difference from subjects with a CC (259PP) genotype. Interestingly, African Americans with a GG genotype had significantly lower MCV values compared to other genotype groups, which contrasted with the overall P259R GG genetic effect. Based on the distribution of MCV by genotype in each race (Table 5 and data not shown), this result may be driven by a race-specific effect in African Americans. The racial differences in our MCV data are consistent with published population-based hematologic studies indicating that African American individuals' hemoglobin and MCV are biologically lower than those of Caucasian individuals (Cheng et al., 2004).

Although this research is the first to report an association between *TCN2* rs1801198 and the MCV trait, previous research has suggested that this SNP exerts a functional biologic effect. Miller, Ramos, Garrod, Flynn, and Green (2002) reported that the rs1801198 genotype influenced serum holoTC and MMA concentrations in a sample of 128 healthy adults at the University of Missouri. A clinical study identified interactions between total serum cobalamin on homocysteine and serum holoTC on homocysteine, as modified by *TCN2* P259R (rs1801198) SNP genotype, in a cohort of 554 community-dwelling elderly Latinos in northern California (Garrod, Allen, Haan, Green, & Miller, 2010). A Norwegian study of 10,601 adult cancer–registry patients identified a positive association between *TCN2* rs1801198 genotype and serum MMA (Fredriksen et al., 2007). And finally, an Irish study of 613 adult men demonstrated that homozygous patients with the C allele for rs1801198 had lower serum cobalamin and homocysteine concentrations (Stanislawska-Sachadyn et al., 2010). Although our results should be interpreted with caution, there is precedent for our P259R finding, as this SNP has been reported to be associated with other cobalamin-related traits.

The work outlined in this study extends the previously published WHAS reports on the evaluation of MMA with SNPs in *TCN2* (Matteini et al., 2008, 2010). In these previous candidate gene analyses, the authors reported no significant associations between MMA levels and *TCN2* SNPs. However, the WHAS sample size of 326 Caucasians in those studies was substantially less compared to the presently reported research (591 Caucasians) and did not include African Americans.

Strengths and Limitations

Strengths of this research include the use of a well-characterized elder adult cohort to ascertain effects of genetic variation on metabolic parameters of cobalamin deficiency. As there was limited quantity of genomic starting material, this study demonstrates that use of whole-genome amplification is a sound technique to augment previous clinical and epidemiological research initiatives on archived samples. Also notable is that this investigation includes examination of genetic variation of two genes coding for protein products that directly interact at the level of the cell.

A key limitation of this study is failure to generate high-quality data for all *TCN2* and *TCblR* SNPs in every study subject. This failure stemmed from implementation of stringent quality control criteria and genotyping challenges associated with using WGA material produced from limited DNA in storage for nearly 20 years. We anticipated this difficulty and designed assays to redundantly cover these genes. Discounting four Caucasian-specific SNPs and nine African American–specific SNPs in these two genes, our coverage was nearly complete at a stringent r^2 threshold of 0.9.

Another limitation to this work is that environmental factors (i.e., medications and comorbidities) known to affect cobalamin deficiency were not included in these analyses. Because many of the SNPs had low allele frequencies in African American and Caucasian subgroups and several covariates were already used, additional covariates would have further reduced power and removed our ability to detect genetic effects.

Finally, this study measured total serum cobalamin as opposed to holoTC concentration; only 20% of the total cobalamin is bound by transcobalamin II, with the remaining 80% bound to haptocorrin. However, previous WHAS investigation of cobalamin metabolites did not include holoTC concentration and there was insufficient participant sample volume to run this test.

Nursing Implications

Despite the limitations of this study, our findings suggest that there are additional biologic factors contributing to an elderly adult's susceptibility to decreased cobalamin nutritional status. In that the estimated frequency of this condition ranges from 6% to more than 40% and the disability trajectories are more severe for these patients, nursing interventions could be designed for individuals at risk of progressing from subclinical disease to full blown deficiency. For example, older adults presenting with vague, heterogeneous preclinical symptoms (e.g., malaise, vertigo, fatigue, mood alterations, peripheral insensitivity, sleeping, and gait disturbances) and demonstrating a higher risk SNP profile in key cobalamin metabolism genes may be candidates for cobalamin-deficiency testing and benefit from early intervention with regular cobalamin supplementation via intramuscular injections.

Presently, it is not known whether elevated cobalamin metabolites are mediated by genetic factors such as the SNPs in this study and an older adult's cobalamin intake. Although genome-wide associations studies have identified new cobalamin-related genes (Hazra et al., 2009; Tanaka et al., 2009), this study focused on evaluating all common variants in two candidate genes involved in cobalamin delivery to cells. Additional nursing research in more highly powered and diverse patient cohorts is needed to replicate the *TCblR* rs2336573 (G220R), *TCN2* rs9621049 (S348F), and *TCN2* rs1801198 SNP (P259R) findings reported here. However, this work may contribute to identification of valuable cobalamin-related targets for future studies, leading to more accurate diagnostics and supplementation regimens for older adults experiencing preclinical symptoms.

Supplemental Materials

The online data supplements are available at http://brn.sagepub.com/supplemental.

References

1000 Genomes Project Consortium, Abecasis, G. R., Auton, A., Brooks, L. D., DePristo, M. A., Durbin, R. M., . . . McVean, G. A. (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature*, *491*, 56–65.

Allen, L. H. (2009). How common is vitamin B-12 deficiency? *American Journal of Clinical Nutrition*, *89*, 693S–696S.

Andres, E., Affenberger, S., Zimmer, J., Vinzio, S., Grosu, D., Pistol, G., . . . Blickle, J. F. (2006). Current hematological findings in cobalamin deficiency. A study of 201 consecutive patients with documented cobalamin deficiency. *Clinical and Laboratory Haematology*, *28*, 50–56.

Aster, J. (2005). Red blood cell bleeding disorders: Anemias of diminished erythropoesis. In V. Kumar, A. Abbas & N. Fausto (Eds.), *Robbins and Cotran pathologic basis of disease* (7th ed., pp. 638–642). Philadelphia, PA: Elsevier Saunders.

Baik, H. W., & Russell, R. M. (1999). Vitamin B12 deficiency in the elderly. *Annual Review of Nutrition*, *19*, 357–377.

Bandeen-Roche, K., Xue, Q. L., Ferrucci, L., Walston, J., Guralnik, J. M., Chaves, P., . . . Fried, L. P. (2006). Phenotype of frailty: Characterization in the women's health and aging studies. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *61*, 262–266.

Bartali, B., Semba, R. D., Frongillo, E. A., Varadhan, R., Ricks, M. O., Blaum, C. S., . . . Fried, L. P. (2006). Low micronutrient levels as a predictor of incident disability in older women. *Archives of Internal Medicine*, *166*, 2335–2340.

Burger, R. L., Schneider, R. J., Mehlman, C. S., & Allen, R. H. (1975). Human plasma R-type vitamin B12-binding proteins. II. The role of transcobalamin I, transcobalamin III, and the normal granulocyte vitamin B12-binding protein in the plasma transport of vitamin B12. *Journal of Biological Chemistry*, *250*, 7707–7713.

Carmel, R. (1985). The distribution of endogenous cobalamin among cobalamin-binding proteins in the blood in normal and abnormal states. *American Journal of Clinical Nutrition*, *41*, 713–719.

Carmel, R. (2000). Current concepts in cobalamin deficiency. *Annual Review of Medicine*, *51*, 357–375.

Chaves, P. H., Ashar, B., Guralnik, J. M., & Fried, L. P. (2002). Looking at the relationship between hemoglobin concentration and prevalent mobility difficulty in older women. Should the criteria currently used to define anemia in older people be reevaluated? *Journal of the American Geriatrics Society*, *50*, 1257–1264.

Chaves, P. H., Carlson, M. C., Ferrucci, L., Guralnik, J. M., Semba, R., & Fried, L. P. (2006). Association between mild anemia and executive function impairment in community-dwelling older women: The women's health and aging study II. *Journal of the American Geriatrics Society*, *54*, 1429–1435.

Chaves, P. H., Garrett, E. S., & Fried, L. P. (2000). Predicting the risk of mobility difficulty in older women with screening nomograms: The women's health and aging study II. *Archives of Internal Medicine*, *160*, 2525–2533.

Chaves, P. H., Semba, R. D., Leng, S. X., Woodman, R. C., Ferrucci, L., Guralnik, J. M., & Fried, L. P. (2005). Impact of anemia and cardiovascular disease on frailty status of community-dwelling older women: The women's health and aging studies I and II. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*, 729–735.

Cheng, C. K., Chan, J., Cembrowski, G. S., & van Assendelft, O. W. (2004). Complete blood count reference interval diagrams derived from NHANES III: Stratification by age, sex, and race. *Laboratory Hematology*, *10*, 42–53.

Christensen, E. I., & Birn, H. (2002). Megalin and cubilin: Multifunctional endocytic receptors. *Nature Reviews Molecular Cell Biology*, *3*, 256–266.

Cockcroft, D. W., & Gault, M. H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*, *16*, 31–41.

de Jager, C. A., Oulhaj, A., Jacoby, R., Refsum, H., & Smith, A. D. (2012). Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: A randomized controlled trial. *International Journal of Geriatric Psychiatry*, *27*, 592–600.

Douaud, G., Refsum, H., de Jager, C. A., Jacoby, R., Nichols, T. E., Smith, S. M., & Smith, A. D. (2013). Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. *Proceedings of the National Academy of Sciences, USA*, *110*, 9523–9528.

Feher, E. P., Larrabee, G. J., & Crook, T. H., 3rd. (1992). Factors attenuating the validity of the geriatric depression scale in a dementia population. *Journal of the American Geriatrics Society*, *40*, 906–909.

Ferrucci, L., Guralnik, J., Bandeen-Roche, K., Lafferty, M. E., Pahor, M., & Fried, L. P. (1995). Physical performance measures. In J. M. Guralnik, L. P. Fried, E. M. Simonsick, J. D. Kasper & M. E. Lafferty (Eds.), *The women's health andaAging study: Health and social characteristics of older women with disability* (NIH Publication No. 95-4009, pp. 35–49). Bethesda, MD: National Institutes of Health, National Institute on Aging.

Ferrucci, L., Kittner, S. J., Corti, C., & Guralnik, J. M. (1995). Neurological conditions. In J. M. Guralnik, L. P. Fried, E. M. Simonsick, J. D. Kasper & M. E. Lafferty (Eds.), *The women's health and aging study: Health and social characteristics of older women with disability* (NIH Publication No. 95-4009, pp. 140–151). Bethesda, MD: National Institutes of Health, National Institute on Aging.

Fredriksen, A., Meyer, K., Ueland, P. M., Vollset, S. E., Grotmol, T., & Schneede, J. (2007). Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. *Human Mutation*, *28*, 856–865.

Fried, L. P., Bandeen-Roche, K., Chaves, P. H., & Johnson, B. A. (2000). Preclinical mobility disability predicts incident mobility disability in older women. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *55*, M43–M52.

Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., . . . McBurnie, M. A. (2001). Frailty in older adults: Evidence for a phenotype. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*, M146–M156.

Gabriel, S., Ziaugra, L., & Tabbaa, D. (2009). SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Current Protocols*

in Human Genetics, *60*, 2. 12:2.12.1–2.12.16. doi:10.1002/0471142905.hg0212s60

Garrod, M. G., Allen, L. H., Haan, M. N., Green, R., & Miller, J. W. (2010). Transcobalamin C776G genotype modifies the association between vitamin B12 and homocysteine in older hispanics. *European Journal of Clinical Nutrition*, *64*, 503–509.

Giannelli, S. V., Patel, K. V., Windham, B. G., Pizzarelli, F., Ferrucci, L., & Guralnik, J. M. (2007). Magnitude of underascertainment of impaired kidney function in older adults with normal serum creatinine. *Journal of the American Geriatrics Society*, *55*, 816–823.

Guralnik, J. M., Fried, L. P., Simonsick, E. M., Bandeen-Roche, K. J., & Kasper, J. D. (1995). Screening the community-dwelling population for disability. In J. M. Guralnik, L. P. Fried, E. M. Simonsick, J. D. Kasper & M. E. Lafferty (Eds.), *The women's health and aging study: Health and social characteristics of older women with disability* (NIH Publication No. 95-4009, pp. 9–18). Bethesda, MD: National Institutes of Health, National Institute on Aging.

Hardlei, T. F., & Nexo, E. (2009). A new principle for measurement of cobalamin and corrinoids, used for studies of cobalamin analogs on serum haptocorrin. *Clinical Chemistry*, *55*, 1002–1010.

Hazra, A., Kraft, P., Lazarus, R., Chen, C., Chanock, S. J., Jacques, P., . . . Hunter, D. J. (2009). Genome-wide significant predictors of metabolites in the one-carbon metabolism pathway. *Human Molecular Genetics*, *18*, 4677–4687.

Herrmann, W., Obeid, R., Schorr, H., & Geisel, J. (2003). Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. *Clinical Chemistry and Laboratory Medicine*, *41*, 1478–1488.

Johnson, M. A., Hawthorne, N. A., Brackett, W. R., Fischer, J. G., Gunter, E. W., Allen, R. H., & Stable, S. P. (2003). Hyperhomocysteinemia and vitamin B-12 deficiency in elderly using Title IIIc nutrition services. *American Journal of Clinical Nutrition*, *77*, 211–220.

Kasper, J. D., & Simonsick, E. M. (1995). Mental health and general well-being. In J. M. Guralnik, L. P. Fried, E. M. Simonsick, J. D. Kasper & M. E. Lafferty (Eds.), *The women's health and aging study: Health and social characteristics of older women with disability* (NIH Publication No. 95-4009, pp. 85–95). Bethesda, MD: National Institutes of Health, National Institute on Aging.

Leng, S. X., Xue, Q. L., Tian, J., Walston, J. D., & Fried, L. P. (2007). Inflammation and frailty in older women. *Journal of the American Geriatrics Society*, *55*, 864–871.

Lievers, K. J., Afman, L. A., Kluijtmans, L. A., Boers, G. H., Verhoef, P., den Heijer, M., . . . Blom, H. J. (2002). Polymorphisms in the transcobalamin gene: Association with plasma homocysteine in healthy individuals and vascular disease patients. *Clinical Chemistry*, *48*, 1383–1389.

Lindenbaum, J., Rosenberg, I. H., Wilson, P. W., Stabler, S. P., & Allen, R. H. (1994). Prevalence of cobalamin deficiency in the Framingham elderly population. *American Journal of Clinical Nutrition*, *60*, 2–11.

Lindgren, A., Kilander, A., Bagge, E., & Nexo, E. (1999). Holotranscobalamin—A sensitive marker of cobalamin malabsorption. *European Journal of Clinical Investigation*, *29*, 321–329.

Maser, R. E., Nielsen, V. K., Bass, E. B., Manjoo, Q., Dorman, J. S., Kelsey, S. F., . . . Orchard, T. J. (1989). Measuring diabetic neuropathy. Assessment and comparison of clinical examination and quantitative sensory testing. *Diabetes Care*, *12*, 270–275.

Matteini, A. M., Walston, J. D., Bandeen-Roche, K., Arking, D. E., Allen, R. H., Fried, L. P., . . . Fallin, M. D. (2010). Transcobalamin-II variants, decreased vitamin B12 availability and increased risk of frailty. *Journal of Nutrition Health and Aging*, *14*, 73–77.

Matteini, A. M., Walston, J. D., Fallin, M. D., Bandeen-Roche, K., Kao, W. H., Semba, R. D., . . . Stabler, S. P. (2008). Markers of B-vitamin deficiency and frailty in older women. *Journal of Nutrition Health and Aging*, *12*, 303–308.

Miller, J. W., Ramos, M. I., Garrod, M. G., Flynn, M. A., & Green, R. (2002). Transcobalamin II 775G>C polymorphism and indices of vitamin B12 status in healthy older adults. *Blood*, *100*, 718–720.

Pangilinan, F., Mitchell, A., VanderMeer, J., Molloy, A. M., Troendle, J., Conley, M., . . . Brody, L. C. (2010). Transcobalamin II receptor polymorphisms are associated with increased risk for neural tube defects. *Journal of Medical Genetics*, *47*, 677–685.

Penninx, B. W., Guralnik, J. M., Ferrucci, L., Fried, L. P., Allen, R. H., & Stabler, S. P. (2000). Vitamin B(12) deficiency and depression in physically disabled older women: Epidemiologic evidence from the women's health and aging study. *American Journal of Psychiatry*, *157*, 715–721.

Pennypacker, L. C., Allen, R. H., Kelly, J. P., Matthews, L. M., Grigsby, J., Kaye, K., & Stabler, S. P. (1992). High prevalence of cobalamin deficiency in elderly outpatients. *Journal of the American Geriatrics Society*, *40*, 1197–1204.

Pfeiffer, C. M., Caudill, S. P., Gunter, E. W., Osterloh, J., & Sampson, E. J. (2005). Biochemical indicators of B vitamin status in the US population after folic acid fortification: Results from the National Health and Nutrition Examination Survey 1999-2000. *American Journal of Clinical Nutrition*, *82*, 442–450.

Refsum, H., Johnston, C., Guttormsen, A. B., & Nexo, E. (2006). Holotranscobalamin and total transcobalamin in human plasma: Determination, determinants, and reference values in healthy adults. *Clinical Chemistry*, *52*, 129–137.

Resnick, H. E., Vinik, A. I., Heimovitz, H. K., Brancati, F. L., & Guralnik, J. M. (2001). Age 85+ years accelerates large-fiber peripheral nerve dysfunction and diabetes contributes even in the oldest-old: The women's health and aging study. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *56*, M25–M31.

Reynolds, E. (2006). Vitamin B12, folic acid, and the nervous system. *Lancet Neurology*, *5*, 949–960.

Rosenblatt, D., & Fenton, W. (2001). Inherited disorders of folate and cobalamin transport and metabolism. In C. Scriver, A. Beaudet, W. Sly, D. Valle, B. Childs, K. Kinzler & B. Vogelstein (Eds.), *The metabolic & molecular bases of inherited disease* (8th ed., vol. 3, pp. 3897–3933). New York, NY: McGraw-Hill.

Semba, R. D., Garrett, E., Johnson, B. A., Guralnik, J. M., & Fried, L. P. (2000). Vitamin D deficiency among older women with and without disability. *American Journal of Clinical Nutrition*, *72*, 1529–1534.

Semba, R. D., Margolick, J. B., Leng, S., Walston, J., Ricks, M. O., & Fried, L. P. (2005). T cell subsets and mortality in older community-dwelling women. *Experimental Gerontology*, *40*, 81–87.

Shane, B., & Stokstad, E. L. (1985). Vitamin B12–folate interrelationships. *Annual Review of Nutrition*, *5*, 115–141.

Smith, A. D., Smith, S. M., de Jager, C. A., Whitbread, P., Johnston, C., Agacinski, G., . . . Refsum, H. (2010). Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: A randomized controlled trial. *PLoS One*, *5*, e12244.

Stabler, S. P. (2013). Clinical practice. Vitamin B12 deficiency. *New England Journal of Medicine*, *368*, 149–160.

Stabler, S. P., Allen, R. H., Fried, L. P., Pahor, M., Kittner, S. J., Penninx, B. W., & Guralnik, J. M. (1999). Racial differences in prevalence of cobalamin and folate deficiencies in disabled elderly women. *American Journal of Clinical Nutrition*, *70*, 911–919.

Stabler, S. P., Lindenbaum, J., & Allen, R. H. (1996). The use of homocysteine and other metabolites in the specific diagnosis of vitamin B-12 deficiency. *Journal of Nutrition*, *126*, 1266S–1272S.

Stanislawska-Sachadyn, A., Woodside, J. V., Sayers, C. M., Yarnell, J. W., Young, I. S., Evans, A. E., . . . Whitehead, A. S. (2010). The transcobalamin (TCN2) 776C>G polymorphism affects homocysteine concentrations among subjects with low vitamin B(12) status. *European Journal of Clinical Nutrition*, *64*, 1338–1343.

Stone, N., Pangilinan, F., Molloy, A. M., Shane, B., Scott, J. M., Ueland, P. M., . . . Brody, L. C. (2011). Bioinformatic and genetic association analysis of microRNA target sites in one-carbon metabolism genes. *PLoS One*, *6*, e21851.

Tabachnik, B., & Fidell, L. (2007). *Using multivariate statistics* (5th ed.). Boston, MA: Allyn & Bacon.

Tanaka, T., Scheet, P., Giusti, B., Bandinelli, S., Piras, M. G., Usala, G., . . . Ferrucci, L. (2009). Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations. *American Journal of Human Genetics*, *84*, 477–482.

Volpato, S., Leveille, S. G., Blaum, C., Fried, L. P., & Guralnik, J. M. (2005). Risk factors for falls in older disabled women with diabetes: The women's health and aging study. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*, 1539–1545.

Walston, J., Arking, D. E., Fallin, D., Li, T., Beamer, B., Xue, Q., . . . Chakravarti, A. (2005). IL-6 gene variation is not associated with increased serum levels of IL-6, muscle, weakness, or frailty in older women. *Exp Gerontol*, *40*, 344–352.

Walston, J., Hadley, E. C., Ferrucci, L., Guralnik, J. M., Newman, A. B., Studenski, S. A., . . . Fried, L. P. (2006). Research agenda for frailty in older adults: Toward a better understanding of physiology and etiology: Summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *Journal of the American Geriatrics Society*, *54*, 991–1001.

Williamson, J. D., Maloney, P., Skinner, E. A., Corti, C., Brancati, F., Ladenson, P. W., . . . Fried, L. P. (1995). Hematologic, biochemical, and hormonal characteristics. In J. M. Guralnik, L. P. Fried, E. M. Simonsick, J. D. Kasper & M. E. Lafferty (Eds.), *The women's health and aging study: Health and social characteristics of older women with disability* (NIH Publication No. 95-4009, pp. 169–177). Bethesda, MD: National Institutes of Health, National Institute on Aging.

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., & Leirer, V. O. (1982). Development and validation of a geriatric depression screening scale: A preliminary report. *Journal of Psychiatric Research*, *17*, 37–49.