Serum Cholinesterase Activities Distinguish between Stroke Patients and Controls and Predict 12-Month Mortality

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To date there is no diagnostic biomarker for mild stroke, although elevation of inflammatory biomarkers has been reported at early stages. Previous studies implicated acetylcholinesterase (AChE) involvement in stroke, and circulating AChE activity reflects inflammatory response, since acetylcholine suppresses inflammation. Therefore, carriers of polymorphisms that modify cholinergic activity should be particularly susceptible to inflammatory damage. Our study sought diagnostic values of AChE and Cholinergic Status (CS, the total capacity for acetylcholine hydrolysis) in suspected stroke patients. For this purpose, serum cholinesterase activities, butyrylcholinesterase-K genotype and inflammatory biomarkers were determined in 264 ischemic stroke patients and matched controls during the acute phase. AChE activities were lower ($P < 0.001$), and butyrylcholinesterase activities were higher in patients than in controls ($P = 0.004$). When normalized to sampling time from stroke occurrence, both cholinergic parameters were correlated with multiple inflammatory biomarkers, including fibrinogen, interleukin-6 and C-reactive protein ($r = 0.713$, $r = 0.607$; $r = 0.421$, $r = 0.341$; $r = 0.276$, $r = 0.255$; respectively; all P values < 0.001). Furthermore, very low AChE activities predicted subsequent nonsurvival ($P = 0.036$). Also, carriers of the unstable butyrylcholinesterase-K variant were more abundant among patients than controls, and showed reduced activity ($P < 0.001$). Importantly, a cholinergic score combining the two cholinesterase activities discriminated between 94.3% matched pairs of patients and controls, compared with only 75% for inflammatory measures. Our findings present the power of circulation cholinesterase measurements as useful early diagnostic tools for the occurrence of stroke. Importantly, these were considerably more distinctive than the inflammatory biomarkers, albeit closely associated with them, which may open new venues for stroke diagnosis and treatment.

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INTRODUCTION

Stroke cases span 780,000 patients annually, accounting for about 1 of every 17 deaths in the United States, with many of the surviving patients displaying severe disabilities (1). Unlike acute coronary syndromes and congestive heart failure with the myocardial isoform of creatine phosphokinase, troponin and B-type natriuretic peptide, there is no single biochemical diagnostic marker available for ischemic stroke that is sufficiently sensitive and specific (2).

Several markers, including basal National Institutes of Health Stroke Scale (NIHSS), infarct volume, early neurological deterioration, immuno-inflammatory profile, growth factors and stem and progenitor cells (2,3) have been explored in efforts to diagnose stroke patients and predict postevent survival. Nevertheless, none of these markers offer a gold standard parameter, especially for the milder stroke cases. One possible link involves the increased poststroke production of proinflammatory cytokines (4,5). Inflammatory markers correlate with brain infarction volume, stroke severity and clinical outcome (6–8), but the underlying neurotransmission pathway(s) and the putative bridges between inflammatory reactions and clinical outcome await further validations.

Recent studies demonstrate causal links between inflammatory pathways

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and cholinergic signaling (9,10). Specifically, the so-called cholinergic antiinflammatory pathway inhibits cytokine synthesis and release through activation of the ± 7 nicotinic acetylcholine (ACh) receptor (11–13). In cortical cells and hippocampal slice cultures, such activation prevents cell damage and cell death during experimental ischemia (14). Correspondingly, acetylcholinesterase (AChE) inhibition attenuates cerebral infarction volume in rats during experimental stroke (15).

Recently, we demonstrated tight interrelationships between inflammatory reactions and the total circulating capacity for ACh hydrolysis (10,12). This suggested that stress-induced increases in this hydrolytic capacity (16) can accentuate the inflammatory response to stroke by counteracting the cholinergic antiinflammatory reaction. Specifically, the cholinergic antiinflammatory pathway may be mitigated by AChE and the closely related enzyme butyrylcholinesterase (BChE), both of which hydrolyze and inactivate ACh and are modulated under inflammatory insults. BChE is the major ACh hydrolyzing enzyme in the circulatory system (17). In addition to the "usual" (BChE-U) form, the Kalow variant (BChE-K) exists in allelic frequencies of 0.13–0.21 (18). BChE-K includes a single nucleotide polymorphism at position 1699 (SNP ID: rs1803274; Alleles: A/G). This leads to an alanine-to-threonine substitution at position 539, 36 residues upstream to the C-terminus of BChE. BChE-K hydrolyzes butyrylthiocholine and acetylthiocholine 30% and 20% less efficiently than BChE-U, respectively. BChE-K carriers would likely sustain improved cholinergic control over inflammation compared with noncarriers; therefore, we hypothesized that BChE-K carriers should be relatively protected from the consequences of ischemic stroke if the poststroke inflammatory reaction is solely damaging, but should inversely be more vulnerable to the occurrence of stroke and to the consequent damages if this inflammatory reaction also entails a certain neuroprotective value.

Elevated inflammatory cytokines can by themselves cause neurological decline (8); consequently, we hypothesized that serum AChE activity would reflect the intensity of the neuroinflammatory response in stroke patients, that the BChE-K variant could affect the risk to experience stroke and that both enzyme activities would predict the risk of poststroke mortality more distinctively than inflammatory markers. To challenge our working hypothesis, we searched for cholinergic predictors of the risk for poststroke survival.

MATERIALS AND METHODS

Subjects

Stroke patients. For this study, we specifically recruited patients with mild to moderate ischemic stroke or TIA, excluding severe strokes and patients who were unable to provide an independent informed consent. The proportion of lacunar strokes among all hospitals in Israel in 2007 was 33% of all ischemic strokes, with much higher prevalence among mild strokes (data from the National Acute Stroke Israeli Survey, NASIS 2007, Ministry of Health, Israel, http://www.israel-neurology.co.il/nasis1207.asp). All participants signed an informed consent when entering the study; therefore, patients suffering from global aphasia were automatically excluded, as were patients who were not interested in participating. All other stroke patients admitted to the Medical Center who met the inclusion criteria were enrolled.

Two hundred and sixty-four patients over 18 years of age with a documented mild to moderate acute ischemic stroke or TIA (neurological deficit lasting less than 24 h) and with suspected symptom onset within the preceding 24 h, were enrolled into the study: 139 with lacunar stroke, 56 with large-artery atherosclerotic stroke, 15 with cardioembolic stroke, 4 with stroke of undetermined etiology, 1 with a stroke of another determined etiology, and 49 with TIA. All patients enrolled were admitted to the Department of Emergency Medicine at the Tel-Aviv Sourasky Medical Center between July 2000 to July 2002 and January 2004 to January 2006. Exclusion criteria included patients with stroke resulting from trauma or an invasive procedure, severe stroke, cerebral hemorrhage, history of malignant tumor, chronic inflammatory disease, autoimmune disease, coagulation disorders, signs and symptoms of acute chronic infection or treatment with antiinflammatory agents, except for acetylsalicylic acid (≤325 mg/day) or inability to provide informed consent. None of the included patients received thrombolytic therapy. A signed informed consent was obtained from all patients enrolled.

Stroke subtyping followed the TOAST classification (19). Survival records for all patients were obtained at the Ministry of Health database. The patients' NIHSS neurological state was assessed by a certified senior vascular neurologist. Table 1 presents the characteristics of patients and matched controls.

Controls. To each patient we matched one neurologically intact control by gender, age (±3 y) and body mass index (BMI). Controls were in routine followup in the various outpatient clinics between September 2000 and September 2004. Exclusion criteria included a history of cerebral or cardiac event during the previous 12 months, known inflammatory diseases, history of acute febrile disease or infection during the previous 3 months, known malignancy, pregnancy, steroidal or nonsteroidal treatment (except for acetylsalicylic acid at a dose of δ325 mg/dL), and invasive procedures (surgery, catheterization, and so on) during the previous 6 months. A written informed consent approved by the local Ethics Committee was obtained from all participants.

Measurements of Serum Cholinesterase Catalytic Activities

AChE and BChE activities, BChE-K or BChE-U genotype and the total serum capacity to hydrolyze acetylcholine were determined retrospectively in frozen serum samples detailed previously (12). All laboratory methods were performed

Table 1. Population characteristics and cholinergic parameters of stroke patients and matched controls.

Variable	Stroke patients at admission	Matched controls	*P* value
n	264	264	
Risk factors			
Age, y (SD)	66.8 (12.7)	66.9 (11.4)	0.37
BMI, kg/m^2 (SD)	26.9 (4.6)	26.9 (4.2)	0.99
Gender, males (%)	64.7	64.7	1.00
Current smokers (%)	25.4	6.4	<0.001
Diabetes (%)	30.7	20.8	0.009
Hyperlipidemia (%)	41.9	49.2	0.089
Hypertension (%)	64.2	51.9	0.004
Inflammatory biomarkers			
hs-CRP, mg/L (SD)	8.9 (22.2)	3.6 (4.4)	<0.001
ESR, mm/H (SD)	27.1 (18.4)	19.9 (14.3)	<0.001
Fibrinogen, g/dL (SD)	341.4 (76)	305.8 (59.8)	<0.001
WBCC, $10^3/\mu L$ (SD)	8.7 (2.6)	6.9 (1.7)	<0.001
hs-IL-6, pg/mL (SD)	4.2 (3.4)		
Medications			
Acetylsalicylic acid, %	46.1[a] (95)	46.1	0.883
β Blockers, %	19.9	26.5	0.065
Angiotensin II Blockers, %	2.2	5.2	0.067
ACE Inhibitors, %	17.6	29.3	<0.001
HMG-CoA reductase inhibitors, %	30.0	29.6	0.929
Fibrates, %	8.7	9.3	0.746
Clopidogrel, %	0.4	3.8	<0.001
Neurological scores			
NIHSS, mean (SD)	4.0 (3.9)		
mRs, mean (SD)	1.9 (1.6)		
Cholinergic activities			
Cholinergic Status total activity, nmol substrate hydrolysed/min*ml (SD)	1705.1 (355.7)	1619.1 (303.8)	0.004
AChE activity, nmol substrate hydrolysed/min*ml (SD)	287.7 (114.3)	502.5 (127.8)	<0.001

[a]Percentage of patients treated with acetylsalicylic acid (aspirin) before the acute stroke/TIA. Parentheses: Percentage of patients treated with aspirin following admission to the emergency room.

by a blinded technician or student, each method by the same person for all measures. In an attempt to minimize intratest variations, exclude day-of-measurement effects and ascertain the authenticity of the observed differences, we used the same reagents throughout the series and remeasured selected samples each test day.

Statistical Analyses

Statistical analyses were conducted under the guidance of David M Steinberg (Tel Aviv). Collected data was summarized and displayed as mean ± standard deviation (SD) using SPSS/WIN (version 13.0, SPSS INC, Chicago, IL, USA). The different biomarkers for patients and controls were compared by Student *t* test for normally distributed variables and the Mann–Whitney *U* test for non-normally distributed variables. To assess associations among categorical variables, we used the χ^2 test. AChE activity, CS and inflammatory biomarker values all were normalized to sampling time from stroke occurrence, and correlations between them determined by the two-tailed Spearman rank test. Linear discrimination analysis (LDA) adapted to paired data was used for discriminating stroke patients from controls based on their cholinergic and inflammatory parameters. The controls were each matched to individual patients by gender, age (±3 y) and BMI. For each data pair, differences were calculated for each variable, then inflammatory scores were derived by applying discriminate analysis (20,21) to the resulting differences. To conduct survival analyses, we used Partek Genomics Suite version 6.3β (6.06.1128) for multifactorial one-way analysis of variance (ANOVA), as instructed. To investigate the association between calculated BChE-K activity and the BChE K genotype, we used multivariate stepwise linear regression. A *P* value of <0.05 was considered statistically significant.

Inflammatory Biomarkers

Venous blood was obtained from all stroke/TIA patients within 24 h from hospital admission (Time 1, average 26.5 + 15.9 h from symptoms onset). The biomarkers tested included the erythrocyte sedimentation rate (ESR) using

Westergreen's method (22), white blood cell count (WBCC) determined by the Coulter STKS electronic analyzer (Beckman Coulter, Nyon, Swiss) and concentrations of high sensitive C-reactive protein (hs-CRP) measured in the Behring BN II Nephelometer (DADE Behring, Marburg, Germany) (23). Plasma high sensitive IL-6 (hs-IL-6) levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). The Clauss' method (24) and a Sysmex 6000 analyzer (Sysmex Corporation, Hyaga, Japan) served to quantify fibrinogen.

All supplementary materials are available online at molmed.org.

RESULTS

Compared to matched controls, stroke patients showed lower serum AChE activities in the acute phase ($P < 0.001$, Table 1). Importantly, matched control values were compatible with those measured in a United States population (25). This suggested either increased risk of stroke in subjects with low AChE, or stroke-associated reductions in serum AChE levels, or both. Significant differences were found in inflammatory biomarkers between patients and controls (Table 1). Of notice, tested stroke patients included more smokers than matched controls (see Supplementary Table 1).

Cholinergic Parameters Discriminate between Stroke Patients and Matched Controls

Physiological parameters relevant for poststroke recovery should discriminate between patients and controls. Linear discriminate analysis demonstrated that the inflammatory score includes the following weights: 0.015 on ESR, 0.201 on the base 10 logarithm of hs-CRP, 0.176 on WBCC and 0.000598 on fibrinogen. Computing this score for each subject and subtracting the stroke patient score from that of a matched control yields a "delta" score for each pair. Figure 1A presents the inflammation score for the paired stroke patients against that for the matched controls. Most of the pairs (75.0%) predictably showed deltas that are negative (higher inflammatory score in the stroke patients). A parallel analysis of the cholinergic scores demonstrated that most patients presented significantly lower AChE activity accompanied by increased total hydrolytic activity (which implies higher BChE activity), and segregated into a completely different group from controls.

Predicting a significant contribution of BChE to the outcome of stroke, we further used the paired linear discriminate analysis to compute a new cholinergic score that includes the following weights: –0.0116 on AChE and 0.00301 on Cholinergic Status (12) (Figure 1B). The average score for this new measure was –2.75 with a standard deviation of 1.66, whereas the average score for the inflammatory measures was –0.50 with SD of 0.70. Correspondingly, the new cholinergic score yielded a sharper discrimination with a negative score for 94.3% of the pairs, as compared with only 75% for the inflammatory measures. Thus the poststroke modulations in cholinesterase activities, both the decrease in AChE and the increase in BChE activity, emerged as valuable biomarkers of stroke. Next, we subjected our findings to a blinded independent validation test by employing the cholinergic status and AChE activity values to blindly assign each subject to a specific group with the highest conditional probability using linear discriminative analysis. This yielded discrimination with specificity of 97% and sensitivity of 80% for the employed tests (Figure 1C).

Total Cholinesterase Activities as Predictors of 12-Month Survival

Upon hospital admission, all stroke patients exhibited lower AChE activity levels than controls. Twenty-one patients died within the study period. To explore differences between survivors and nonsurvivors, we performed a multifactorial one-way ANOVA analysis that included cardiovascular risk factors, inflammatory biomarkers and the cholinergic parameters. Total cholinesterase activities, age, hyperlipidemia, and smoking emerged as the only significant parameters ($P < 0.05$) to distinguish between stroke survivors and nonsurvivors. Among these variables, age and hyperlipidemia were the most prominent factors ($P < 0.01$). Of note, 12-month nonsurvivors also presented lower calculated BChE activity during the acute phase of stroke compared with 12-month survivors (1271.6 ± 343 versus 1426.5 ± 315.4, $P = 0.031$, Figure 1D). When matched controls were included in the analysis, AChE activity was the most significant parameter to distinguish between groups ($P = 5.5*10–10$) with hs-CRP, WBCC and diabetes history following far behind ($P = 0.012$, $P = 0.014$ and $P = 0.029$, respectively). Next, we explored the possible tissue and cell type origins of AChE. Affymetrix exon array and GeneCard microarray tests demonstrated that the *AChE* gene, known to be primarily expressed in nerve and muscle also is expressed in high levels in nucleated blood cells (Figure 1E), suggesting that serum AChE levels reflect both central and peripheral contributions.

Cholinesterase Associations with the Inflammatory Response

During the acute phase, stroke patients predictably presented significantly elevated average levels of all the investigated inflammatory biomarkers measured when compared with matched controls ($P < 0.001$, Table 1), indicating a marked yet transient inflammatory response. However, the increases in particular inflammatory biomarkers showed large interindividual variability; therefore, when all of these markers were grouped together for each subject, they did not segregate as a single group (data not shown). In contrast, total acetylcholine hydrolysis activity (contributed mostly by BChE [17]) was significantly higher in stroke patients compared with controls ($P = 0.004$, Table 1). Correspondingly, both AChE and BChE activities were directly and prominently correlated with multiple inflammatory biomarkers, provided that they were normalized to sampling time from stroke occurrence. For example, AChE and BChE activity values, as well as inflammatory

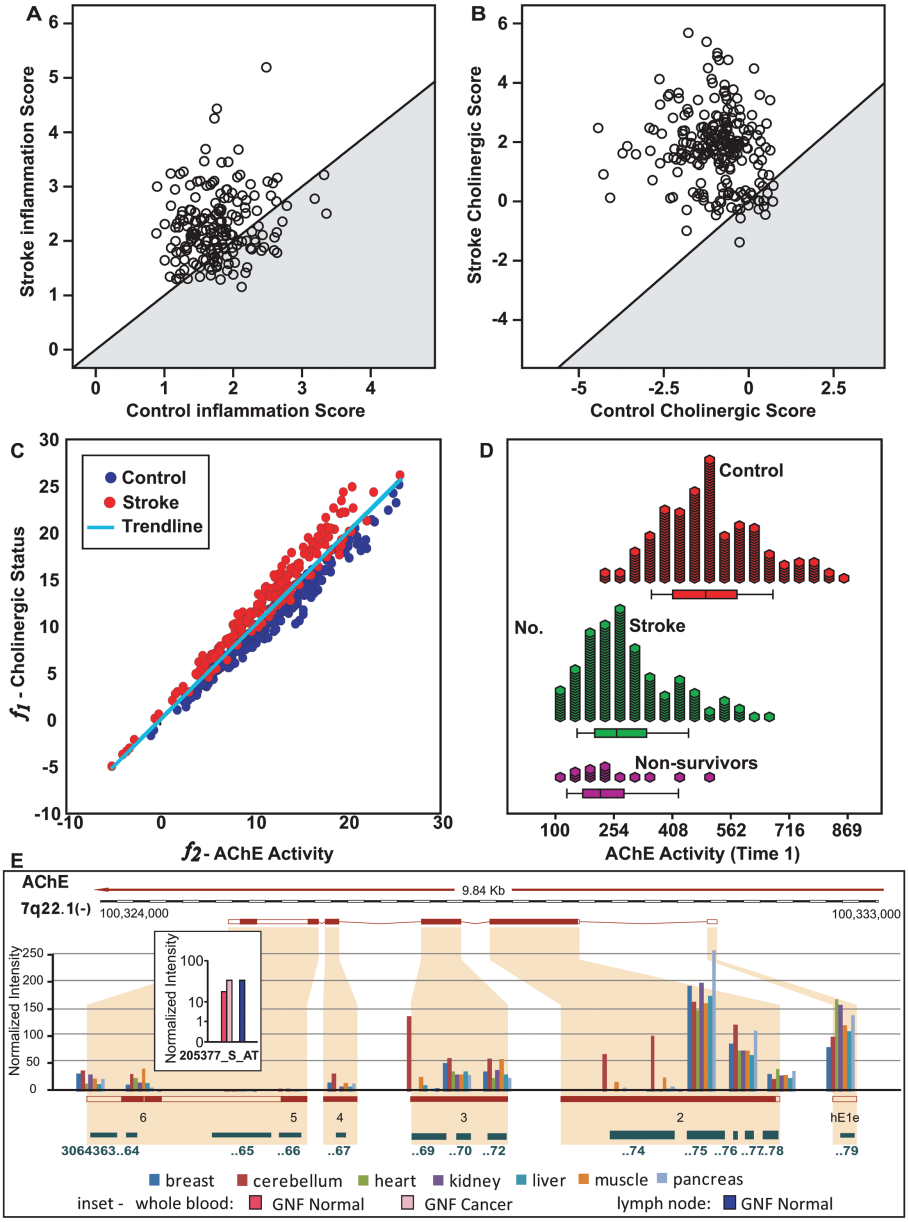

Figure 1. AChE modulations in poststroke patients. (A) The inflammation score and (B) the cholinergic score for each of the stroke patients against that for the matched control. Reference lines represent x = Y. Pairs above the line showed positive differences between patients and controls and vice versa. Note that comparison of A to B displays superiority of the cholinergic over the inflammatory biomarkers in distinguishing stroke patients from control subjects. (C) Linear Discriminative Analysis (LDA) based on cholinergic parameters. Note clear separation of stroke patients and controls to two groups. The axes are the discriminative functions of total acetylthiocholine hydrolytic activity (F1, *y* axis) and AChE activity (F2, *x* axis). (D) Histogram of poststroke AChE reductions segregate patients (green) from controls (red) and survivors from nonsurvivors (purple). (E) Tissue sources expressing AChE include blood leukocytes. Shown are the results of microarray tests presenting the exonic expression of the *AChE* gene, located on the reverse genomic strand of chromosome No 7. The corresponding HG-U133_PLUS_2 probe set ID: AChE - 205377_S_AT numbers are noted from right to left and known genomic annotations (found by ensemble (http://www.ensemblestudios.com)) are marked. Bar graph: Tissues and cell types with highest expression levels of the human *AChE* gene. Total signal intensity of AChE mRNA exons was measured by Affymetrix exon arrays (human s_t v1) sample data sets in selected tissues (raw data downloaded from www.affymetrix.com and normalized using the Affymetrix expression console tool with PLIER algorithm). Inset: whole blood and lymph node data adopted from GeneCards' GNF SymAtlas (Human GeneAtlas GNF1H, v1.2., Build 20080303-1059, developed by the Bioinformatics Team at GNF (http://symatlas.gnf.org) and normalized by gcRMA). Note prominent logarithmically normalized values in nucleated blood cells and that probe sets interrogating the constitutive exons 2 and 3 are labeled at considerably higher levels than other exons.

biomarker levels, were each divided by the number of hours from the reported onset of the stroke event to the sampling time for each patient (i.e., serum CRP level/h from stroke onset, fibrinogen level/h from stroke onset). Total hydrolytic values showed stronger correlations with each of the inflammation markers than AChE alone; specifically, both cholinergic parameters were correlated with fibrinogen, interleukin-6, and C-reactive protein ($r = 0.713$, $r = 0.607$; $r = 0.421$, $r = 0.341$; $r = 0.276$, $r = 0.255$; for total cholinergic status and AChE respectively; all P values < 0.001) (Figure 2A–D).

The BChE K Variant Associates with Higher Stroke Risks

The *BChE* gene is expressed primarily in the liver, but microarray tests show prominent expression levels in nucleated blood cells as well (Figure 3A). That total cholinesterase activities discriminated between stroke patients and matched controls better than AChE measurements alone predicted active contribution of BChE to either the risk of stroke occurrence, its outcome or both. To distinguish between these possibilities, we genotyped patients and controls in search for the prevalence of the BChE-K variant that presents reduced hydrolytic activity compared with the "usual" BChE-U variant (Figure 3B). The observed genotype distribution in controls was 69.1% UU, 25.8% UK and 5.1% KK variants, consistent with the Hardy-Weinberg equilibrium. This distribution significantly differed from that of the stroke patients, who included 62.5% UU, 35.2% UK and 2.3% KK variants. Thus, the stroke cohort included 37.5% carriers of BChE-K compared with 30.9% in controls ($\chi^2 = 8.8$, $P = 0.012$), demonstrating a 20% difference between BChE-K and BChE-U carriers in patients.

The corresponding mean calculated acetylthiocholine hydrolytic activities of serum BChE for the UU, UK and KK carrier stroke patients were 1464.5 ± 317.9, 1349.3 ± 297.4 and 941.2 ± 226.4 (p between alleles = 0.001), whereas activities for controls were 1103.3 + 219.3, 1031.2 + 198.6 and 941.1 + 203.4, respectively (P between alleles = 0.028) (see Figure 3B and data not shown). Thus homozygous BChE-K carriers failed to induce BChE following stroke, possibly indicating yet larger instability or mal-expression for BChE-K in stroke patients compared with controls. Subsequently, a multiple linear regression model was constructed in both the stroke patients and controls with calculated BChE activity as the dependent variable and age, gender, current smoking, body mass index (BMI), baseline NIHSS score, vascular risk factors and BChE-K as independent variables. The variables which remained significantly associated with calculated BChE activity were BMI and BChE-K genotype for both cohorts ($P = 0.001$; Table 2).

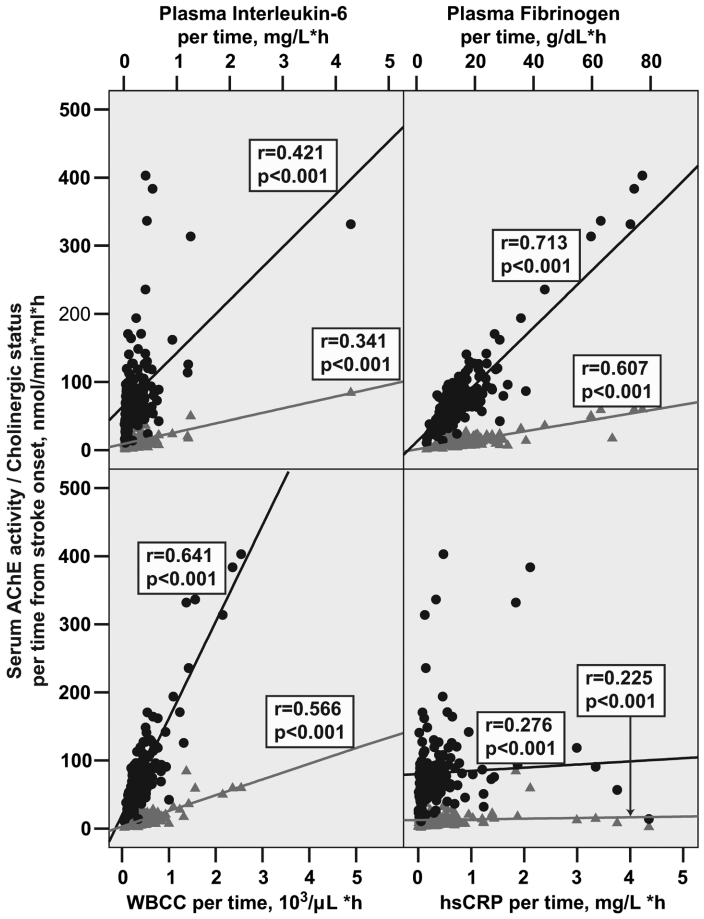

Figure 2. Inflammatory biomarkers correlate with serum AChE and BChE activities normalized to sampling time from stroke occurrence. Measured inflammatory biomarkers and AChE and BChE activities were divided by the time from stroke symptoms occurrence (in hours). Note that the cholinergic status (namely, total acetylthiocholine hydrolytic activity in serum) consistently showed higher correlation to each of the measured inflammatory markers.

DISCUSSION

Our study addressed the risks of stroke occurrence and nonsurvival and identified prominent yet distinct interrelationships for all of these risks with cholinergic parameters. Serum cholinesterase activities associated with modified risk of stroke occurrence and provided useful diagnostic and prognostic insights, considerably more distinctive than the inflammatory biomarkers. Stroke-induced reduction in AChE and overproduction of BChE and inflammatory markers may involve different kinetic parameters. Yet when normalized to sampling time from onset of symptoms, we found a tight correlation between cholinesterase activities

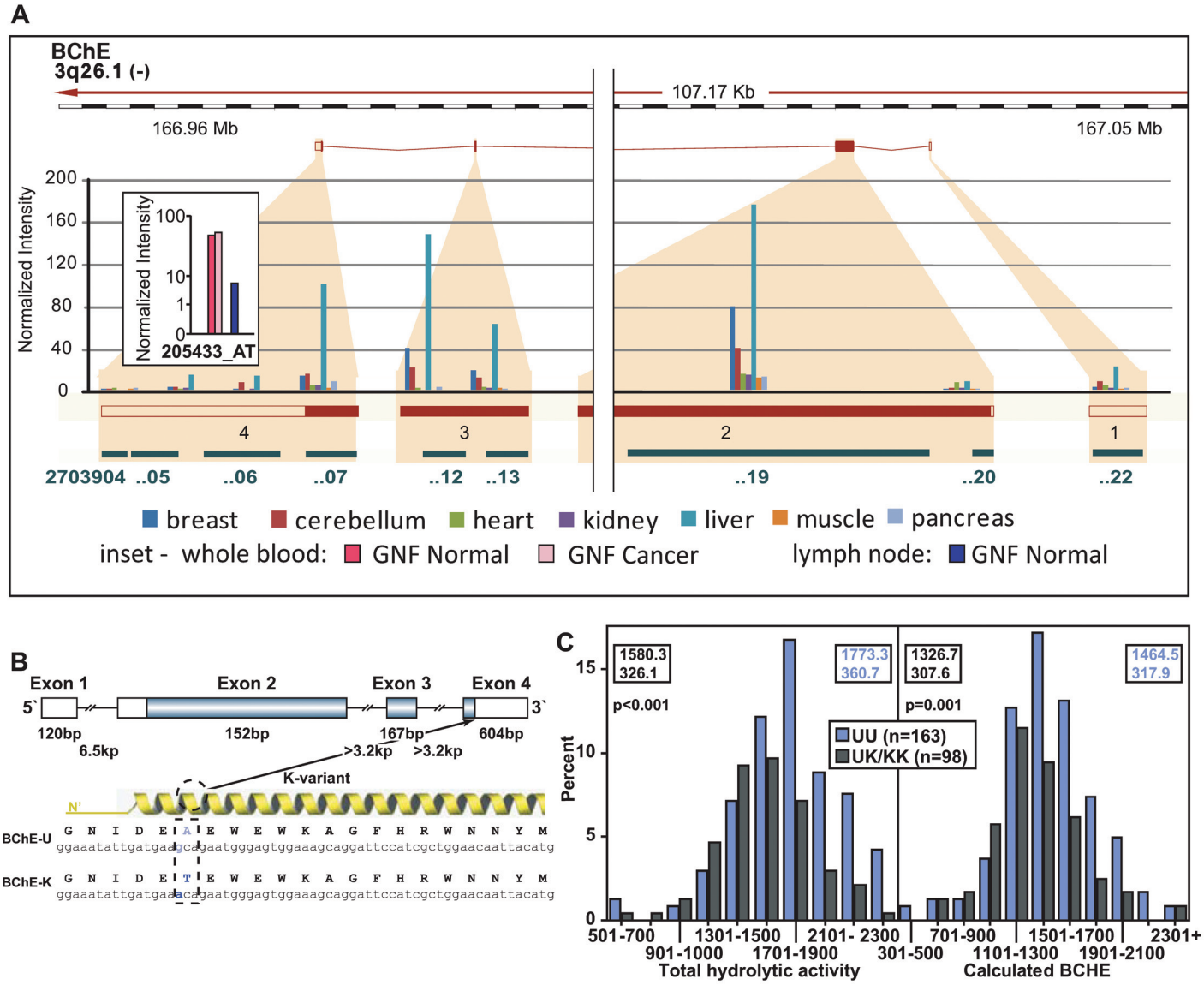

Figure 3. BChE-K prevelance in stroke patients. (A) Cell and tissue sources expressing the *BChE* gene, located on the reverse genomic strand of chromosome 3 and corresponding HG-U133_PLUS_2 probe sets. ID: BChE – 205433_AT probe set numbers are noted from right to left and known genomic annotations (found by ensemble (http://www.ensemblestudios.com)) are marked. Bar graph and inset: Tissues and cell types with highest expression levels of the human *BChE* gene. Shown is total signal intensity of BChE mRNA measured by Affymetrix exon arrays (human s_t v1) sample data sets in selected tissues (raw data downloaded from www.affymetrix.com and normalized using the Affymetrix expression console tool with the PLIER algorithm). Inset: whole blood and lymph node data adapted from GeneCards' GNF SymAtlas (Human GeneAtlas GNF1H, v1.2., Build 20080303-1059, developed by the Bioinformatics Team at GNF (http://symatlas.gnf.org) and normalized by gcRMA). Note variable intensity of labeling for different probe sets and the prominent logarithmically normalized labeling values in nucleated blood cells. The *BChE* gene yields only one transcript. (B) *BChE* gene structure, exonic composition and the 3'-terminal DNA and C-terminal amino acid sequences of BChE-U and -K. The amino acid substitution (framed) is shown above the DNA sequence. (C) Histograms of measured BChE activity in stroke patient carriers of the UU, UK and KK genotypes and matched controls. Units are nmole acetylthiocholine hydrolized/min/mL serum.

and individual inflammatory markers. Our findings support the hypothesis of AChE and BChE in plasma directly antagonizing vagal cholinergic signaling at the macrophage level (9,10,12) and promoting the systemic inflammatory response, albeit at different intensities for AChE and BChE (Figure 4).

Reduction in serum AChE activity of the stroke patients likely reflects stroke-induced cholinergic hyperexcitation, which potentially can serve as protection

Table 2: Multivariate stepwise linear regression model for calculated BChE activity

	Independent variables[a]	β[b]	SE (β)[c]	*P* value	Model R^2
Stroke patients	BMI	0.29	4.38	<0.001	0.388
	BChE-K carriage	−0.262	37.469	<0.001	
Controls	BMI	0.28	2.72	<0.001	0.334
	BChE-K carriage	−0.21	20.82	<0.001	

[a]Multiple linear regression analysis with the calculated BChE activity as the dependent variable. A predictive model was derived by stepwise linear regression analysis. The following independent variables were assessed: age, gender, BMI, diabetes mellitus, hypertension, current smoking, NIHSS and BChE-K genotype. Only those variables with a *P* value of < 0.05 were included in the final fitted model.
[b]β indicates linear regression coefficient.
[c]SE(β) = standard error of β.

from uncontrolled inflammatory reactions (10). However, compatible with the potentially lethal condition defined as cholinergic crisis (26), patients with lowest AChE values included most of the nonsurvivors. These findings suggest that mild stroke-induced decreases in AChE activity associate with mitigated inflammatory reactions. Indeed, moderate AChE inhibition improves recovery from cerebral infarction in rats with experimental stroke (15). Future studies will be required to explore the possible involvement in this decline of the AChE mRNA-targeted microRNA-132 (10).

Stroke patients show anxiety feelings (27), and the inverse association between circulating AChE activity and anxiety scores (25) highlight AChE activity as a putative link also following stroke. At the translational research level, these findings indicate that diagnostic measurements of serum AChE activity in patients potentially could be of value for improving the recovery prospects of stroke patients with low AChE levels.

BChE activity increased in poststroke patients, and patients with the unstable, less active BChE-K variant were somewhat more prevalent in stroke patients, suggesting that higher BChE activities reflected a better recovery process. BChE-K increases the risk for early onset cardiac disease (28), suggesting that other, rare BChE mutations with yet lower hydrolytic activities (for example, "atypical" D70G BChE [17]; or null BChE mutations [29]) may entail yet poorer recovery. Thanks to the slower hydrolytic pace of BChE compared with AChE, this increase can protect from the lethal consequences of cholinergic crisis due to AChE decline while preventing the debilitating outcome of failed cholinergic neurotransmission.

When studied alone, average values of specific inflammatory biomarkers showed statistically significant differences between stroke patients and carefully matched controls. However, no such differences were observed when the various inflammatory biomarkers of each subject were grouped together. This implies that the composite inflammatory status of stroke patients is both complex and variable in nature (30). In contrast, the cholinergic biomarkers provide clear segregation between stroke patients and controls, possibly due to their limited number.

Several limitations of our study need to be addressed. First, our current findings are limited to patients with mild to moderate ischemic stroke or TIA. This introduced

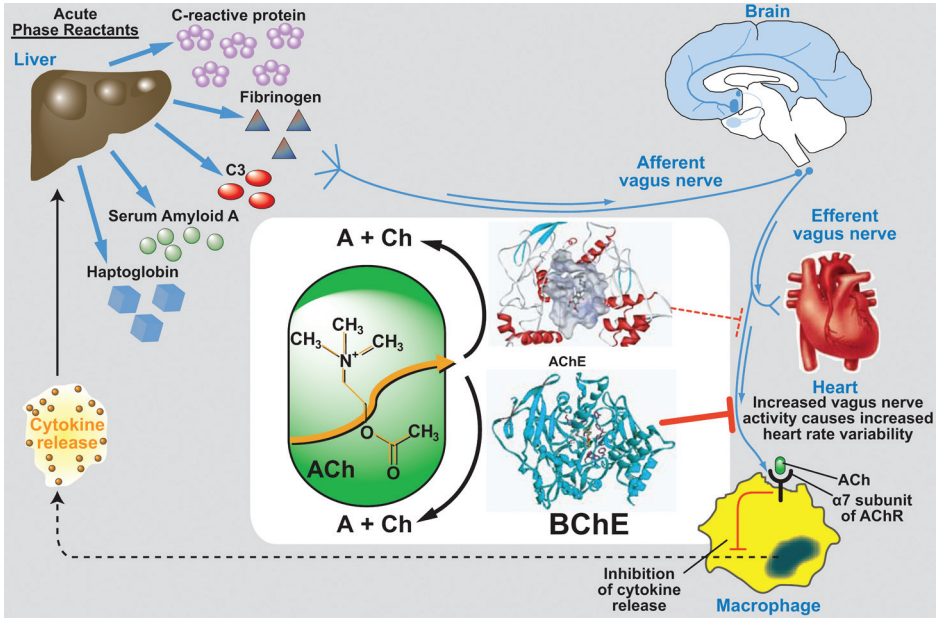

Figure 4. The cholinergic inflammatory mediators of stroke. Acute ischemic stroke triggers focal and systemic inflammatory responses. Vagal ACh signaling inhibits cytokine production; circulating ACh is hydrolyzed by both circulating AChE and BChE, with the latter constituting a more prominent effecter of the inflammatory consequences of stroke. Macrophages and other cytokine-producing cells express ACh receptors, which induce intracellular signals that inhibit cytokine synthesis. Afferent inflammatory signals activate an opposing cholinergic response that suppresses cytokine production and can limit inflammation.

a certain selection bias, although every effort was made to include all of the patients admitted to the Medical Center within the recruitment period. Second, the inflammatory biomarkers selected were few, and we did not perform serial measurements of inflammatory or cholinergic biomarkers and define the kinetics of changes in these values. In our current study we measured the cholinergic parameters within the same time points as the inflammatory markers. Since both are interconnected and regulate each other, our findings emphasize the importance of serial measurements of both markers simultaneously, while normalizing the data to sampling time from onset of stroke symptoms.

While considering the inclusion bias and differences in baseline parameters between stroke and control volunteers, our findings are compatible with the hypothesis that cholinergic modulations monitor the individual prospects of survival from ischemic stroke. In-depth understanding of the association between cholinergic hyperarousal and inflammatory reactions, and delineating in experimental animals their causal effects on the neurological state in acute stroke may foreshadow previously nonperceived strategies for early stroke diagnosis and treatment. Further studies also are needed to validate these measurements as a diagnostic tool, and extensive genetic analysis studies should be performed to test the possibility that BChE-K is a risk factor for stroke.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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