Inflammatory cytokines and ischemic stroke risk

The REGARDS cohort

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Abstract

Objective

We studied circulating interleukin (IL)–6, IL-8, and IL-10 concentrations and incident ischemic stroke risk in a biracial cohort, and determined if these cytokines mediated the racial disparity in stroke incidence affecting the black population.

Methods

The Reasons for Geographic and Racial Differences in Stroke study enrolled 30,237 black and white men and women age ≥ 45 in 2003–2007. We measured baseline IL-6, IL-8, and IL-10 in a case-cohort study of 557 participants with incident stroke over 5.4 years and 951 participants in a cohort sample.

Results

IL-6, but not IL-8 or IL-10, was higher in cases compared to the cohort sample (mean 4.5 vs 3.7 ng/mL; $p < 0.001$). Only IL-6 was associated with stroke risk factors. Adjusting for age, sex, and race, the hazard ratio (HR; 95% confidence interval) for incident stroke for the highest vs lowest quartile of IL-6 was 2.4 (1.6–3.4). HRs for the highest vs lowest quartiles of IL-8 and IL-10 were 1.5 (1.0–2.1) and 1.4 (1.0–1.9), respectively. After additional adjustment for stroke risk factors, only higher IL-6 remained associated with stroke risk (HR 2.0; 1.2–3.1). Associations did not differ by race. Mediation analyses showed that IL-6 mediated the black–white disparity in stroke risk, but mediation was via IL-6 associations with stroke risk factors.

Conclusions

In this biracial population-based sample, IL-6 was strongly associated with risk of incident stroke and mediated the racial disparity in stroke via inflammatory effects of risk factors. Further study on the clinical utility of IL-6 measurement in stroke risk assessment would be helpful.

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Glossary

CI = confidence interval; CRP = C-reactive protein; CV = coefficient of variation; CVD = cardiovascular disease; HR = hazard ratio; IL = interleukin; REGARDS = Reasons for Geographic and Racial Differences in Stroke.

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The black population in the United States has a persistent disparity compared to other racial groups for stroke mortality. Higher stroke incidence and case fatality contribute to this disparity, which is most apparent between ages 45 and 64 years.¹ Blacks have higher levels of inflammation-sensitive proteins,^{2,3} so inflammation is a pathway that might explain part of this racial disparity.

Many studies have examined associations of inflammation biomarkers with cardiovascular and cerebrovascular disease, with relatively few examining stroke specifically.⁴ Proinflammatory cytokines interleukin (IL)-6 and IL-8 increase in stroke patients compared to controls, while IL-10, an anti-inflammatory cytokine, may increase or decrease.^{5–8} Studies of incident stroke are limited. The Health, Aging and Body Composition study reported higher IL-6 was associated with incident stroke,⁹ while lower IL-10 was associated with increased stroke risk in the Leiden 85-Plus Study¹⁰ and People's Republic of China/United States of America Collaborative Study of Cardiovascular and Cardiopulmonary Epidemiology.¹¹ Causal relationships with ischemic stroke in Asians were suggested in genetic association studies for IL-10^{12,13} and IL-6.¹⁴ Finally, the Northern Manhattan Study reported

on the interrelation of IL-6 and C-reactive protein (CRP) in relation to ischemic stroke.¹⁵

As IL-6, IL-8, and IL-10 represent different facets of the inflammatory/immune response, study of these biomarkers may improve understanding of inflammatory/immune processes in stroke. We examined associations of these cytokines with incident stroke in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study and addressed whether they mediate the racial disparity in stroke incidence between blacks and whites.

Methods

Population

REGARDS is a population-based cohort study in the United States on racial and geographic differences in stroke mortality.¹⁶ From January 2003 to October 2007, investigators enrolled 30,239 black and white individuals age 45 and older by telephone. The response rate was 33% and cooperation rate 49%, as seen in other cohort studies.¹⁷ Black participants and those living in the stroke belt and buckle were oversampled¹⁶;

45% men, 55% women, 58% white, 42% black, 56% stroke belt residents, 44% non-stroke belt residents. We defined the stroke belt as the coastal plains of North Carolina, South Carolina, and Georgia, and the stroke belt as the rest of North and South Carolina and Georgia as well as Tennessee, Mississippi, Alabama, Louisiana, and Arkansas. The non-stroke belt was the remaining contiguous United States. Facilitated by a computer-assisted interview, examiners collected extensive participant information on risk factors and initial verbal informed consent. Written informed consent, blood pressure, physical measures, blood samples, ECG, and medication inventory were then obtained at an examination in participants' homes.¹⁶

Standard protocol approvals and registrations

Institutional review boards at each study institution reviewed and approved the study protocol.

Measurements and definitions

Systolic blood pressure ≥ 140 mm Hg, diastolic pressure ≥ 90 mm Hg, or self-reported hypertension plus use of anti-hypertensive medications defined hypertension. Diabetes was defined by self-report of use of antidiabetic medications, fasting glucose >126 mg/dL, or nonfasting glucose >200 mg/dL. ECG criteria defined left ventricular hypertrophy.¹⁸ Atrial fibrillation was classified by self-report or based on ECG. Participants with myocardial infarction on ECG, or who reported myocardial infarction, bypass, angioplasty, or stenting, had prebaseline coronary disease. We defined prebaseline stroke based on self-report. Participants reporting orthopnea or paroxysmal nocturnal dyspnea had heart failure.¹⁹

Stroke ascertainment

We contacted participants or their proxies every 6 months by telephone.¹ Medical records were obtained for death or suspicion of stroke or TIA.²⁰ After prereview by a trained stroke clinician, a committee of stroke physicians reviewed records to validate strokes. Validation required focal neurologic symptoms lasting more than 24 hours or nonfocal symptoms with positive imaging for stroke. Stroke classification was as ischemic or hemorrhagic, and secondarily by ischemic subtypes.²¹ The outcomes of interest in this analysis were fatal and nonfatal ischemic strokes through October 31, 2014.

Case-cohort sample

We used a case-cohort study design to evaluate associations of biomarkers with stroke risk.²² Mean follow-up was 5.4 years. We excluded participants with prebaseline stroke. Cases were 557 participants with incident ischemic stroke. We selected a cohort random sample using stratified sampling to be inclusive of high-risk groups so that the sample contained 50% blacks, 50% whites, 50% women, 50% men, and for the following age groups: 20% 45–54, 20% 55–64, 25% 65–74, 25% 75–84, and 10% ≥ 85 . Of 1,067 cohort random sample participants, 87 with prebaseline stroke were excluded. Incident stroke occurred in 29 of the 980 remaining members of the cohort random sample.

Laboratory methods

Baseline blood was drawn and stored using standardized methods. As described previously, trained technicians followed detailed instructions for phlebotomy and sample processing using centrally assembled kits. Technicians drew blood from fasting participants in the morning using butterfly needle systems and processing samples within 120 minutes of phlebotomy. Technicians shipped samples overnight on frozen gel ice packs to the University of Vermont Laboratory for Clinical Biochemistry Research.²³ In the case-cohort sample, laboratory technicians measured IL-6 by ultrasensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). The laboratory analytical coefficient of variation (CV) was 6.3%. Technicians measured IL-8 using the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc.; St. Charles, MO), with an analytical CV of 10.1%. They measured IL-10 using the Milliplex MAP Human Cardiovascular Disease (CVD) Panel 3 (Millipore Corporation; Billerica, MA) run as a single-plex assay. The laboratory analytical CV was 8.1%.

Statistical methods

We weighted all analyses according to the inverse sampling probability in the cohort random sample. Multivariable linear regression calculated cross-sectional associations of IL-6, IL-8, and IL-10 with individual stroke risk factors. Associations were examined in the cohort sample.

We examined the risk of stroke for sex- and race-specific quartiles of each individual biomarker using weighted Cox proportional hazards models. We used the weighted distribution of each biomarker in the cohort random sample to define quartiles. Potential race interactions (biomarker * race) were tested.

Analyses were minimally adjusted (age, sex, race, age-by-race interaction²⁴), then adjusted for additional variables: region, income, education, body mass index, alcohol use history, smoking history, physical activity levels, systolic blood pressure, clinical history of coronary artery disease, hypertension, heart failure, diabetes, chronic kidney disease, and use of statins, antihypertensives, and nonsteroidal anti-inflammatory drugs. A final model added CRP as it is a recognized cardiovascular risk marker reflecting inflammation. Sensitivity analyses assessed differences in associations by age, for ischemic stroke subtypes, and after excluding participants above the 98th percentile of values for each biomarker.

We studied the mediating effect of IL-6 on the association of race with stroke risk by examining the change in the black-white hazard ratio (HR) for stroke before and after including IL-6 in the model. We calculated the 95% confidence interval (CI) of the percent mediation using bootstrapping (1,000 replicate samples with replacement). We qualitatively compared these analyses using the minimally adjusted model and the fully adjusted model.

Table 1 Baseline characteristics by case status^a

Variable	Cohort random sample ^b	Stroke cases	p Value
Age, y	64.7 (9.3)	70.0 (8.3)	<0.001
Male	485 (45)	272 (52)	0.02
White race	497 (59)	314 (59)	0.96
Region			0.57
Stroke buckle	181 (19)	108 (20)	
Other stroke belt	333 (34)	188 (36)	
Other US regions	466 (47)	232 (44)	
Annual household income, \$			<0.001
>20,000	178 (16)	100 (19)	
20,000–34,000	237 (23)	165 (31)	
35,000–74,000	278 (31)	160 (30)	
≥75,000	146 (17)	43 (8)	
Not identified	141 (13)	60 (11)	
Education level			0.002
Less than high school	147 (12)	80 (15)	
High school graduate	223 (23)	161 (30)	
Some college	262 (28)	127 (24)	
College graduate	348 (37)	160 (30)	
Smoking status			0.01
Never smoker	474 (48)	216 (41)	
Former smoker	366 (38)	212 (40)	
Current smoker	136 (13)	98 (19)	
Alcohol use			0.16
Never drinker	291 (29)	158 (30)	
Former drinker	187 (18)	114 (22)	
Current drinker	502 (53)	256 (48)	
Physical activity frequency			0.85
None	343 (34)	176 (34)	
1–3 times per wk	347 (36)	189 (37)	
4 or more times per wk	279 (30)	148 (29)	
Body mass index, kg/m ²	29.3 (5.9)	28.7 (5.8)	0.12
Waist circumference, cm	95.7 (14.6)	97.5 (14.4)	0.03
Clinical history			
Coronary artery disease	166 (16)	154 (30)	<0.001
Hypertension	615 (61)	417 (79)	<0.001
Diabetes	239 (25)	163 (31)	0.03

Table 1 Baseline characteristics by case status^a (continued)

Variable	Cohort random sample ^b	Stroke cases	p Value
Heart failure	149 (16)	92 (18)	0.50
Chronic kidney disease	129 (9)	96 (18)	<0.001
Medication use			
Statins	295 (33)	183 (35)	0.47
Antihypertensives	501 (52)	346 (67)	<0.001
NSAIDs	134 (14)	70 (13)	0.61
Systolic blood pressure, mm Hg	127 (17)	133 (17)	<0.001
Lipids			
Total cholesterol, mg/dL	191 (40)	191 (43)	0.97
HDL, mg/dL	51 (17)	50 (17)	0.06
LDL, mg/dL	113 (33)	113 (36)	0.96
C-reactive protein, mg/L	4.2 (7.0)	5.7 (8.9)	0.001
IL-6, ng/mL	3.7 (2.6)	4.5 (3.1)	<0.001
IL-8, ng/mL	3.4 (5.3)	3.6 (4.5)	0.52
IL-10, ng/mL	28.5 (192.0)	15.6 (38.4)	0.09

Abbreviations: HDL = high-density lipoprotein; IL = interleukin; LDL = low-density lipoprotein; NSAID = nonsteroidal anti-inflammatory drug. Values are n (%) or mean (SD).

^a The stroke buckle was defined as the coastal plains of North Carolina, South Carolina, and Georgia. The stroke belt was defined as the rest of North and South Carolina and Georgia and Tennessee, Mississippi, Alabama, Louisiana, and Arkansas.

^b Weighted to analytic cohort.

Data availability

The data used in these analyses include potentially identifying participant information and therefore are not publicly available due to legal and ethical restrictions. Qualified investigators may request access from the University of Alabama at Birmingham to obtain de-identified data (regardsadmin@uab.edu).

Results

Participant characteristics

Table 1 shows baseline participant characteristics by case status. Over an average (SD) follow-up of 5.4 ± 2.2 years, there were 557 cases of incident ischemic stroke; 314 in white (59%) and 240 in black (41%) participants. Individuals with incident strokes were more likely to be older, male, from the stroke belt or buckle, have lower education and household income levels, be a current smoker, have stroke risk factors (coronary artery disease history, hypertension, diabetes, chronic kidney disease), and have higher levels of CRP and IL-6. There was no difference between cases and the cohort random sample for other characteristics.

Table 2 Comparisons of interleukin (IL)–6, IL-8, and IL-10 by stroke risk factors in multivariable models

Stroke risk factor	IL-6, ng/mL		IL-8, ng/mL		IL-10, ng/mL	
	Mean (SD)	<i>p</i> Value	Mean (SD)	<i>p</i> Value	Mean (SD)	<i>p</i> Value
Age, y						
<64	3.6 (2.8)	<0.001	3.2 (4.6)	0.34	16.2 (138.5)	0.04
64–74	4.1 (2.8)		3.5 (4.6)		35.4 (138.6)	
75+	4.7 (2.8)		3.7 (4.7)		15.8 (139.0)	
Sex						
Female	4.2 (2.8)	0.28	3.3 (4.6)	0.29	28.1 (138.7)	0.13
Male	4.0 (2.8)		3.6 (4.7)		17.0 (139.2)	
Race						
Black	4.4 (2.8)	<0.001	3.5 (4.6)	0.97	22.3 (139.0)	0.93
White	3.9 (2.8)		3.5 (4.6)		22.9 (139.0)	
Region						
Buckle	4.2 (2.8)	0.02	3.6 (4.7)	0.19	22.3 (139.4)	0.16
Belt	4.3 (2.8)		3.2 (4.7)		13.7 (139.3)	
Other ^a	3.9 (2.8)		3.6 (4.6)		29.2 (138.9)	
Income						
<\$20,000	4.7 (2.9)	<0.001	3.6 (4.7)	0.73	32.8 (141.3)	0.09
\$20,000–34,000	4.2 (2.8)		3.3 (4.7)		13.2 (139.2)	
\$35,000–74,000	3.8 (2.9)		3.6 (4.7)		33.5 (141.3)	
\$≥75,000	3.5 (2.9)		3.5 (4.8)		13.0 (144.8)	
Not stated	4.2 (2.8)		3.2 (4.7)		12.0 (139.7)	
Education						
<HS ^b	4.9 (2.9)	<0.001	3.7 (4.7)	0.60	17.0 (141.9)	0.85
HS graduate	4.4 (2.8)		3.6 (4.6)		20.0 (139.2)	
Some college	4.0 (2.8)		3.2 (4.7)		23.7 (139.5)	
College graduate	3.7 (2.8)		3.4 (4.7)		26.2 (141.1)	
BMI, kg/m²						
<25	3.7 (2.8)	<0.001	3.8 (4.7)	0.21	33.6 (141.6)	0.19
25–30	4.0 (2.8)		3.5 (4.7)		17.4 (141.0)	
>30	4.5 (2.8)		3.2 (4.7)		20.0 (141.7)	
Alcohol use						
Never	4.2 (2.9)	<0.001	3.4 (4.8)	0.21	21.6 (142.5)	0.86
Former	4.7 (2.8)		3.9 (4.7)		19.5 (140.1)	
Current	3.8 (2.8)		3.3 (4.7)		24.4 (141.3)	
Smoking status						
Never	3.9 (2.8)	<0.001	3.8 (4.7)	0.20	26.6 (140.7)	0.59
Former	4.0 (2.8)		3.2 (4.7)		18.5 (141.1)	
Current	5.0 (2.9)		3.5 (4.8)		21.0 (142.8)	

Continued

Table 2 Comparisons of interleukin (IL)–6, IL-8, and IL-10 by stroke risk factors in multivariable models (*continued*)

Stroke risk factor	IL-6, ng/mL		IL-8, ng/mL		IL-10, ng/mL	
	Mean (SD)	<i>p</i> Value	Mean (SD)	<i>p</i> Value	Mean (SD)	<i>p</i> Value
Physical activity						
None	4.5 (2.8)	<0.001	3.5 (4.7)	0.91	21.0 (140.7)	0.94
1–3 times/wk	4.0 (2.8)		3.4 (4.7)		24.3 (140.2)	
≥4 times/wk	3.8 (2.8)		3.4 (4.7)		22.6 (140.7)	
Statin use						
No	4.1 (2.8)	0.58	3.5 (4.6)	0.29	25.2 (138.9)	0.28
Yes	4.1 (2.9)		3.3 (4.7)		16.8 (140.1)	
Antihypertensive use						
No	3.8 (2.8)	0.001	3.3 (4.8)	0.28	20.2 (144.7)	0.55
Yes	4.3 (2.8)		3.6 (4.7)		24.9 (142.0)	
Systolic blood pressure, mm Hg						
<120	3.9 (2.9)	0.20	3.5 (4.8)	0.18	29.1 (142.3)	0.51
120–135	4.1 (2.8)		3.7 (4.6)		19.2 (139.2)	
>135	4.3 (2.8)		3.2 (4.7)		20.4 (140.2)	
NSAID use						
No	4.1 (2.8)	0.57	3.5 (4.6)	0.20	23.6 (139.2)	0.48
Yes	4.0 (2.8)		3.1 (4.7)		16.2 (139.8)	
Coronary artery disease						
No	4.0 (2.8)	<0.001	3.3 (4.6)	0.10	19.2 (140.4)	0.07
Yes	4.6 (2.9)		3.8 (4.7)		35.8 (143.3)	
Hypertension						
No	3.8 (2.9)	0.004	3.4 (4.8)	0.72	21.6 (143.7)	0.86
Yes	4.3 (2.9)		3.5 (4.7)		23.0 (139.8)	
Diabetes						
No	4.0 (2.8)	0.001	3.4 (4.7)	0.15	20.2 (140.0)	0.29
Yes	4.5 (2.8)		3.7 (4.6)		29.0 (139.1)	
Heart failure						
No	4.0 (2.8)	0.001	3.5 (4.6)	0.66	22.7 (139.5)	0.91
Yes	4.7 (2.8)		3.3 (4.7)		21.6 (140.1)	
Chronic kidney disease						
No	4.0 (2.8)	0.007	3.4 (4.7)	0.62	22.6 (140.6)	0.93
Yes	4.6 (2.9)		3.6 (4.8)		23.6 (144.8)	
C-reactive protein, mg/L						
<1.3	2.9 (2.6)	<0.001	3.4 (4.7)	0.23	24.7 (141.0)	0.20
1.3–4.0	3.7 (2.6)		3.3 (4.7)		13.9 (139.7)	
>4.0	5.8 (2.6)		3.8 (4.7)		29.7 (140.5)	

Abbreviations: BMI = body mass index; HS = high school; NSAID = nonsteroidal anti-inflammatory drug.

^a The stroke buckle was defined as North Carolina, South Carolina, and Georgia. The stroke belt was defined as the stroke buckle and the other stroke belt states: Tennessee, Mississippi, Alabama, Louisiana, and Arkansas. Other comprises the remaining contiguous US states.

^b Analyses adjusted for age, sex, and race.

Table 3 Hazard ratios (HRs) for stroke by quartiles of interleukin (IL)–6, IL-8, and IL-10

	Model ^a	N	HR (95% confidence interval) ^b			
			Quartile 1	Quartile 2	Quartile 3	Quartile 4
IL-6	1	1,502	1.0 (ref)	1.4 (1.0, 2.1)	1.8 (1.3, 2.7)	2.4 (1.6, 3.5)
	2	1,370	1.0 (ref)	1.3 (0.8, 2.1)	1.7 (1.1, 2.7)	2.0 (1.2, 3.1)
	3	1,350	1.0 (ref)	1.2 (0.8, 2.0)	1.6 (1.0, 2.5)	1.6 (1.0, 2.6)
IL-8	1	1,506	1.0 (ref)	1.2 (0.9, 1.8)	1.3 (0.9, 1.9)	1.5 (1.0, 2.1)
	2	1,378	1.0 (ref)	1.1 (0.7, 1.7)	1.0 (0.7, 1.6)	1.0 (0.7, 1.6)
	3	1,375	1.0 (ref)	1.1 (0.7, 1.6)	1.0 (0.7, 1.6)	1.0 (0.6, 1.5)
IL-10	1	1,502	1.0 (ref)	1.1 (0.7, 1.5)	1.1 (0.8, 1.6)	1.4 (1.0, 2.0)
	2	1,376	1.0 (ref)	1.1 (0.7, 1.7)	1.0 (0.6, 1.6)	1.2 (0.8, 1.8)
	3	1,373	1.0 (ref)	1.1 (0.7, 1.8)	1.0 (0.7, 1.6)	1.2 (0.8, 1.8)

^a Model 1: adjusted for age, sex, race, and age * race; model 2: model 1 with region, income, education, body mass index, alcohol use, smoking status, physical activity, statin use, antihypertensive use, systolic blood pressure, use of nonsteroidal anti-inflammatory drugs, clinical history of coronary artery disease, hypertension, diabetes, heart failure, and chronic kidney disease; model 3: model 2 with C-reactive protein.

^b HRs compared participants in each successive quartile of each cytokine to participants in the first quartile.

Associations of IL-6, IL-8, and IL-10 with stroke risk factors

In multivariable models adjusting for sex, higher IL-6 was significantly associated with age, black race, region, income, education level, body mass index, smoking status, alcohol use, and physical activity (table 2). IL-6 was higher with hypertension, prevalent coronary artery disease, heart failure, diabetes, kidney disease, and CRP. IL-10 only differed by age,

being higher in the middle tertile of age compared to older and younger age groups. IL-8 was not significantly associated with any of the risk factors.

Associations of IL-6, IL-8, and IL-10 with incident ischemic stroke

Table 3 shows the HRs of incident ischemic stroke for increasing quartiles of IL-6, IL-8, and IL-10. In models adjusted

Table 4 Mediation of racial difference in stroke incidence by interleukin (IL)–6

Age, y	Without IL-6	With IL-6	% Mediation (95% CI)
Model 1^a hazard ratios (95% CI)			
45	2.2 (1.0, 5.0)	1.9 (0.8, 4.3)	28 (0, >100)
55	2.1 (1.2, 3.5)	1.8 (1.1, 3.2)	20 (1, 63)
65	1.9 (1.4, 2.6)	1.8 (1.3, 2.5)	11 (2, 35)
75	1.8 (1.3, 2.4)	1.8 (1.3, 2.4)	0 (<0, 31)
85	1.7 (1.0, 2.9)	1.8 (1.0, 3.0)	0 (<0, 46)
Model 2^a hazard ratios (95% CI)			
45	1.9 (0.8, 4.89)	1.9 (0.8, 4.9)	2
55	1.8 (1.0, 3.3)	1.8 (1.0, 3.3)	0
65	1.6 (1.1, 2.3)	1.7 (1.1, 2.4)	0
75	1.5 (1.0, 2.2)	1.5 (1.1, 2.2)	0
85	1.4 (0.7, 2.5)	1.4 (0.8, 2.6)	0

Abbreviation: CI = confidence interval.

^a Model 1: adjusted for age, sex, race, and age * race. Model 2: adjusted for model 1, region, income, education, body mass index, alcohol use, smoking status, physical activity, statin use, antihypertensive use, systolic blood pressure, use of nonsteroidal anti-inflammatory drugs, clinical history of coronary artery disease, hypertension, diabetes, heart failure, and chronic kidney disease. Models only include participants with complete information (n = 1,370).

for age, sex, race, and age * race, comparing upper quartiles to quartile 1, ischemic stroke risk was monotonically increased across IL-6 quartiles 2, 3, and 4. With additional adjustment for risk factors, there was minimal attenuation. Adding CRP to the full model also minimally attenuated HRs (table 3). There were no associations of IL-8 or IL-10 with risk of incident stroke even in minimally adjusted models. There were no significant differences in associations of IL-6, IL-8, or IL-10 with stroke risk by race (all *p* for interaction ≥ 0.2), and no effect on results if participants above the 98th percentile for each biomarker were excluded from analysis (data not shown). In secondary analyses evaluating stroke subtypes (cardioembolic, large vessel, small vessel, other, and unknown), results were similar to the overall results (data not shown).

The association of IL-6 with incident stroke differed by age in white but not black participants, being larger in younger people (*p* for age * race * IL-6 = 0.047; *p* for age * IL-6 in white participants = 0.04 and in black participants = 0.79). Specifically, in white participants aged 45, 55, 65, 75, and 85, the successive HRs of stroke for IL-6 in the 4th compared to 1st quartile were 8.4, 4.7, 2.7, 1.5, and 0.9. These HRs were all 2.0–2.1 in black participants. Similar patterns were not observed for IL-8 or IL-10.

Since IL-6 was higher in black than white participants, we next studied whether IL-6 mediated the racial disparity in stroke risk. In models adjusted for age, sex, race, and age * race, IL-6 mediated 28% of the racial disparity (95% CI <0%, >100%) at age 45, 20% (1%, 63%) at age 55, and 11% (2%, 35%) at age 65 (table 4). In fully adjusted models, there was no mediation of the racial disparity by IL-6 at any age.

Discussion

This study of black and white participants from the REGARDS study showed a strong, graded association of higher IL-6 at baseline with risk of future ischemic stroke. After full adjustment, those in the third vs first quartile of IL-6 had a 70% increased risk while those in the fourth quartile had a doubling of risk. By contrast, there was no association of baseline levels of IL-8 or IL-10 with stroke risk. IL-6 mediated the race disparity in stroke risk affecting black participants, but this mediation was via traditional stroke risk factors, supporting the importance of inflammation in the relationship of these risk factors to the racial disparity in stroke.

In older black and white adults (aged 70–79 years) in the Health, Aging and Body Composition study, higher IL-6 was associated with increased risk of ischemic stroke; the adjusted relative risk (95% CI) for those in the third compared to first tertile of IL-6 was 3.70 (1.67–8.21).⁹ Similarly, in older black and white adults in the Cardiovascular Health Study (aged 65 years and older), IL-6 was associated with increased risk of stroke and TIA; the adjusted HR (95% CI) for the fifth vs first

quintile was 1.78 (1.35–2.35).²⁵ Our results confirm these studies and extend these findings to a younger, population-based biracial cohort. IL-6 is a well-characterized proinflammatory cytokine with multiple roles including regulation of inflammation, immune, and acute phase responses.²⁶ In addition to stroke,^{5,7} circulating levels of IL-6 are associated with risk of coronary heart disease,²⁷ CVD,²⁸ and other inflammatory diseases²⁹ including cognitive dysfunction.³⁰ Our results support the potential predictive ability of IL-6 for cerebrovascular disease.

While we did not formally compare the associations of IL-6 and CRP with stroke in this analysis, the association of IL-6 with stroke risk in REGARDS was more robust than the association of CRP with stroke in REGARDS (Cahill et al, personal communication). Of note, IL-6 showed similar prediction in black and white participants in REGARDS while CRP at the guideline-based clinical cutoff of ≥ 3 mg/L performed differently in black and white participants. In white participants, CRP ≥ 3 mg/L (approximately the top tertile) was associated with a 40% increased risk of stroke in fully adjusted models. However, in black participants, CRP levels of 9 mg/L were required to reach a 40% increased risk of stroke. In sum, based on REGARDS data, IL-6 would be a more robust stroke biomarker than CRP.

We also report lack of association between IL-8 and incident stroke risk. This extends previous data on IL-8 and prevalent stroke.⁷ IL-8 is an acute phase proinflammatory cytokine that serves as a chemoattractant for neutrophils and natural killer, T, and other cell types as well as mobilizing hematopoietic stem cells in angiogenesis.²⁶ While IL-8 may not be predictive of incident stroke risk, IL-8 appears to play a role in poststroke physiology. In a study that examined genetic profiles of peripheral blood mononuclear cells in ischemic stroke patients compared to healthy controls, the IL-8 gene was identified as a key network enriched in stroke patients.³¹ In addition, IL-8 has been reported to be predictive of mortality in patients with severe TBI³² and to be associated with severe cortical atrophy in older adults.³³ IL-8 may be a marker of long-term changes in the brain either subsequent to an injury or as a result of aging.

IL-10 was examined previously in 2 studies as a marker for incident stroke.^{10,11} Some evidence supports the importance of IL-10 after stroke as well.^{5–8} IL-10 is a key anti-inflammatory cytokine, inhibiting proinflammatory cytokine expression and regulating the innate immune response. In rat models, IL-10 reduced the extent of ischemic brain injury subsequent to occlusion of cerebral arteries.³⁴ IL-10 may serve a neuroprotective role in human stroke patients as well³⁵ and may predict neurologic outcome after stroke.^{5,36} Our study of black and white men and women with an average age of 68 years showed no association of circulating IL-10 levels with risk of incident ischemic stroke; although 2 previous studies did report associations with stroke risk. One study examined 599 adults ≥ 85 years of age from Leiden,

Netherlands,¹⁰ and one comprised residents of the Shijingshan district of Beijing.¹¹ The discrepancies between our results and those reported previously may be due to chance or may be due to differences in age and ethnic background among the cohorts.

Strengths of our study are the prospective study design and large population-based cohort of white and black men and women. An expert committee adjudicated strokes and cohort retention was high. We measured multiple cytokines for a comprehensive analysis. Limitations require consideration. Stroke ascertainment was dependent on telephone contact, so we may have missed some cases. Measurements of cytokines at a single time point may have resulted in misclassification. These factors would tend to lead to underestimation of risk associations. Residual uncontrolled confounding due to unmeasured confounders or misclassification of our covariates is also possible. We lack data on other racial groups and our findings may not generalize to other groups. IL-6 assays are research assays with no currently accepted standardization that might allow clinical application. Finally, we recommend caution in interpretation of the observed interactions of IL-6 by race and age given the sample size and unclear explanation.

In the REGARDS cohort, higher circulating IL-6 was associated with stroke risk factors and a substantially increased risk of incident stroke, independent of these factors. There was no evidence of variation in association by race. IL-6 mediated the racial disparity in stroke risk in REGARDS, but this mediation was via traditional stroke risk factors, suggesting the proinflammatory nature of these risk factor relationships with stroke. IL-8 and IL-10 showed no associations with stroke risk factors or risk of incident stroke in this cohort. Determination of the clinical utility of IL-6 for stroke risk assessment requires additional research and better standardization of IL-6 assays.

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Disclosure

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Peter W. Callas, PhD	University of Vermont, Burlington	Author	Analyzed data, revised the manuscript for intellectual content
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Brett Kissela, MD	University of Cincinnati	Author	Revised the manuscript for intellectual content
Neil A. Zakai, MD	University of Vermont, Burlington	Author	Revised the manuscript for intellectual content
Mary Cushman, MD	University of Vermont, Burlington	Author	Major role in acquisition of data, interpreted data, revised the manuscript for intellectual content

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