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Atherosclerosis Progression in the APPLE Trial Can Be Predicted in Young People With Juvenile-Onset Systemic Lupus Erythematosus Using a Novel Lipid Metabolomic Signature

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Objective. Patients with juvenile-onset systemic lupus erythematosus (JSLE) have increased atherosclerosis risk. This study investigated novel atherosclerosis progression biomarkers in the Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial, the largest investigator-led randomized control trial of atorvastatin versus placebo for atherosclerosis progression in JSLE, using carotid intima-media thickness (CIMT) as the primary outcome.

Methods. Unsupervised clustering of baseline CIMT and CIMT progression over 36 months was used to stratify patients with JSLE. Disease characteristics, cardiovascular risk scores, and baseline serum metabolome were investigated in CIMT-stratified patients. Machine learning techniques were used to identify and validate a serum metabolomic signature of CIMT progression.

Results. Baseline CIMT stratified patients with JSLE (N = 151) into three groups with distinct high, intermediate, and low CIMT trajectories irrespective of treatment allocation, despite most patients having low cardiovascular disease risk based on recommended assessment criteria. In the placebo group (n = 60), patients with high versus low CIMT progression had higher total (P = 0.001) and low-density lipoprotein (LDL) (P = 0.002) cholesterol levels, although within the reference range. Furthermore, a robust baseline metabolomic signature predictive of high CIMT progression was identified in the placebo arm (area under the curve, 80.7%). Patients treated with atorvastatin (n = 61) had reduced LDL cholesterol levels after 36 months, as expected; however, despite this, 36% still had high atherosclerosis progression, which was not predicted by metabolomic biomarkers, suggesting nonlipid drivers of atherosclerosis in JSLE with management implications for this subset of patients.

Conclusion. Significant baseline heterogeneity and distinct subclinical atherosclerosis progression trajectories exist in JSLE. Metabolomic signatures can predict atherosclerosis progression in some patients with JSLE with relevance for clinical trial stratification.

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INTRODUCTION

Juvenile-onset systemic lupus erythematosus (JSLE) accounts for approximately 15% to 20% of patients with systemic lupus erythematosus (SLE). JSLE is a rare disease, with ~10,000 and ~200,000 children and young people (CYP) estimated to live with the disease in the UK and the US, respectively.[1,2] JSLE is characterized by a more severe clinical phenotype compared to SLE in adults, leading to comorbidity burden, including a significantly increased risk of developing cardiovascular disease (CVD). The impact of augmented CVD risk from early onset of SLE has considerable individual and societal implications. In addition, there are recognized ethnic disparities in relation to SLE incidence and prevalence rates (2–3 times higher in people of Black race and Asian descent compared to White individuals)[3] and ethnic differences in clinical presentation and severity of JSLE.[4]

Notably, patients with JSLE have an estimated 100- to 300-fold increased CVD-related mortality compared to age-matched healthy CYP.[5] Subclinical atherosclerosis (chronic inflammation of the large arteries with a long asymptomatic course, which is a major cause of CVD) was detected in ~32% of patients with JSLE.[6] A retrospective analysis of the large UK JSLE cohort (n = 413) identified 12 CVD-related events, which occurred at a median age of 16 years and a median disease duration of only 2 years.[7] However, despite strong evidence of increased CVD risk in patients with JSLE, comorbidity-tailored recommendations or research directed toward stratifying and managing patients with JSLE based on CVD risk are limited.[8,9] Notably, a growing body of evidence, including data generated by our group, support that circulating biomarkers can predict CVD risk in healthy CYP[10,11] and CYP with JSLE.[12,13]

Carotid intima-media thickness (CIMT) is a measure of atherosclerosis that can be used to predict CVD-related events from childhood into middle age[14] and improve the performance of traditional risk factors used for CVD risk classification.[15] Various studies have found a significant increase in CIMT in CYP with JSLE.[6,16] The Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial was a randomized double-blind placebo-controlled study of atorvastatin for subclinical atherosclerosis prevention in JSLE.[17] The trial failed to meet its primary end point, which was a significant decrease in the rate of CIMT progression between atorvastatin and placebo arms, although it showed rates of CIMT progression in the placebo group comparable to those reported in CYP with familial hypercholesterolemia.[18] A secondary analysis identified that atorvastatin-treated postpubertal patients with elevations in baseline high-sensitivity C-reactive protein (hsCRP) had lower CIMT rates of progression,[18] suggesting that heterogeneity of patients with JSLE contributed to the negative results in the primary analysis. Future clinical trials success may depend on correct patient stratification for targeted interventions.

We hypothesized that patients with JSLE recruited to the APPLE trial could be stratified based on biomarkers, with potential utility for tailored CVD risk management strategies yielding better patient selection for clinical trials. To address this, we performed an in-depth analysis of patient, disease, and lipid metabolic factors that underpin CVD risk heterogeneity in patients with JSLE, using data and serum samples collected in the APPLE trial.

PATIENTS AND METHODS

APPLE cohort. Access to clinical, serological, and vascular scan data, as well as matched serum samples from the JSLE cohort enrolled in the APPLE trial, was facilitated by an international collaboration with the Childhood Arthritis and Rheumatology Research Alliance (CARRA) and APPLE trial investigators (US). The APPLE trial was a prospective multicenter cohort of 221 CYP with JSLE (aged 10–18 at inclusion) recruited from various sites in North America and observed for 36 months.[17] Participants were randomized 1:1 to receive either placebo (n = 108) or atorvastatin (n = 113). All participants met well-defined inclusion criteria as per published protocol.[17]

In this study, we performed only complete case analyses and investigated a trial subcohort consisting of 151 patients with JSLE (77 atorvastatin arm; 74 placebo arm; Table 1) with complete baseline data and matched serum samples. In addition, we investigated CIMT progression over 36 months in another subcohort of 121 of 151 patients with JSLE (60 placebo arm, 61 atorvastatin arm; Tables 2 and 3, respectively) who completed the APPLE trial and had complete data sets to enable the analysis. Data related to various patient and disease-related features were available as collected per the APPLE trial protocol.

CIMT measurements in the APPLE cohort. The APPLE investigators provided relevant CIMT measurements collected as per trial protocol,[17,18] which included assessment of the thickness of 12 vascular sites using similar ultrasound machines and a central reader.[17] The mean of the mean common CIMT (MMeanIMT) measurement was the revised primary end point of the APPLE trial.[17] CIMT measures were collected at different time points: baseline, 6 months, 12 months, and 36 months (end of trial). CIMT progression (ΔCIMT) was calculated by subtracting the mean of each of 12 CIMT measurements at 36 months from the corresponding 12 CIMT measurements at baseline.

CVD risk score calculation at baseline. The Framingham risk score[19] (FRS) 2008 and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) score[20] were calculated in R, the QRISK3 score[21] was calculated using R package "QRISK3" (https://CRAN.R-project.org/package=QRISK3), and the atherosclerotic cardiovascular disease (ASCVD) score was calculated with R package "CVrisk" (https://CRAN.R-project.org/package=CVrisk). The risk stratification cutoffs for each score are provided in Supplementary Table 1.

Table 1. Demographic comparison between baseline CIMT groups from APPLE cohort*

		Baseline CIMT assessment			
	Total (N = 151)	High (n = 44)	Intermediate (n = 64)	Low (n = 43)	*P* value[a]
Female, n (%)	128 (84.8)	34 (77.3)	53 (82.8)	41 (95.3)	0.054
Postpuberty at baseline, n (%)	96 (63.6)	30 (68.2)	42 (67.7)	24 (55.8)	0.375
Age, mean ± SD y	15.60 ± 2.67	16.53 ± 2.72	15.30 ± 2.55	15.11 ± 2.63	**0.021**[b]
Race and ethnicity, n (%)					**0.044**
White	74 (49.0)	18 (40.9)	27 (42.2)	29 (67.4)	
Black	39 (25.8)	16 (36.4)	16 (25.0)	7 (16.3)	
Asian	10 (6.6)	3 (6.8)	7 (10.9)	0 (0.0)	
Other	28 (18.5)	7 (15.9)	27 (42.2)	7 (16.3)	
Annual household income, n (%)					0.44
<$25,000	42 (27.8)	10 (22.7)	22 (34.4)	10 (23.3)	
$25,000–49,999	39 (25.8)	11 (25.0)	17 (26.6)	11 (25.6)	
$50,000–74,999	22 (14.6)	6 (13.6)	6 (9.4)	10 (23.3)	
$75,000–99,999	17 (11.3)	8 (18.2)	6 (9.4)	3 (7.0)	
$100,000–150,000	14 (9.3)	4 (9.1)	6 (9.4)	4 (9.3)	
>$150,000	7 (4.6)	1 (2.3)	5 (7.8)	1 (2.3)	
Patient and disease characteristics at baseline					
BMI, mean ± SD	24.23 ± 5.27	24.83 ± 6.10	24.52 ± 5.29	23.20 ± 4.20	0.304
Disease duration, mean ± SD mo	29.52 ± 29.37	39.75 ± 35.45	25.98 ± 25.44	24.30 ± 25.84	**0.021**[c]
SLEDAI, mean ± SD	4.71 ± 4.17	4.32 ± 4.12	4.91 ± 4.23	4.81 ± 4.21	0.76
SLICC DI, mean ± SD	0.35 ± 0.70	0.50 ± 0.82	0.34 ± 0.70	0.21 ± 0.56	0.156
Hypertension, n (%)	49 (32.5)	17 (38.6)	22 (34.4)	10 (23.3)	0.282
History of smoking, n (%)	2 (1.3)	1 (2.3)	0 (0.0)	1 (2.3)	0.474
dsDNA antibody positive, n (%)	122 (80.8)	37 (84.1)	52 (81.2)	33 (76.7)	0.680
Creatinine clearance, mean ± SD mL/min/m^2	138.80 ± 31.72	139.70 (28.03)	145.27 (33.43)	128.23 (30.55)	**0.023**[d]
C3, mean ± SD mg/dL	102.03 ± 27.02	102.93 ± 29.34	100.03 ± 26.18	104.18 ± 26.30	0.721
C4, mean ± SD mg/dL	15.24 ± 7.47	16.02 ± 7.26	14.81 ± 6.99	15.11 ± 8.45	0.714
Medications at baseline (past 30 days), n (%)					
Aspirin	102 (67.5)	33 (75.0)	45 (70.3)	24 (55.8)	0.133
Hydroxychloroquine	149 (98.7)	44 (100.0)	62 (96.9)	43 (100.0)	0.252
Multivitamin	111 (73.51)	33 (75.0)	45 (70.3)	33 (76.7)	0.734
Corticosteroids	124 (82.12)	38 (86.4)	54 (84.4)	32 (74.4)	0.287
Cyclophosphamide	23 (15.23)	6 (13.6)	11 (17.2)	6 (14.0)	0.848
Mycophenolate mofetil	34 (22.52)	11 (25.0)	15 (23.4)	8 (18.6)	0.754
Azathioprine	23 (15.23)	10 (22.7)	9 (14.1)	4 (9.3)	0.207
Methotrexate	19 (12.58)	7 (15.9)	6 (9.4)	6 (14.0)	0.573
Rituximab	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
NSAIDs	46 (30.46)	15 (34.1)	19 (29.7)	12 (27.9)	0.809
ACE inhibitor	33 (21.85)	12 (27.3)	16 (25.0)	5 (11.6)	0.153
Serum biomarkers at baseline, mean ± SD					
hsCRP, mg/L	2.53 ± 7.53	2.38 ± 5.65	3.19 ± 9.90	1.58 ± 4.03	0.57
Homocysteine, μmol/L	7.27 ± 3.32	7.41 ± 2.95	7.24 ± 3.15	7.16 ± 4.00	0.941
Lipid levels at baseline, mean ± SD mg/dL					
Total cholesterol[e]	153.90 ± 39.96	151.83 ± 33.71	152.73 ± 43.34	158.03 ± 41.06	0.75
HDL cholesterol[e]	45.55 ± 12.60	45.38 ± 13.62	45.72 ± 12.87	45.46 ± 11.24	0.99
LDL cholesterol[e]	85.76 ± 32.7	86.24 ± 28.57	84.90 ± 36.69	86.64 ± 30.74	0.961
Triglycerides	115.42 ± 74.28	101.17 ± 50.11	116.09 ± 87.63	129.67 ± 70.97	0.226
Lipoprotein A	21.68 ± 26.16	29.60 ± 26.92	19.89 ± 28.39	16.10 ± 19.21	0.051

Note: *P* values < 0.05 (considered significant) are in bold.
* ACE, angiotensin-converting enzyme; APPLE, Atherosclerosis Prevention in Pediatric Lupus Erythematosus; BMI, body mass index; CIMT, carotid intima-media thickness; dsDNA, double-stranded DNA; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NSAID, nonsteroidal anti-inflammatory drug; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC DI, Systemic Lupus International Collaborating Clinics Damage Index.
[a] Chi-squared test, one-way analysis of variance, or Tukey's range test. Tanner stages 4–5 are classified as postpuberty.
[b] High vs low CIMT group: *P* = 0.033; high vs intermediate: *P* = 0.045.
[c] High vs low CIMT group: 0.036; high vs intermediate: *P* = 0.042.
[d] Intermediate vs low CIMT group: *P* = 0.017.
[e] The recommended lipid levels in people younger than 18 years of age (per APPLE trial inclusion criteria) are total cholesterol <170 mg/dL, HDL cholesterol >45 mg/dL, and LDL cholesterol <110 mg/dL. Lipid levels fluctuate and are not usually monitored during puberty.

Metabolomics. Measures of 250 serum biomarkers were acquired with an established nuclear magnetic resonance (NMR) spectroscopy platform (Nightingale Health, https://nightingalehealth.com/).[22] Analyzed serum samples had not been exposed to any freeze and thaw cycle, and previous research showed that this platform

Table 2. Demographic comparison between the high CIMT progression group and low CIMT progression group in the APPLE study placebo-treated participants (n = 60)*

	Total placebo (n = 60)	High CIMT progression group (n = 35)	Low CIMT progression group (n = 25)	*P* value[a]
Female, n (%)	51 (85.0)	29 (82.9)	22 (88.0)	0.855
Postpuberty at baseline, n (%)	38 (63.3)	21 (60.0)	17 (68.0)	0.564
Age, mean ± SD y	15.50 ± 2.48	15.46 ± 2.49	15.56 ± 2.52	0.876
Race and ethnicity, n (%)				0.848
White	35 (58.33)	19 (54.29)	16 (64.0)	
Black	13 (21.67)	8 (22.86)	5 (20)	
Asian	4 (6.67)	3 (8.57)	1 (4.0)	
Other	8 (13.33)	5 (14.29)	3 (12)	
Annual household income, n (%)				0.763
<$25,000	16 (26.67)	9 (25.71)	7 (28)	
$25,000–49,999	17 (28.33)	9 (25.71)	8 (32)	
$50,000–74,999	7 (11.67)	5 (14.29)	2 (8)	
$75,000–99,999	8 (13.33)	6 (17.14)	2 (8)	
$100,000–150,000	6 (10)	2 (5.71)	4 (16)	
>$150,000	3 (5)	2 (5.71)	1 (4)	
Patient and disease characteristics at baseline				
BMI, mean ± SD	24.51 ± 6.19	24.91 ± 6.60	23.94 ± 5.66	0.555
Duration of lupus, mean ± SD, mo	28.05 ± 30.11	27.89 ± 34.68	28.28 ± 22.88	0.961
SLEDAI, mean ± SD	4.02 ± 3.96	4.51 ± 3.98	3.32 ± 3.90	0.253
SLICC DI, mean ± SD	0.333 ± 0.774	0.457 ± 0.886	0.160 ± 0.554	0.144
Hypertension, n (%)	23 (38.3)	16 (45.7)	7 (28.0)	0.262
History of smoking, n (%)	0 (0)	0 (0)	0 (0)	-
dsDNA antibody positive, n (%)	45 (75.0)	24 (68.6)	21 (84.0)	0.290
Creatinine clearance, mean ± SD mL/min/m^2	133.18 ± 28.66	134.59 ± 28.24	131.21 ± 29.7	0.891
C3, mean ± SD mg/dL	106.2 ± 25.24	110.50 ± 24.53	100.05 ± 25.50	0.121
C4, mean ± SD mg/dL	16.95 ± 7.72	17.85 ± 8.18	15.63 ± 6.96	0.282
Medications at baseline (past 30 days), n (%)				
Aspirin	43 (71.67)	24 (68.57)	19 (76)	0.735
Hydroxychloroquine	59 (98.33)	34 (97.14)	25 (100)	1
Multivitamin	42 (70)	23 (65.71)	19 (76)	0.568
Corticosteroids	48 (80)	29 (82.86)	19 (76)	0.743
Cyclophosphamide	10 (16.67)	6 (17.14)	4 (16)	1
Mycophenolate mofetil	11 (18.33)	8 (22.86)	3 (13.04)	0.463
Azathioprine	11 (18.33)	7 (20)	4 (16)	0.955
Methotrexate	8 (13.33)	5 (14.29)	3 (12)	1
Rituximab	0 (0.0)	0 (0.0)	0 (0.0)	-
NSAIDs	19 (31.67)	9 (25.71)	10 (40)	0.373
ACE inhibitor	17 (28.33)	11 (31.43)	6 (24)	0.735
Serum biomarkers at baseline, mean ± SD				
hsCRP, mg/L	2.88 ± 6.50	2.93 ± 6.13	2.82 ± 7.11	0.953
Homocysteine, μmol/L	7.52 ± 4.24	8.08 ± 4.97	6.76 ± 2.91	0.24
Lipid levels at baseline, mean ± SD mg/dL				
Total cholesterol[b]	144.59 ± 31.3	156.97 ± 32.91	127.76 ± 19.12	**<0.001**
HDL cholesterol[b]	45.92 ± 12.71	48.38 ± 13.53	42.56 ± 10.88	0.082
LDL cholesterol[b]	74.09 ± 26.75	83.24 ± 27.98	62.00 ± 19.71	**0.002**
Triglycerides	128.12 ± 94.52	136.62 ± 115.75	116.56 ± 54.09	0.425
Lipoprotein A	12.25 ± 16.04	14.82 ± 17.61	8.76 ± 13.17	0.153

Note: $P < 0.05$ were considered significant and presented in bold.
* ACE, angiotensin-converting enzyme; APPLE, Atherosclerosis Prevention in Pediatric Lupus Erythematosus; BMI, body mass index; CIMT, carotid intima-media thickness; dsDNA, double-stranded DNA; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NSAID, nonsteroidal anti-inflammatory drug; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC DI, Systemic Lupus International Collaborating Clinics Damage Index.
[a] Chi-squared test or Wilcoxon signed rank test. Tanner stages 4–5 are classified as postpuberty.
[b] The recommended lipid levels in people younger than 18 years of age (as per APPLE trial inclusion criteria) are total cholesterol <170 mg/dL, HDL cholesterol >45 mg/dL, and LDL cholesterol <110 mg/dL. Lipid levels fluctuate and are not usually monitored during puberty.

has good accuracy in detecting metabolites in samples stored for ≥15 years,[23,24] as was the case with the APPLE trial samples. Measures included absolute concentrations (millimoles per liter), ratios, and percentages of lipoprotein composition of numerous metabolites (Supplementary Table 2). Data imputation was performed using the Nearest Neighbor Hot Deck Imputation method after removing metabolites with more than 10% missing data (5 metabolites were removed), leaving a total of 245 metabolites per sample for analysis.

Table 3. Demographic comparison between the high, intermediate, and low CIMT progression groups in the APPLE study atorvastatin-treated participants (N = 61)*

		CIMT progression groups			
	Total (n = 61)	High (n = 22)	Intermediate (n = 24)	Low (n = 25)	*P* value[a]
Female, n (%)	49 (80.3)	17 (77.3)	21 (87.5)	11 (73.3)	0.503
Postpuberty, n (%)	35 (57.4)	13 (60.1)	13 (54.2)	9 (60.0)	0.919
Age, mean ± SD, y	15.34 ± 2.72	14.87 ± 2.51	15.21 ± 2.93	16.24 ± 2.63	0.314
Race and ethnicity, n (%)					0.677
White	23 (37.7)	9 (40.9)	9 (37.5)	5 (33.3)	
Black	16 (26.23)	6 (27.3)	5 (20.8)	5 (33.3)	
Asian	5 (8.2)	0 (0.0)	3 (12.5)	2 (13.3)	
Other	17 (27.87)	7 (31.8)	7 (29.2)	3 (20.0)	
Annual household income, n (%)					0.167
<$25,000	17 (27.87)	4 (18.2)	12 (50.0)	1 (7.1)	
$25,000–49,999	15 (24.59)	8 (36.4)	3 (12.5)	4 (28.6)	
$50,000–74,999	6 (9.84)	3 (13.6)	3 (12.5)	0 (0.0)	
$75,000–99,999	7 (11.48)	2 (9.1)	2 (8.3)	3 (21.4)	
$100,000–150,000	7 (11.48)	2 (9.1)	2 (8.3)	3 (21.4)	
>$150,000	4 (6.56)	1 (4.5)	1 (4.2)	2 (14.3)	
Patient and disease characteristics at baseline					
Body mass index, mean ± SD	24.17 ± 4.73	22.97 ± 4.38	24.57 ± 5.40	25.31 ± 3.91	0.298
Duration of lupus, mean ± SD mo	28.26 ± 29.94	25.68 ± 20.37	28.79 ± 28.34	31.20 ± 43.37	0.858
SLEDAI, mean ± SD	5.38 ± 4.74	6.55 ± 5.83	4.38 ± 3.62	5.27 ± 4.45	0.303
SLICC DI, mean ± SD	0.393 ± 0.714	0.23 ± 0.53	0.42 ± 0.72	0.60 ± 0.91	0.295
History of hypertension, n (%)	17 (27.9)	5 (22.7)	7 (29.2)	5 (33.3)	0.766
History of smoking, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	-
dsDNA antibody positive, n (%)	51 (83.6)	18 (81.8)	19 (79.2)	14 (93.3)	0.489
Creatinine clearance, mean ± SD mL/min/m^2	147.25 ± 34.40	158.09 ± 45.41	141.95 ± 22.07	139.82 ± 29.76	0.179
C3, mean ± SD mg/dL	99.57 ± 28.05	84.28 ± 36.44	92.55 ± 41.53	96.53 ± 34.33	0.608
C4, mean ± SD mg/dL	13.87 ± 6.36	11.76 ± 5.26	14.04 ± 6.84	16.89 ± 6.25	0.058
Medications at baseline (past 30 days), n (%)					
Aspirin	36 (59.02)	11 (50.0)	15 (62.5)	10 (66.7)	0.543
Hydroxychloroquine	60 (98.36)	22 (100.0)	23 (95.8)	15 (100.0)	0.457
Multivitamin	44 (72.13)	17 (77.3)	16 (66.7)	11 (73.3)	0.72
Corticosteroids	51 (83.61)	20 (90.9)	17 (70.8)	14 (93.3)	0.093
Cyclophosphamide	8 (13.11)	3 (13.6)	2 (8.3)	3 (20.0)	0.574
Mycophenolate mofetil	15 (24.59)	5 (22.7)	6 (25.0)	4 (26.7)	0.962
Azathioprine	8 (13.11)	3 (13.6)	4 (16.7)	1 (6.7)	0.664
Methotrexate	5 (8.2)	1 (4.5)	3 (12.5)	1 (6.7)	0.598
Rituximab	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
NSAIDs	20 (32.79)	7 (31.8)	7 (29.2)	6 (40.0)	0.776
ACE inhibitor	13 (21.31)	5 (22.7)	4 (16.7)	4 (26.7)	0.744
Serum biomarkers at baseline, mean ± SD					
hsCRP, mg/L	2.87 ± 9.66	2.11 ± 3.56	4.44 ± 15.00	1.48 ± 2.16	0.59
Homocysteine, μmol/L	7.17 ± 2.52	7.25 ± 2.85	6.88 ± 2.59	7.52 ± 1.95	0.731
Lipid levels at baseline, mean ± SD mg/dL					
Total cholesterol[b]	158.48 ± 41.74	165.41 ± 43.55	157.88 ± 44.72	149.27 ± 34.22	0.519
HDL cholesterol[b]	44.93 ± 12.68	44.00 ± 13.78	45.17 ± 12.47	45.93 ± 12.07	0.899
LDL cholesterol[b]	92.21 ± 32.7	99.14 ± 37.65	91.12 ± 31.38	83.80 ± 26.18	0.373
Triglycerides	106.62 ± 55.85	111.09 ± 51.70	107.92 ± 63.33	98.00 ± 51.55	0.78
Lipoprotein A	27.15 ± 31.6	29.95 ± 33.27	23.33 ± 31.66	29.13 ± 30.52	0.754

* ACE, angiotensin-converting enzyme; APPLE, Atherosclerosis Prevention in Pediatric Lupus Erythematosus; CIMT, carotid intima-media thickness; dsDNA, double-stranded DNA; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; NSAID, nonsteroidal anti-inflammatory drug; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC DI, Systemic Lupus International Collaborating Clinics Damage Index.
[a] Chi-squared test, one-way analysis of variance, or Tukey's range test. Tanner stages 4–5 are classified as postpuberty.
[b] The recommended lipid levels in people younger than 18 years of age (per APPLE trial inclusion criteria) are total cholesterol <170 mg/dL, HDL cholesterol >45 mg/dL, and LDL cholesterol <110 mg/dL. Lipid levels fluctuate and are not usually monitored during puberty.

Statistical analysis. Statistical tests were performed in R and GraphPad Prism. Data were assessed for normality and analyzed with parametric or nonparametric tests, as appropriate. The chi-square test and Fisher's exact test were used for comparison among categorical variables. Details of statistical tests and parameters accounted for in the analyses are given in the figure legends. $P < 0.05$ was considered statistically significant. Bonferroni correction was applied for multiple testing.

Unsupervised hierarchical clustering was performed with ClustVis (https://biit.cs.ut.ee/clustvis/). This method was used to stratify patients with JSLE at baseline (using 12 CIMT measurements at the beginning of the trial) and based on their CIMT progression over 36 months in the atorvastatin versus placebo arms, using the 12 CIMT progression (ΔCIMT measurements). The data analysis pipeline is detailed in Supplementary Figure 1.

The Duke Clinical Research Institute (Durham, North Carolina) served as the data coordinating center and provided oversight of all aspects of the study's conduct, management, and statistical analysis. The study was conducted at 21 CARRA sites in North America. Local institutional review board approval was obtained, and all patients or their guardians gave informed consent and assent following local guidelines. The ClinicalTrials.gov identifier is NCT00065806, and the chief investigator was author Laura E. Schanberg.

The APPLE clinical trial study protocol and results are publicly available.[17] Preliminary analyses of this study are also available at Social Science Research Network (SSRN) (https://ssrn.com/abstract=4336159 or https://doi.org/10.2139/ssrn.4336159). The study has been reported according to the **Consolidated Standards of Reporting Trials** reporting guidelines.[17] Data used for all the complementary analyses included in this article and the analytic codes are available on request.

RESULTS

Baseline CIMT measurements stratify patients with JSLE into three groups, each associated with distinct CIMT trajectories irrespective of treatment allocation. The baseline CIMT heterogeneity of patients with JSLE recruited to the APPLE clinical trial was assessed in a subcohort of 151 patients with a mean age of 15.6 years (range 10.3–21.7 years, 85% female). A summary of baseline characteristics is depicted in Table 1.

Unsupervised hierarchical clustering was used to stratify the cohort using 12 CIMT measures at baseline. Three groups were identified with relatively high (n = 44), intermediate (n = 64), and low (n = 43) baseline CIMT measurements (Figure 1A). Compared to patients in the low and intermediate CIMT groups, patients with JSLE with high baseline CIMT were significantly older ($P = 0.021$) and had longer disease duration ($P = 0.021$) (Table 1). Female patients were more frequently identified in the low baseline CIMT group (95.3%) compared to the high (77.3%) and intermediate (68.2%) groups ($P = 0.054$) (Table 1). No significant differences among various patient and disease-related parameters, including lipid serum levels, were found (Table 1), except for creatinine clearance estimations, which were significantly higher in the intermediate compared to the low baseline CIMT groups ($P = 0.017$) (Table 1).

As a validation, the baseline MMeanIMT (primary end point of the trial) was significantly different among the three groups (high vs intermediate, $P < 0.0001$; high vs low, $P < 0.0001$; intermediate vs low, $P < 0.0001$) (Figure 1B), thus supporting significant CIMT heterogeneity across the JSLE cohort that was maintained across the time frame of the study (Figure 1C). In support, there were distinct CIMT trajectories over 36 months of the three patient groups in which patients did not cross over (Figure 1D), irrespective of treatment allocation. Together, these data demonstrate significant CIMT heterogeneity at baseline and CIMT progression at 36 months, despite minimal differences in demographic and disease features, supporting further investigation of factors contributing to distinct CIMT progression rates in JSLE.

Patients with JSLE in the placebo arm of the APPLE trial stratified into two groups based on their CIMT trajectories over 36 months. To examine the natural progression of subclinical atherosclerosis, the change in the 12 CIMT measures from baseline to 36 months (ΔCIMT) was assessed in all patients allocated to the placebo arm of the APPLE study (n = 60) (Table 2). Unsupervised hierarchical clustering stratified patients into two groups based on ΔCIMT with high (n = 35) and low (n = 25) CIMT progression (Figure 2A). A significant increase in MMeanIMT was seen in the high CIMT progression group ($P < 0.0001$), whereas a significant decrease in MMeanIMT ($P = 0.001$) characterized the low CIMT progression group (Figure 2B).

Importantly, there were no significant differences in age, sex, puberty stages, and race between the high and low CIMT progression groups (Table 2). Unsurprisingly, routinely measured serum total cholesterol ($P = 0.0004$) and low-density lipoprotein (LDL) cholesterol ($P = 0.002$) levels, known to be associated with atherosclerosis development, were significantly elevated in the high compared to low CIMT progression group (Table 2), although the mean ± SD values were within the recommended range for both groups (total cholesterol 156.97 ± 32.91 vs 127.76 ± 19.12 mg/dL and LDL cholesterol 83.24 ± 27.98 vs 62.00 ± 19.71 mg/dL in the high vs low CIMT progression groups, respectively). In addition, baseline serum total cholesterol and LDL cholesterol and homocysteine levels positively correlated with ΔCIMT progression in the placebo group (Supplementary Figure 2A). There were also positive correlations between CIMT progression and various JSLE-related biomarkers at baseline, such as creatinine and C4 levels, and negative correlation with the spot urine protein/creatinine ratio (PCR). The Systemic Lupus International Collaborating Clinics (SLICC) Damage Index was also positively associated with CIMT progression (Supplementary Figure 2A). Taken together, these findings indicate that various measures of chronic inflammation at baseline differentially correlate with subclinical atherosclerosis progression over 36 months (as higher C4 levels and a lower urine PCR reflect better disease control at baseline, whereas increased damage reflects the opposite), and altered lipid metabolism (reflected by

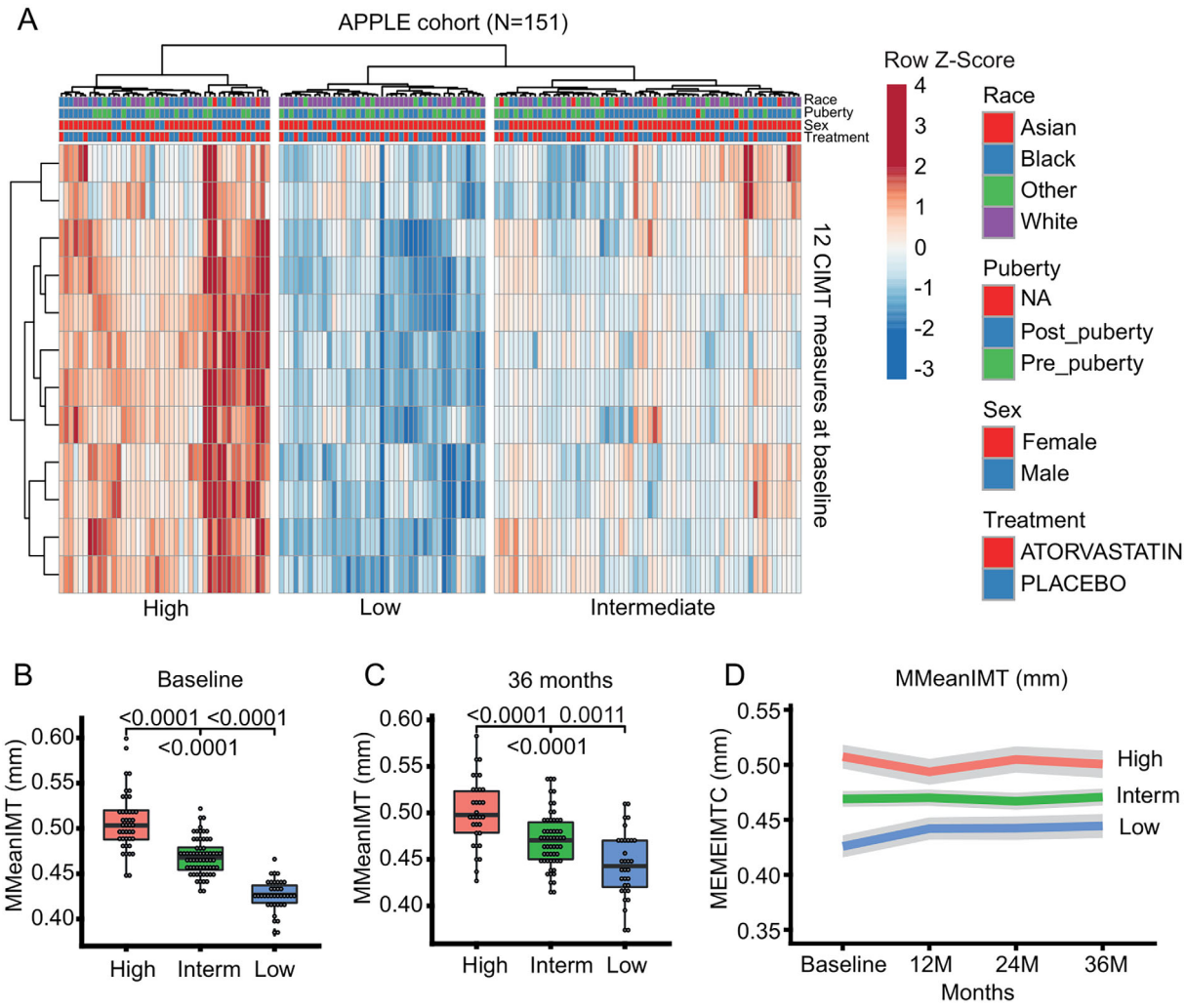

Figure 1. Juvenile-onset systemic lupus erythematosus (JSLE) stratification (all APPLE patients with complete baseline data, N = 151) by baseline CIMT (12 measures). (A) Baseline CIMT measures of patients with JSLE were stratified using unsupervised hierarchical clustering. All 12 CIMT measures were standardized within each row by Z score and plotted as a heat map representing the relationship to the mean of the group (red represents relatively high CIMT measures, and blue represents relatively low CIMT measures). Each column represents a patient with JSLE. Three groups of patients with distinct baseline CIMT profiles were identified. (B and C) Box and whisker plots show baseline and 36-month MMeanIMT measurements (APPLE primary outcome) in the identified high, intermediate, and low baseline CIMT groups. Comparisons between groups were performed using the Wilcoxon signed rank test. (D) Distinct longitudinal MMeanIMT progression from baseline to 36 months of the high, intermediate, and low CIMT progression groups (mean, 95% confidence interval), irrespective of treatment allocation. Only patients with JSLE with completed CIMT data at 36 months were included in (C) and (D) (n = 121). APPLE, Atherosclerosis Prevention in Pediatric Lupus Erythematosus; CIMT, carotid intima-media thickness; MMeanIMT; mean of the mean common CIMT.

differences in the lipid profile and homocysteine levels) may contribute in different ways to atherosclerosis progression in JSLE.

Patients with JSLE treated with atorvastatin in the APPLE trial stratified into three groups based on their CIMT trajectories over 36 months. CIMT progression over 36 months (ΔCIMT) was also assessed in the atorvastatin arm of the APPLE trial (n = 61) (Table 3). Unsupervised cluster analysis (using the 12 ΔCIMT measures as described in Patients and Methods) identified three distinct groups: high (n = 22), intermediate (n = 24), and low (n = 15) CIMT progression groups (Figure 2C). Notably, 36% of patients with JSLE in the atorvastatin group (n = 22 of 61) had high CIMT progression over 36 months despite treatment. Significant changes in MMeanIMT over 36 months were observed in high (increased, $P < 0.0001$) and low (decreased, $P = 0.002$) CIMT progression groups, whereas the intermediate group ($P = 0.51$) had almost stable MMeanIMT measurements over 36 months (Figure 2D). As observed in the placebo group, no significant differences in clinical and demographic measures were observed in patients in the atorvastatin arm across the three CIMT progression groups at baseline (Table 3), and few correlations between CIMT and clinical

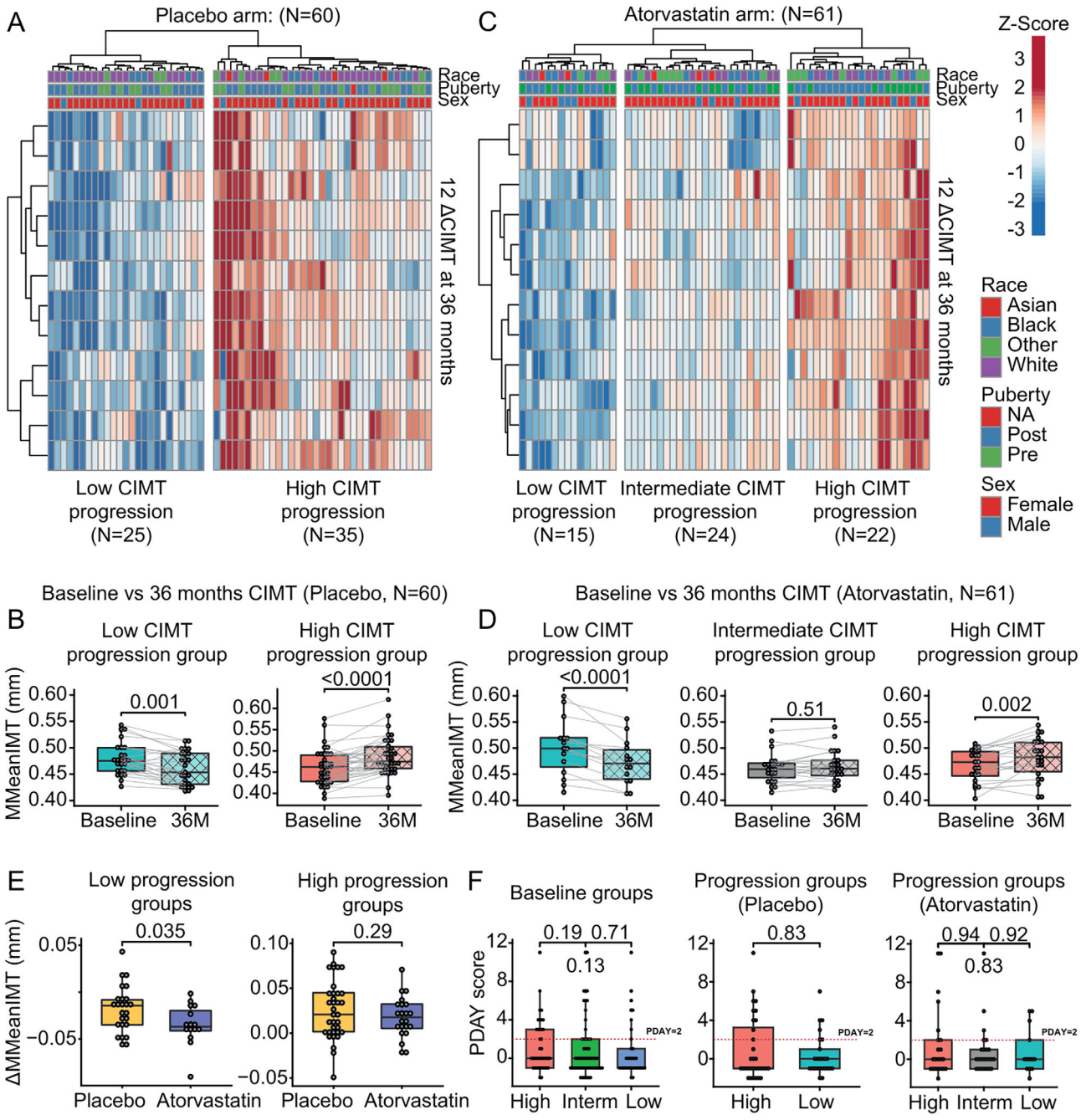

Figure 2. Juvenile-onset systemic lupus erythematosus (JSLE) stratification by ΔCIMT (12 measurements) at baseline versus 36 months in the placebo and atorvastatin arms. (A) Heat map displaying ΔCIMT (Z scored) from patients with JSLE from the placebo arm (full CIMT data set, n = 60) stratified by unsupervised hierarchical clustering. Each column represents a patient with JSLE. High and low ΔCIMT progression groups were discovered over 36 months. (B) Box and whisker plots showing comparisons of MMeanIMT between groups from (A) at baseline and 36 months. (C) Heat map displaying ΔCIMT (Z scored) from patients with JSLE from the atorvastatin arm (full CIMT data set, n = 61) stratified by unsupervised hierarchical clustering. Each column represents a patient with JSLE. High, intermediate, and low ΔCIMT progression groups were discovered over 36 months. (D) Box and whisker plots showing comparison of MMeanIMT between groups from (C) at baseline and 36 months. (E) Box and whisker plots showing comparison of MMeanIMT between high and low CIMT progression groups between patients in the placebo and atorvastatin arms. Wilcoxon's signed rank test or *t*-test. (F) Box and whisker plots showing comparisons of PDAY scores between baseline, placebo, and atorvastatin progression groups. CIMT, carotid intima-media thickness; MMeanIMT, mean of the mean common CIMT; PDAY, Pathobiological Determinants of Atherosclerosis in Youth.

measures were identified (Supplementary Figure 2B). Most notably, there were no correlations among 12 CIMT progression measures and baseline serum lipids, likely due to the impact of atorvastatin treatment on the CIMT trajectories of some patients with JSLE, even if the trial did not show overall benefit. Furthermore, the correlation analysis suggests that atorvastatin treatment disrupted the association between various biomarkers and CIMT progression observed in the placebo group (Supplementary Figure 2B). Interestingly, complement fractions C3 and C4, biomarkers of serological activity in JSLE, were inversely associated with CIMT progression similar to an independent analysis of the APPLE trial,[25] indicating that disease-related factors may drive CIMT progression in JSLE despite statin treatment normalizing the lipid profile.

Interestingly, when the MMeanIMT progression over 36 months (ΔMMeanIMT) was compared in the low progression groups across both treatment arms, atorvastatin-treated patients had significantly reduced CIMT progression over 36 months compared with the placebo-treated patients (Figure 2E). This suggests that patients with JSLE allocated to the statin treatment group with low CIMT progression over 36 months (24.5%, n = 15, based on the unsupervised cluster analysis) benefited from treatment with statins because their CIMT progression was significantly reduced compared to patients with JSLE in the placebo arm with low CIMT progression (41.6%, n = 25), despite no differences in the baseline lipid profiles between the low versus high progression groups in the statin arm (Table 3). Conversely, no difference in ΔMMeanIMT was seen between the high CIMT progression groups in the two treatment arms, suggesting that atorvastatin did not influence the CIMT progression rate in these high CIMT progression patients (58.3% [n = 35 of 60] in the placebo arm and 36% [n = 22 of 61] in the atorvastatin arm) (Figure 2E).

Finally, to confirm the pharmacological effect of atorvastatin in JSLE, although serum LDL cholesterol levels did not significantly decrease in the placebo arm ($P = 0.61$; Supplementary Figure 3), we found a significant reduction in routinely measured serum LDL cholesterol levels at 36 months in the atorvastatin-treated patients ($P < 0.0001$; Supplementary Figure 3). Thus, despite the decrease of serum LDL cholesterol levels with atorvastatin treatment, a sizable proportion of patients (n = 22, 36.1%) continued to CIMT progression, suggesting that CIMT progression was driven by factors independent from dysregulation of lipid metabolism.

Validated CVD risk scores misclassified patients with JSLE in the APPLE trial. Because no significant differences between individual traditional CVD risk factors (age, sex, blood pressure, diabetes, body mass index [BMI], smoking) were identified between distinct CIMT trajectories in either the placebo or the atorvastatin arms of the APPLE trial (with the exception of total and LDL cholesterol levels in the placebo arm) (Tables 2 and 3), we explored the classification accuracy of four of the most commonly used CVD risk scores for stratification in general population: the FRS,[19] validated from age 20; the QRISK3 score,[21] validated from age 25, the only CVD risk score that includes "SLE" as well as "steroid treatment" as individual items; and the PDAY score,[20] the only score proposed for use in CYP from age 14, with scores >2 indicating a high risk for coronary artery calcium progression in 25 years.[26]

Applying the various CVD risk scores to the APPLE trial JSLE cohort at baseline as per data availability, we found that very few patients were identified as high risk (Supplementary Table 1). The FRS score classified all patients with JSLE as low risk (<5%, n = 144), and the ASCVD score classified a large proportion of patients with JSLE as low risk (<5%, n = 92 of 99), a small proportion as borderline to moderate risk (5%–19.9%, n = 7 of 99), and none as high risk at baseline. Only the QRISK3 and PDAY scores identified a very small number of patients with JSLE as high risk (>20% and >10 points, n = 2 of 144 and n = 3 of 138, respectively), whereas the largest number of patients were classified as low risk (<5%, n = 120 of 144 and n = 98 of 138, respectively) and the remaining were classified as borderline to moderate risk (Supplementary Table 1).

Furthermore, very few of the patients with JSLE were correctly classified as high risk when compared to their stratification based on CIMT at baseline or CIMT progression pattern over 36 months in the placebo or atorvastatin arm (Supplementary Figure 4). The QRISK3 score classified 1 of 63 and 1 of 39 patients with JSLE with intermediate and low CIMT, respectively, as high risk at baseline (QRISK3 score >20%), but only 2 of 35 patients with JSLE with a high-progression CIMT pattern in the placebo group were correctly identified as high risk (Supplementary Figure 4).

Similarly, there was no conformity between the PDAY score and baseline CIMT or CIMT progression stratifications (Figure 2F); no significant difference was seen between groups stratified for high versus low baseline CIMT or CIMT progression, although patients with PDAY score >2 (at least borderline risk) were identified in all the CIMT-stratified groups (Figure 2F). Thus, most patients with high CIMT at baseline or high CIMT progression over 36 months in the APPLE trial were misclassified by four different CVD risk scores, suggesting that none of these tools perform well in CYP with JSLE.

Novel serum metabolomic signature predicts high CIMT progression in the placebo arm but not in the atorvastatin arm. Because the commonly used CVD risk scores failed to accurately classify patients with high CIMT progression and high CIMT progression in patients with JSLE in the placebo arm was positively associated with higher routinely measured serum LDL and total cholesterol levels (Table 2, Supplementary Figure 2A), a more detailed NMR metabolomic analysis was performed (250 serum lipid-based metabolites; full list Supplementary Table 2) at baseline (n = 60).

Forty-eight metabolites were significantly up-regulated in the high compared to the low CIMT progression group in the placebo arm (Figure 3A). The top six significantly increased metabolites selected after stringent Bonferroni correction were total esterified

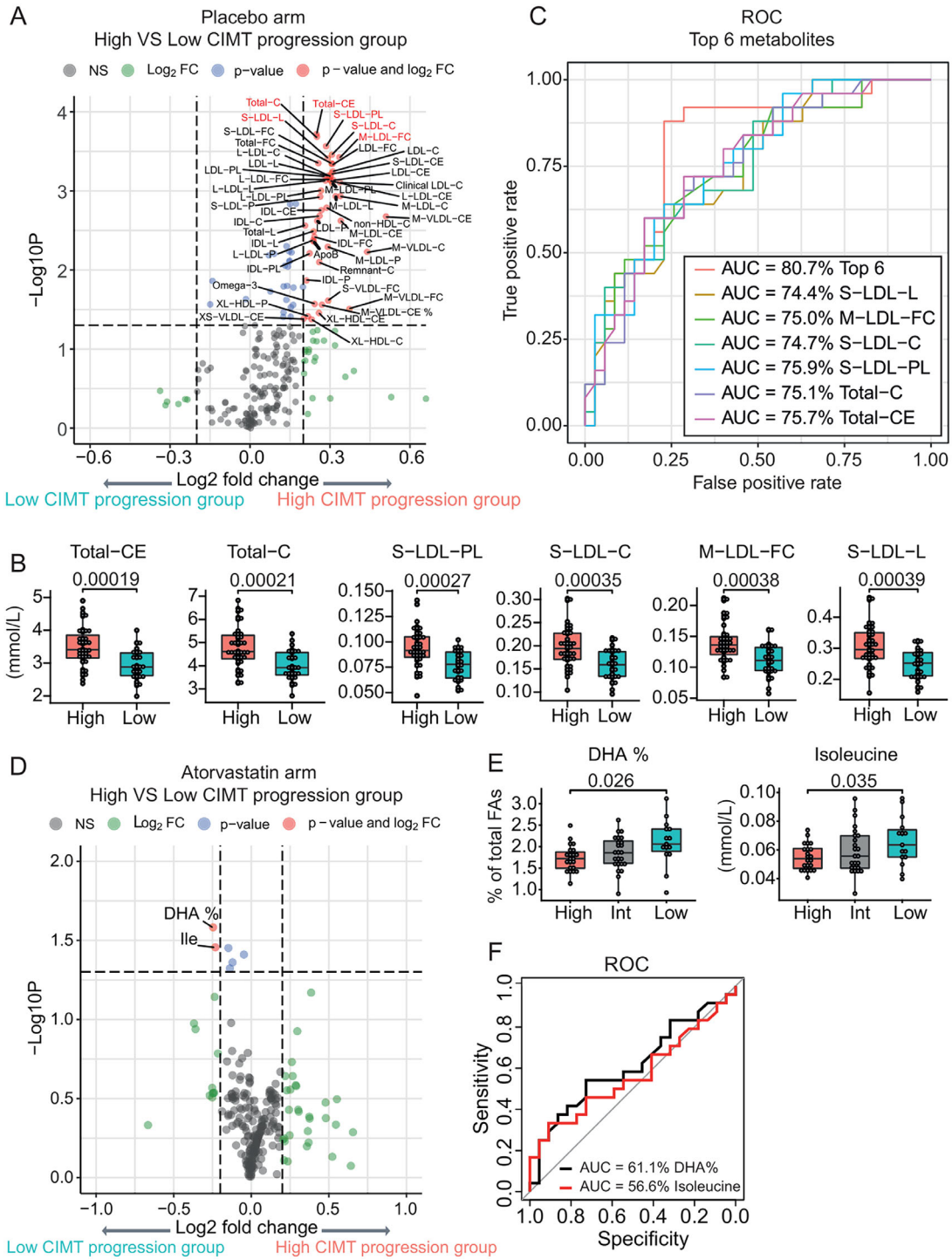

Figure 3. Baseline serum metabolomics (n = 245 after data cleaning) comparisons between CIMT progression groups: placebo and atorvastatin arms. (A) Volcano plot displaying fold change of all metabolites and Log_{10} *P* values comparing high (n = 35) and low (n = 25) CIMT progression groups: placebo arm ($P < 0.01$; log_2 [fold change] >0.2). Top six significant metabolites (Bonferroni correction) are highlighted in red. (B) Box and whisker plots showing the top six metabolite levels of the high versus low CIMT progression groups: placebo arm. Unpaired *t*-test. (C) ROC analysis for discriminating high versus low CIMT progression groups using the top six metabolites combined and separately by AUC. (D) Volcano plot displaying fold change of all metabolites and Log_{10} *P* values comparing high (n = 22) and low (n = 15) CIMT progression groups: atorvastatin arm ($P < 0.05$; log_2 [fold change] >0.2). Top two significant metabolites (Bonferroni correction) are highlighted in red. (E) Box and whisker plots showing the top two metabolite levels of the high versus low CIMT progression groups: atorvastatin arm. Unpaired *t*-test. (F) ROC analysis for discriminating high versus low CIMT progression groups using percentage of docosahexaenoic acid and isoleucine by AUC. AUC, area under the curve; CIMT, carotid intima-media thickness; ROC, receiver operating characteristic curve; S-LDL-L, total lipids in small low density lypoproteins (LDL); M-LDL-FC, free cholesterol in medium LDL; S-LDL-C, cholesterol in small LDL; S-LDL-PL, phospholipids in small LDL; Total-C, total cholesterol; Total – CE, total cholesteryls esters.

cholesterol, total cholesterol, phospholipids in small LDL, cholesterol in small LDL, free cholesterol in medium LDL, and total lipids in small LDL (Figure 3A [red labels] and B). This suggests that patients with JSLE in the high CIMT progression group had a distinct, proatherogenic lipid metabolomic profile, dominated by cholesterol and LDL subsets. Using the six-metabolite signature combined, receiver operating characteristic curve (ROC) analysis in multivariate logistic regression showed an area under the curve (AUC) of 80.7%, higher than that for the individual metabolites alone (AUC range 74.4%–75.9%) (Figure 3C). This was also higher than the AUC for total cholesterol (AUC 76.3%) and LDL cholesterol (AUC 72.5%) levels measured in the APPLE trial (Supplementary Figure 5A), suggesting that these six metabolites could provide a biomarker signature for predicting CIMT progression in JSLE.

To support these findings, univariate logistic regression analysis was performed on all metabolites, comparing the high and low CIMT progression groups in the placebo arm and accounting for clinical and treatment features. All six selected metabolites were increased in the high CIMT progression group (Supplementary Figure 5B). These results were further confirmed using supervised machine learning approaches. The optimized sparse partial least squares discriminant analysis showed separation between the two CIMT progression groups and identified similar metabolites (highlighted in red) in the first component of the model driving the high versus low CIMT progression stratification (Supplementary Figure 5C and D). Together, the additional analysis validated the six-metabolite predictive signature of CIMT progression in the placebo arm (Figure 3A–C).

The same NMR metabolomics analysis pipeline was applied to the atorvastatin arm of the APPLE trial. Only two metabolomic markers (the ratio of docosahexaenoic acid to total fatty acids and isoleucine) were significantly different between the high and low CIMT progression groups (Figure 3D and 3E), with poor performance under ROC analysis (Figure 3F). Thus, no distinct baseline metabolomic signature was found between the high and low CIMT progression groups in the atorvastatin treatment arm. Because neither routine serum lipid measures (Supplementary Figure 2B) nor the in-depth metabolomic signature correlated with CIMT progression, these results show that in atorvastatin-treated patients, baseline lipid signatures do not predict CIMT progression and that statin treatment abrogated the predictive signature of CIMT progression found in the placebo group.

DISCUSSION

The current study included a novel patient stratification and biomarker identification analysis of the APPLE trial data and samples to improve CVD risk assessment in JSLE, aiming to address the unmet clinical need for early identification and tailored CVD risk management. Patients with JSLE recruited to the APPLE trial, despite being young, already had different degrees of subclinical atherosclerosis. This study further explored subclinical atherosclerosis heterogeneity by stratifying patients into distinct groups and by defining distinct CIMT progression rates over 36 months, irrespective of treatment allocation. The only significant predictors of baseline CIMT unsupervised patient stratification were age, disease duration, and creatinine clearance, supporting previous findings that longer SLE duration is associated with increased CVD risk.[27,28] However, the other predictors of baseline CIMT identified by the multivariable analysis of the APPLE trial[29] (minority status, higher BMI, male sex, higher lipoprotein A levels, proteinuria, azathioprine use, and prednisone dose) did not differ between the baseline CIMT patient groups derived from this current unsupervised cluster analysis. No patient- or disease-related significant differences were identified between the high versus low CIMT progression groups in the placebo arm either, apart from the increased levels of total and LDL cholesterol in the high progression group. These findings are difficult to appreciate at the individual patient level because most patients with JSLE had normal lipid profiles, even if there were statistically significant differences between the high and low CIMT progression groups.

Although the previous secondary analysis of the APPLE trial showed that hsCRP and pubertal status predicted response to atorvastatin, our unsupervised cluster analysis did not identify these markers as being different between patients with JSLE stratified on CIMT at baseline or according to the rate of their progression over 36 months,[18] which could be explained by the limitations posed by the available sample size, as well as differences in the methodologic approach of our analyses. Our approach allowed us to identify predictors directly related to the APPLE trial primary outcome while accounting for the CIMT patient heterogeneity at baseline as well as heterogeneity in their CIMT progression pattern, which in turn ensures a more comprehensive investigation of potential biomarkers of subclinical atherosclerosis progression. Conversely, the previous secondary analysis categorized patients based on markers such as hsCRP and pubertal status, while not taking into account their CIMT heterogeneity at the beginning of the trial, which was the most significant predictor of treatment response in our analysis (Figure 2E shows that only patients with low CIMT progression benefited from statin treatment, suggesting that CIMT stratification at baseline could have led to a positive outcome of the APPLE trial).

Patients with JSLE allocated to the placebo arm provided the opportunity to examine untreated CIMT progression, as a validated measure for CVD risk,[30,31] and led to the identification of two patterns of CIMT progression and a robust serum lipid signature that defined the patients with JSLE who progressed at a higher rate, despite routinely measured lipid profiles being within the reference limits in both groups. Not surprisingly, none of the validated CVD risk scores used in the general population performed well in the APPLE trial cohort because almost all patients with JSLE were classified as low risk. Five conventional CVD risk scores underestimated the CVD risk in adult-onset SLE by 50%,

whereas three "lupus adapted" scores (QRISK3 and modified FRS/SCORE risk scores) misclassified 25% patients with SLE as low risk.[32] This emphasizes the need for additional high-performance biomarkers for CVD risk identification in SLE across age.

Lipid metabolomics is extensively used for atherosclerosis risk prediction in SLE because it provides more in-depth information that routinely measured lipids (including particle size and components). A machine learning model using the same metabolomic platform we employed in this study identified a lipidomic signature that distinguished patients with adult-onset SLE with versus without atherosclerosis plaques on vascular scans with a good performance (AUC 80%),[33] whereas a high apolipoprotein B:A1 ratio, linked with high $CD8^+$ T cell phenotyping and transcriptomic profile, was identified as potential marker for atherogenic progression in JSLE.[12] In our study, the six-biomarker lipid signature outperformed the LDL cholesterol and total cholesterol (used in routine practice) in identifying patients with JSLE with high rates of natural CIMT progression. This metabolomic signature provides an opportunity to explore future validation in external JSLE cohorts, which we will be pursuing.

Three of six metabolites defining the CIMT progression signature in the placebo arm are lipid components of small and dense LDL particles. Thus, in addition to predictive power, our signature also provides mechanistic insight because the association between the size of LDL particles and atherosclerosis has been explored before, with studies providing evidence for prolonged retention in plasma and enhanced ability to penetrate the arterial wall of small LDL particles.[34–36] Lipid-lowering drugs with smaller LDL targeted reduction properties, such as rosuvastatin, may represent a better targeted treatment choice for atherosclerosis prevention[37] for patients with JSLE, highlighting the need for more precise patient stratification to address the statin response heterogeneity found in JSLE.

Although accelerated atherosclerosis has been linked to many autoimmune rheumatic diseases, the association between JSLE disease activity and CIMT progression remains controversial, with some studies finding an association,[6] whereas others did not.[38] In our analysis, the untreated CIMT progression correlated positively with a proatherogenic lipid profile and presence of SLICC JSLE damage, suggesting that JSLE severity contributes to atherosclerosis, similar to previous reports.[39] However, we acknowledge the limitations of our correlation analyses between CIMT progression and baseline biomarkers due to the exploratory nature of these analyses and lack of multiple testing correction despite the use of a more stringent P value cutoff (<0.01), as well as inability to account for the potential impact of the variation of these biomarkers over 36 months, which is also likely to have influenced the CIMT progression in both the placebo and atorvastatin arms. This suggests a limited predictive value of individual baseline biomarkers for a disease that is recognized to fluctuate significantly over time. The observed differences between the direction of correlation of various JSLE markers reflecting disease activity and CIMT progression in both the placebo and atorvastatin arms highlight the need for a more comprehensive understanding of the interplay between lipid regulation, chronic inflammation, JSLE treatment, and traditional CVD factors in determining the pattern of CIMT progression in JSLE.

One possible explanation for the APPLE trial not meeting its primary end point is offered by the CIMT progression stratification in the atorvastatin arm, which identified a subgroup of patients with JSLE who progressed at a high rate despite atorvastatin successfully lowering their proatherogenic lipid profile. This indicates alternative mechanisms underpinning their atherosclerosis progression because the high CIMT progressors receiving statin treatment were not defined at baseline by the metabolomic signature, which characterized the high progressors in the placebo group. Together, these findings support the hypothesis of complementary atherosclerosis mechanisms in JSLE, very likely related to dysregulated lipid metabolism, chronic inflammation, and endothelial dysfunction, possibly modulated in distinct ways in the high versus low CIMT progression groups. The investigation of molecular mechanisms of atherosclerosis in JSLE or that of anti-inflammatory and metabolic therapeutic benefits of atorvastatin are beyond the scope of this article.

As with many other CVD measures, CIMT alone is not an ideal measure for predicting CVD risk in CYP because of challenges of standardization across ages. Factors contributing to the heterogeneity of the CIMT measures include variable ultrasound probe positioning and potential individual heterogeneity in the context of pubertal growth during the trial, despite the use of a standardized vascular ultrasound protocol and use of a central reader in the APPLE trial. These factors, in addition to lifestyle advice provided to all patients and other unidentified factors, might explain why some patients surprisingly experienced CIMT regression over time in the low progression groups in both the placebo and statin arms. There is an increasing body of evidence that atherosclerosis can be regressed in both human and animal studies, with the most accepted possible mechanisms being related to mobilization of apolipoprotein B lipoproteins from the arterial wall, combined with efflux of cholesterol, other lipids, and foam cells, as well as influx of healthy phagocytes that remove necrotic debris and macrophage phenotypic changes, all potentially leading to atherosclerosis lesions reversal.[40,41] Despite no convincing evidence that a specific therapy can promote atherosclerosis regression, there are increasing efforts in targeting the inflammatory mechanisms of atherosclerosis.[42]

This novel analysis of the APPLE trial provides evidence for the limitations of restricting CVD risk factor assessment to traditional CVD variables in patients with JSLE who have distinct trajectories of subclinical atherosclerosis progression. In addition, demographic and disease characteristics, as well as routine lipid profiling, did not identify patients with JSLE with increased CVD risk, and although effective in lowering serum lipids, atorvastatin

did not prevent subclinical atherosclerosis progression in many at-risk patients with JSLE. Further research into the mechanisms driving the unique lipidomic signature predictive of CIMT progression that we identified in the untreated patients, as well as investigation of other proinflammatory and metabolic proatherosclerotic mechanisms not influenced by statins, may potentially support future personalized therapeutic strategies to address the increased CVD risk in JSLE.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Mr. Peng and Prof. Ciurtin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Peng, Donnes, Ardoin, Schanberg, Lewandowski, Robinson, Jury, Ciurtin.

Acquisition of data. Peng, Ardoin, Schanberg, Lewandowski, Robinson, Jury, Ciurtin.

Analysis and interpretation of data. Peng, Donnes, Robinson, Jury, Ciurtin.

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APPENDIX A: COLLABORATORS

The following collaborators participated in this study by enrolling patients at sites or by performing study procedures at sites: Stacy Ardoin, Esi Morgan Dewitt, C. Egla Rabinovich, Janet Ellis, Kelly Mieszkalski, Janet Wootton (Duke University Medical Center, Durham, North Carolina); Peter Chira, Joyce Hsu, Tzielan Lee, Christy Sandborg, Jan Perea (Stanford University School of Medicine, Palo Alto, California); Beth Gottlieb, Patricia Irigoyen, Jennifer Luftig, Shaz Siddiqi, Zhen Ni, Marilynn Orlando, Eileen Pagano (Cohen Children's Medical Center, New Hyde Park, New York); Andrew Eichenfield, Lisa Imundo, Deborah Levy, Philip Kahn, Candido Batres, Digna Cabral (Morgan Stanley Children's Hospital of New York–Presbyterian, New York, New York); Kathleen A. Haines, Yukiko Kimura, Suzanne C. Li, Jennifer Weiss, Mary Ellen Riordan, Beena Vaidya (Hackensack University Medical Center, Hackensack, New Jersey); Emily von Scheven, Michelle Mietus-Snyder (University of California at San Francisco Medical Center, San Francisco, California); Earl Silverman, Lawrence Ng (Hospital for Sick Children, Toronto, Ontario, Canada); Suzanne Bowyer, Susan Ballinger, Thomas Klausmeier, Debra Hinchman, Andrea Hudgins (Indiana University School of Medicine, Indianapolis, Indiana); Marilynn Punaro, Shirley Henry, Shuzen Zhang (Texas Scottish Rite Hospital for Children, Dallas, Texas); Nora G. Singer, Elizabeth B. Brooks, Stacy Miner, Nancy Szabo, Lisabeth Scalzi (University Hospitals/Case Medical Center, Cleveland, Ohio); David Sherry, Libby Dorfeld, Sarajane Wilson, Jenna Tress (Children's Hospital of Philadelphia, Philadelphia, Pennsylvania); Deborah McCurdy, Tatiana Hernandez, Jyotsna Vitale (University of California Los Angeles Medical Center, Los Angeles, California); Marisa Klein-Gitelman, Angela Kress, Nicole Lowe, Falguni Patel (Children's Memorial Hospital, Chicago, Illinois); Carol Wallace, Stephanie Hamilton (Seattle Children's Hospital and Regional Medical Center, Seattle, Washington); Richard Silver, Katie Caldwell, Diane Kamen (Medical University of South Carolina, Charleston, South Carolina); Linda Wagner-Weiner, Becky Puplava, Atanas Lonchev (University of Chicago, Chicago, Illinois); Gloria Higgins, Monica Bacani (Nationwide Children's Hospital, Columbus, Ohio); Hermine Brunner, Cynthia Rutherford, Jamie Meyers-Eaton, Shannen Nelson, Alexei Grom (Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio); Larry Jung, Teresa Conway, Lacey Frank, Lori Kuss (Creighton University Medical Center, Omaha, Nebraska); Jenny Soep, Hazel Senz (University of Colorado, Aurora, Colorado); and Ann Reed, Thomas Mason, Jane Jaquith, Diana E. Paepke-Tollefsrud (Mayo Clinic, Rochester, Minnesota).