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Soluble Low-density Lipoprotein Receptor-related Protein 1 in Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objectives.*** This study aimed to expand knowledge about soluble low-density lipoprotein receptor-related protein 1 (sLRP1) in juvenile idiopathic arthritis (JIA) by determining associations of sLRP1 levels in nonsystemic JIA patients with clinical and inflammatory biomarker indicators of disease activity.

Methods. Plasma sLRP1 and 44 inflammation-related biomarkers were measured at enrollment and 6 months later in a cohort of 96 newly diagnosed Canadian patients with nonsystemic JIA. Relationships between sLRP1 levels and indicators of disease activity and biomarker levels were analyzed at both visits.

Results. At enrollment, sLRP1 levels correlated negatively with age and active joint counts. Children showed significantly higher levels of sLRP1 than adolescents (mean ranks: 55.4 and 41.9, respectively; $P = 0.02$). Participants with 4 or fewer active joints, compared to those with 5 or more active joints, had significantly higher sLRP1 levels (mean ranks: 56.2 and 40.7, respectively; $P = 0.006$). At enrollment, considering the entire cohort, sLRP1 correlated negatively with the number of active joints ($r = -0.235$, $P = 0.017$). In the entire cohort, sLRP1 levels at enrollment and 6 months later correlated with 13 and 6 pro- and antiinflammatory biomarkers, respectively. In JIA categories, sLRP1 correlations with inflammatory markers were significant in rheumatoid factor–negative polyarticular JIA, oligoarticular JIA, enthesitis-related arthritis, and psoriatic arthritis at enrollment. Higher sLRP1 levels at enrollment increased the likelihood of absence of active joints 6 months later.

Conclusion. Plasma sLRP1 levels correlate with clinical and biomarker indicators of short-term improvement in JIA disease activity, supporting sLRP1 as an upstream biomarker of potential utility for assessing JIA disease activity and outcome prediction.

Key Indexing Terms: inflammation, juvenile idiopathic arthritis, lipoprotein receptors

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Low-density lipoprotein receptor-related protein 1 (LRP1) is a transmembrane lipoprotein receptor that mediates binding and endocytosis of extracellular ligands and modulates certain intracellular inflammatory and immune signaling pathways[1]. LRP1 is also known as cluster of differentiation antigen 91, α_2-macroglobulin (α_2M) receptor, and apolipoprotein E receptor. LRP1 binds many ligands, including lipoproteins, proteases, extracellular matrix proteins, growth factors, bacterial toxins, and viruses[2], and mediates endocytosis of cellular debris released after cell death[3]. Heat shock proteins, which chaperone antigenic peptides, bind to LRP1[4]. Further, peptides bound to activated α_2M are internalized by LRP1 for presentation to T cells[5].

LRP1 comprises 2 chains: a 515-kDa α-chain and an 85-kDa β-chain. The 85-kDa β-chain comprises an extracellular ectodomain, a transmembrane domain, and an intracellular tail[6,7]. The α-chain is extracellular and is attached to the ectodomain of the β-chain. In the presence of inflammation, the α-chain and part of the β-chain ectodomain are shed into the circulation as soluble LRP1 (sLRP1)[7]. Thus, sLRP1 is a circulating segment released from the intact cell-bound LRP1 receptor. The shedding of sLRP1 is both a consequence of inflammation and integral to modulating the inflammatory process, including influencing the release of cytokines. Like cell-bound LRP1, circulating sLRP1 also might mediate antigen sequestration and presentation.

Elevated levels of sLRP1 are found in adults with rheumatoid arthritis (RA) and systemic lupus erythematosus[7]. Previously, we reported that sLRP1 levels were higher in children from most juvenile idiopathic arthritis (JIA) subsets[8,9] and that sLRP1 was a component of a panel of clinical and biomarker attributes that collectively could predict short-term JIA outcomes[10]. It was this earlier discovery of sLRP1's contribution to the predictive panel that prompted us, in this current study, to investigate more thoroughly sLRP1's specific associations with clinical and biomarker features in JIA.

We hypothesized that elevated sLRP1 levels in JIA correlate with clinical and biomarker indicators of disease activity and may predict short-term improvement in arthritis activity.

MATERIALS AND METHODS

The study population comprised an inception cohort of 96 newly diagnosed patients with JIA (exclusive of the systemic JIA subset) who consented to participate in the Biologically Based Outcome Predictor (BBOP) study. Participants, recruited from 11 participating Canadian pediatric rheumatology clinics between 2008 and 2012, were assessed at enrollment and 6 months postenrollment. To avoid overrepresentation of oligoarticular JIA, the most prevalent JIA subset, and underrepresentation of the less common polyarticular and systemic JIA subsets, the eligibility criteria for the first 6 months of the BBOP study required more than 4 active joints at first presentation. After 6 months, all JIA subsets were eligible for recruitment. The disease was diagnosed, and participants were categorized in accord with the International League of Associations for Rheumatology classification criteria[11]. As participants were evaluated at enrollment, assignments of oligoarticular or polyarthritis categories were presumptive, since all patients were enrolled at 6 months or less after diagnosis. All JIA categories, except systemic JIA, and all those with available plasma sLRP1 measurements were eligible for enrollment into the present BBOP substudy. Of the 96 participants comprising the cohort reported in this study, 58 (60.4%) were included in the cohort reported in our earlier study[10].

The study was approved by each of the participating sites (Biomedical Research Ethics Board [REB], University of Saskatchewan: 07-86; Clinical REB, University of British Columbia: H07-01204; Health REB, University of Alberta: 6984; Biomedical REB, University of Manitoba: H2007:111; REB, Hospital for Sick Children: 1000011118; REB, Children's Hospital of Eastern Ontario: 09-16E; Biomedical REB, McGill University: PED-07-020; Research Ethics Committee, Université Laval: 123.05.09; Research Ethics Committee of the Centre Hospitalier Universitaire de Sherbrooke: 07-119; IWK Health Centre REB: 1001241; Human Investigation Committee, Memorial University: 06.047). We obtained the participants' consents to publish the material.

Blood was collected by venipuncture into P100 Vacutainer tubes (BD Biosciences) through previously described protocols[12]. Samples were kept at 4°C until shipped on the day of collection by overnight courier at ambient temperature to the central biobank laboratory. On arrival at the destination laboratory, blood was centrifuged (1000 g × 15 min) within 24 hours of collection, and the plasma stored at −80°C until batch assayed.

Of the 96 study participants, all had sLRP1 levels measured at enrollment, and 58 had sLRP1 levels measured 6 months postenrollment. sLRP1 was assayed in duplicate by ELISA as follows: 96-well microtiter plates (Microlon, Greiner Bio-One Inc.) were coated overnight at 4°C with 100 µL per well of monoclonal antibody specific for sLRP1 (clone α2-MRα2; Genway Biotech), 1 µg/mL diluted in carbonate-bicarbonate (15 mM Na_2CO_3, 35 mM $NaHCO_3$). Plates were then washed 3 times with 0.1% phosphate-buffered saline containing 0.1% Tween 20 (PBST), and then 100 µL of plasma diluted 1/500 in PBST were added to duplicate wells (or 1/1000, as needed to bring the sample ELISA values within the standard curve). After incubation at 37°C for 1 hour, the plates were washed 3 times with PBST and 100 µL biotin-labeled antihuman LRP1 monoclonal antibody (clone α2-MRα2; Pierce, Thermo Scientific) diluted 1:2500 in PBST were added. The plates were incubated for 1 hour at 37°C, then washed 3 times with PBST. Then, 100 µL of horseradish peroxidase (HRP)-conjugated Avidin (Vector Laboratories) were added at a 1:5000 dilution for 30 minutes at 20°C. Plates were washed 3 times with PBST, and 100 µL of substrate (2 mM o-phenylenediamine 0.02 M citric acid, 0.05 M Na_2HPO_4, 0.012% H_2O_2) added after. After a 30-minute incubation, the reactions were terminated by the addition of 100 µL of 4M H_2SO_4. Optical densities were measured at 492 nm (Universal Microplate Reader EL800, Bio-Tek Instruments Inc.). Concentrations of sLRP1 were calculated based on a standard curve, which had a sensitivity of 0.1 ng/mL. The standard curve was generated using sLRP1 purified by affinity chromatography, using anti-sLRP (Genway Biotech) linked to Pierce NHS-Activated Agarose Slurry (Thermo Scientific).

Inflammation-related biomarker concentrations (cytokines, chemokines, growth factors, and metzincins) were measured using magnetic bead-based single and multiplex panels (EMD Millipore) with the following product codes: receptor activator of NF-κB ligand (HBN51K1RANKL), RANTES (regulated on activation of normal T cells expressed and secreted; HCYTOMAG-60K-01), osteoprotegerin (HBN1B-51K-01), tissue inhibitor of metalloproteinase (TIMP) 1/2 (HTIMP1-54K-02), TIMP3/4 (HTIMP2-54K-01), matrix metalloproteinase (MMP) 3/12/13 (HMMP1-55K-03), MMP1/2/7/9/10 (HMMP2-55K-05), MMP8 (HSP2MAG-63K-01), 29-plex cytokine/chemokine panel (HCYTMAG-60K-PX29), and fibroblast growth factor 2 (HCYTOMAG-60K-01). These analytes were analyzed on a Luminex100 LabMAP system (Luminex; Analytical Facility for Bioactive Molecules, Hospital for Sick Children) according to the manufacturer's instructions.

Despite logarithmic transformation, sLRP1 was not normally

The authors declare no conflict of interest

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distributed. Therefore, nonparametric tests were applied to the data. Spearman correlations were used to identify relationships between sLRP1 levels and indicators of disease activity (active joint count, erythrocyte sedimentation rate [ESR], and C-reactive protein [CRP]). At enrollment, differences in sLRP1 levels in relation to sex, age group (children < 10 and adolescents > 10 yrs[13]), and duration of morning stiffness were analyzed by Mann-Whitney *U* test. Comparisons of sLRP1 across JIA categories were assessed by the Kruskal-Wallis test. Logistic regression was applied to model the relationship between sLRP1 levels at enrollment as a predictor of the absence of active joints, 6 months after enrollment. Juvenile arthritis disease activity scores (JADAS-71) were calculated for the 6-month visit using the number of active joints, ESR, CRP, physician global assessment, and parent global assessment. The association between sLRP1 levels at enrollment and JADAS-71 scores at the 6-month visit were evaluated using linear regression[14]. False discovery rate (FDR) correction for multiple comparisons was applied to the correlation analyses[15].

RESULTS

The distributions of JIA categories and demographic characteristics of the 96 study participants at enrollment are shown in Table 1. Of the 96 participants, 66 (68.8%) were female. The median age at enrollment was 10.0 years (IQR 4.0–14.0 yrs). The median time from diagnosis to enrollment was 7.0 days (IQR 0.0–20.0 days). Six participants received medication before enrollment. Of these 6 participants, 3 received a nonsteroidal antiinflammatory drug (NSAID); of these 3, two received an NSAID 30 days before enrollment and 1 received an NSAID 10 days before enrollment. One participant received a disease-modifying antirheumatic drug (DMARD; methotrexate [MTX]) 10 days before enrollment, 1 participant received corticosteroids and MTX 7 days before enrollment, and 1 participant received corticosteroids 7 days before enrollment. At the 6-month visit, patients were receiving NSAIDs (68%), DMARDs (50%), corticosteroids (16%), and biologic agents (9%).

Enrollment sLRP1 levels ranged from 0.10 to 636.8 ng/mL (median 35.4, IQR 14.2–97.6 ng/mL) and for the 6-month follow-up visit, from 0.6 to 582.6 ng/mL (median 30.8, IQR 12.5–65.7 ng/mL).

The levels of sLRP1 did not differ significantly between females and males ($P = 0.20$) (Table 2). Children showed significantly higher levels of sLRP1 than adolescents (children: mean rank 55.4, median 53.3, IQR 21.1–135.5; adolescents: mean rank 41.9, median 20.9, IQR 9.9–53.4, $P = 0.02$). Considering the number of active joints regardless of JIA categories, patients with ≤ 4 active joints compared to those with ≥ 5 active joints had significantly higher sLRP1 levels (mean rank 56.2, median 50.2, IQR 20.0–104.5, and mean rank 40.7, median 24.3, IQR 9.7–64.0, respectively, $P = 0.006$).

Table 1. Demographics of the study population.

JIA Category	n (%)	Sex, F:M	Age at Enrollment, Yrs, Median (IQR)
Polyarthritis RF–	41(37.3)	30:11	11.0 (4.0–14.0)
Oligoarticular	26 (23.6)	17:9	8.0 (2.0–12.0)
Psoriatic arthritis	9 (8.2)	6:3	6 (4.5–13.5)
Polyarthritis RF+	8 (7.3)	5:3	11.5 (7.5–15.0)
ERA	8 (7.3)	6:2	13.5 (10.0 –14.0)
Undifferentiated	4 (3.6)	2:2	9.5 (6.0–13.7)
Total	96 (100)	66:30	10.0 (4.0–14.0)

ERA: enthesitis-related arthritis; JIA: juvenile idiopathic arthritis; RF: rheumatoid factor.

Table 2. Comparison of sLRP1 levels according to study participants' sex, age, and number of active joints.

		Median (IQR)	Mean Rank	*P*
Sex	Male	47.8 (17.3–127.5)	53.8	
	Female	31.1 (11.7–73.4)	46.1	0.20
Age group	Children	53.3 (21.1–135.5)	55.4	
	Adolescents	20.9 (9.9–53.4)	41.9	0.02
No. active joints	≤ 4	50.2 (20.0–104.5)	56.2	
	> 4	24.3 (9.7–64.0)	40.7	0.006

sLRP1: soluble low-density lipoprotein receptor-related protein 1.

Table 3 shows the number of active joints and sLRP1 levels at both visits. Although levels were highest in the oligoarthritis and psoriatic arthritis (PsA) categories (median 52.8, IQR 19.7–183.2, and median 70.7, IQR 21.0–261.6, respectively) and lowest in the rheumatoid factor–positive polyarticular (RF+ polyarthritis) JIA category (median 20.5, IQR 8.4–45.5), the Kruskal-Wallis test showed no significant differences among JIA categories ($P = 0.38$).

After FDR correction, age showed a negative correlation with sLRP1 levels at both visits (Table 4). At the enrollment visit, when the entire cohort was considered, sLRP1 correlated negatively with the number of active joints ($r = -0.235$, $P = 0.02$); that is, the higher the sLRP1 level, the fewer the number of active joints. However, 6 months later, there was no correlation between sLRP1 and the number of active joints.

To assess if young age and fewer active joints were each independently related to higher sLRP1 levels, we compared young children < 5 years of age with oligoarthritis to those with polyarthritis. In the group of 12 young children with polyarthritis, there was a negative but insignificant correlation with active joint count ($r = -0.156$, $P = 0.65$). In the group of 11 young children with oligoarthritis, there was a positive but insignificant correlation with joint count ($r = 0.632$, $P = 0.06$). The Mann-Whitney U test showed no significant difference between the 2 groups (data not shown).

There was no significant association between sLRP1 levels and the duration of morning stiffness at either visit. There were significant positive correlations between sLRP1 with 13 pro- or 6 antiinflammatory biomarker levels at enrollment and at 6-month visits, respectively (Table 4). In both visits, there was no correlation between sLRP1 levels with ESR, CRP, or antinuclear antibody (ANA). There was no significant association between sLRP1 at enrollment with JADAS-71 after 6 months.

Considering JIA categories, sLRP1 correlations with pro- and antiinflammatory biomarkers were significant in RF-negative polyarticular JIA (RF– polyarthritis), oligoarticular JIA, PsA, and enthesitis-related arthritis (ERA) categories at enrollment (Table 5).

There was no significant difference in sLRP1 levels at

Table 3. The number of active joints and sLRP1 levels at enrollment and 6-month visit.

JIA Category	No. Active Joints at Enrollment, Median (IQR)	No. Active Joints at 6-month Visit, Median (IQR)	sLRP at Enrollment, ng/mL, Median (IQR)	sLRP at 6-month, ng/mL, Median (IQR)
Polyarthritis RF–	9.0 (5.0–18.0)	2.5 (0.0–4.7)	26.9 (9.5–87.1)	26.8 (13.7–59.6)
Oligoarthritis	2.0 (1.0–3.0)	1.0 (0.0–2.0)	52.8 (19.7–183.2)	43.2 (11.4–183.1)
PsA	3.0 (2.0–9.0)	0.0 (0.0–2.0)	70.7 (21.0–261.6)	25.7 (1.1–139.0)
Polyarthritis RF+	19.0 (7.0–27.0)	0.0 (0.0–2.5)	20.5 (8.4–45.5)	17.9 (6.6–43.8)
ERA	3.0 (1.0– 8.0)	0.0 (0.0–1.0)	24.7 (7.1–56.5)	30.4 (2.8–52.4)
Undifferentiated	2.0 (1.0–7.0)	0.0 (1.0–5.0)	27.5 (20.0–84.1)	18.1 (16.0–75.8)
Total	5.0 (2.0–12.0)	1.0 (0.0–3.0)	35.4 (14.2–82.2)	27.5 (12.9–64.5)

ERA: enthesitis-related arthritis; PsA: psoriatic arthritis; RF: rheumatoid factor; sLRP1: soluble low-density lipoprotein receptor-related protein 1.

Table 4. Significant correlations of sLRP1 with biomarkers and clinical variables at enrollment and 6 months after enrollment.

	Enrollment Visit, n = 96		6-month Visit, n = 58	
	Median (IQR)	r (*P*)	Median (IQR)	r (*P*)
Age, yrs	**10.0 (6.0–14.0)**	**–0.309 (0.002)**	**9.8 (5.0–14.3)**	**–0.324 (0.01)**
No. active joints	5.0 (2.0–12.0)	–0.235 (0.02)	1.0 (0.0–3.0)	
IFN-α[a]	**27.1 (16.0–46.9)**	**0.443 (0.001)**	**29.1 (19.2–45.8)**	**0.359 (0.006)**
IFN-γ[b]	7.0 (4.0–15.2)	0.335 (0.001)	7.0 (4.0–15.2)	
IL-10[c]	7.1 (1.8–16.2)	0.315 (0.005)	6.5 (3.3–14.2)	
IL-12p40[c]	**22.7 (9.4–47.9)**	**0.428 (0.001)**	**7.1 (1.8–16.2)**	**0.322 (0.02)**
IL-12p70[b]	5.1 (2.1–9.2)	0.303 (0.004)	9.2 (5.1–19.0)	
IL-13[b]	**4.2 (0.0–14.2)**	**0.572 (0.001)**	**22.7 (9.4–47.9)**	**0.416 (0.005)**
IL-15[b]	1.5 (0.0–7.6)	0.383 (0.002)	27.9 (11.6–48.5)	
IL-1Ra[c]	50.1 (5.2–129.9)	0.329 (0.003)	5.1 (2.1–9.2)	
IL-1a[b]	**13.8 (3.3–39.3)**	**0.530 (0.001)**	**4.9 (3.3–17.7)**	**0.477 (0.001)**
IL-2[c]	1.9 (0.0–7.2)	0.412 (0.001)	4.2 (0.0–14.2)	
IL-4[c]	**1.9 (0.0–15.0)**	**0.609 (0.001)**	**6.2 (0.6–18.2)**	**0.446 (0.005)**
IL-6[b]	**7.4 (2.0–14.2)**		**1.5 (0.0–7.6)**	**0.457 (0.001)**
IL-7[b]	7.7 (2.6–14.7)	0.370 (0.001)	2.6 (0.0–8.9)	
TNF-β[b]	4.2 (0.0–11.7)	0.395 (0.001)	50.1 (5.2–129.9)	

The biomarkers that correlated significantly with sLRP1 at both study visits are in bold. [a] Mediators having both pro- and antiinflammatory actions. [b] Proinflammatory mediator. [c] Antiinflammatory mediator. IFN: interferon; IL: interleukin; sLRP1: soluble low-density lipoprotein receptor-related protein 1; TNF: tumor necrosis factor.

enrollment and 6 months postenrollment for the 58 subjects who had sLRP1 levels at both time points (Wilcoxon signed-rank test, $P = 0.20$). Logistic regression analysis (Table 6) showed that higher sLRP1 levels at enrollment increase the likelihood of an absence of active joints 6 months after enrollment, with an accuracy rate of 73.4%. There was no significant association between levels of sLRP1 at enrollment with JADAS-71 scores after 6 months ($P = 0.45$).

Analyzing data after removing the DMARD- and steroid-treated participants did not change the regression analysis estimate (0.35), and the *P* value changed from 0.024 to 0.025, an infinitesimal difference. Therefore, the 3 study participants who received a DMARD/steroids before enrollment were retained in the analyses.

DISCUSSION

To our knowledge, this is the first report of sLRP1 in JIA that reveals correlations of sLRP1 with clinical characteristics and inflammation-related biomarkers. sLRP1 levels were elevated in patients with oligoarticular JIA, PsA, polyarthritis RF–, and ERA. In these subsets, sLRP1 correlated with certain pro- and antiinflammatory biomarkers. At enrollment, but not at the 6-month follow-up, sLRP1 was inversely correlated with the number of active joints.

Antiinflammatory effects are exerted by LRP1's ability to modulate production of proinflammatory mediators, decrease the availability of certain cell surface receptors that have signaling effects, and phagocytosis[6,16]. Specifically, LRP1 affects inflammation by the following mechanisms: (1) intracellular LRP1 can competitively bind pathogenic peptides and, by scavenging the peptide into an endosome for destruction, reduce its availability; (2) the intracellular domain of LRP1, released after proteolysis, reduces the inflammatory effects of lipopolysaccharide-induced signaling[17]; (3) reduced levels of LRP1 are associated with increased expression of tumor necrosis factor- α (TNF-α) receptor, thus potentiating the binding of TNF-α and augmenting its proinflammatory actions[18]; and (4) LRP1 promotes phagocytosis, particularly of apoptotic cells[19], and modulates the proinflammatory, immune, cell differentiation, and angiogenic effects of transforming growth factor β[16].

Table 5. Correlation coefficient (r) and *P* values of sLRP1 with inflammatory biomarkers for each JIA category at enrollment.

Biomarker	JIA Categories			
	RF– Polyarthritis, n = 41	Oligoarthritis, n = 26	PsA, n = 9	ERA, n = 8
	r (*P*)			
Age		–0.469 (0.02)		
Exotoxin[a]		0.465 (0.02)		
MMP-2[a]				–0.881 (0.004)
MMP-10[a]				–0.905 (0.002)
MMP-12[a]			0.850 (0.004)	
MMP-13[a]			0.733 (0.03)	
G-CSF[a]		0.550 (0.004)		
GM-CSF[a]		0.474 (0.02)		
FGF-2[a]	0.326 (0.046)	0.455 (0.02)		
IFN-α[b]	0.484 (0.002)	0.611 (0.001)	0.867 (0.002)	
IFN-γ[a]	0.400 (0.01)	0.518 (0.007)		
IL-10[c]		0.674 (< 0.001)		
IL-12p40[c]		0.534 (0.009)		
IL-12p70[a]		0.512 (0.009)		
IL-13[a]	0.519 (0.003)	0.734 (< 0.001)	0.857 (0.01)	
IL-15[a]		0.611 (0.02)		
IL-17[a]			0.667 (0.05)	
IL-1Ra[c]		0.700 (0.003)		
IL-1a[a]	0.447 (0.009)	0.764 (< 0.001)		
IL-2[c]	0.552 (0.01)	0.679 (0.008)		
IL-4[c]		0.719 (0.001)		
IL-5[a]		0.779 (< 0.001)		
IL-6[a]		0.502 (0.03)		
IL-7[a]	0.411 (0.04)	0.412 (0.04)	0.905 (0.002)	
IL-8[a]				–0.764 (0.03)
TNF-α[a]		0.554 (0.004)		
TNF-β[a]		0.800 (< 0.001)	0.886 (0.02)	
VEGF[a]		0.440 (0.02)		

Biomarker levels are reported in ng/mL. Significance level was < 0.05. Only significant correlations are shown. [a] Proinflammatory mediator. [b] Mediators having both pro- and antiinflammatory actions. [c] Antiinflammatory mediator. ERA: enthesitis-related arthritis; FGF-2: fibroblast growth factor 2; G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon; IL: interleukin; JIA: juvenile idiopathic arthritis; MMP: matrix metalloproteinases; PsA: psoriatic arthritis; sLRP1: soluble low-density lipoprotein receptor-related protein 1; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

Table 6. Logistic regression results.

Logistic regression analysis							
Predictor	Intercept	SE	Wald	df	*P*	OR	95% CI
Constant	–0.30	0.55	0.30	1	0.59	0.74	
sLRP1	0.35	0.16	5.09	1	0.02	1.42	1.047–1.92
Description of the data for logistic regression							
		Code 0	Code 1	Percentage Correct		Cox & Snell R^2 = 0.058, Nagelkerke R^2 = 0.08, –2 log likelihood = 108.92, chi-square (Hosmer and Lemeshow test) = 5.99 with *P* = 0.65	
Code 0		4	24	14.3			
Code 1		1	65	98.5			
Overall percentage				73.4			

Code 0: absence of active joint at 6-month visit. Code 1: presence of active joint at 6-month visit. df: degrees of freedom; SE: standard error of the intercept; sLRP1: soluble low-density lipoprotein receptor-related protein 1.

sLRP1 is biologically active by mediating cell signaling and promoting expression of regulatory cytokines by macrophages[7]. The signaling pathways triggered by sLRP1 in macrophages result in expression of the mRNAs[7] for TNF-α, interleukin (IL)-10, and chemokine (C-C motif) ligand 2. Like membrane-bound LRP1, sLRP1 may exert both pro- and antiinflammatory effects. sLRP1 may function as an endogenous inhibitor of inflammation by scavenging pathogenic peptides[7]. In certain situations, it may exert proinflammatory effects. Gorovoy, *et al* showed that the levels of sLRP1 in adult patients with RA were significantly higher than the levels in patients with osteoarthritis[7]. Brifault, *et al* showed that, in contrast to membrane-anchored LRP1, sLRP1 is a potent proinflammatory protein that induces release of proinflammatory cytokines in microglia cells[20].

Strengths of the present study include the prospective design, enrollment of patients soon after diagnosis, and longitudinal follow-up.

The present study showed at study enrollment (time of diagnosis) that there was an inverse correlation of sLRP1 with active joint counts and significantly higher levels of sLRP1 in patients with 4 or fewer active joints compared to those with more than 5 active joints. This observation suggests that, in the context of JIA, sLRP1's biologic effects may be skewed toward antiinflammation.

Previously, we reported that baseline sLRP1 is among 8 predictors of inactive disease in JIA 18 months after enrollment, with an accuracy rate of 83%[10]. In that study, higher levels of sLRP1 together with the number of active and effused joints, age, ANA positivity, IL-10, IL-12p70, and vitamin D levels at enrollment were associated with improved outcomes 18 months later. In the present study, higher sLRP1 levels at enrollment were also predictive of active joint counts of zero after 6 months. Together, these clinical correlations further suggest the possibility of sLRP1's biologic effect in JIA as favoring antiinflammation. Focusing on sLRP1 in this study, rather than on other biomarkers from among the panel we identified previously, is important because sLRP1 is a distinctly different type of biomarker: It is not an inflammatory cytokine produced and secreted from within cells. Rather, sLRP1 is a biomarker that is shed from a cell surface receptor responding to and mediating inflammation, and might be involved with the processing of etiologically important antigenic ligands.

In the context of clinical indicators supporting sLRP1's antiinflammatory effects, the correlations of sLRP1 with proinflammatory biomarkers might be perceived as incongruous. However, this apparent incongruity could reflect the complex interactions of pro- and antiinflammatory influences in JIA and the challenges in translating statistical correlations to biologic processes. It is conceivable that sLRP1 is shed in response to proinflammatory influences and serves to counteract their effects. This study also illustrated the dynamic nature of sLRP1 as correlations changed over time. There was no significant difference between sLRP1 levels at enrollment and 6 months postenrollment, an observation that might have been influenced by the smaller sample size at the follow-up visit. Future large-scale studies are required to ascertain the influence of pharmacotherapy on sLRP1 levels.

The BBOP study aimed to ensure adherence to rigorous standard operating procedures for all aspects of the study, including collecting, processing, transporting, and storing biospecimens. The P100 tube was selected for blood collection, as the tube contains a proprietary protease inhibitor, to help mitigate cytokine degradation; sample freezing occurred within 24 hours of collection. Plasma separation from P100 tubes immediately after sample collection might be a preferable protocol. However, as samples collected for BBOP were all handled in the same standardized way, we feel the results should still be informative and comparisons among participants reliable.

Storing of plasma/serum samples at –150°C might be preferable to storing at –80°C to ensure integrity of cytokine assays, as attrition of certain cytokines (IL-1α, IL-1β, IL-8, IL-10, IL-15, as examples) can occur with prolonged storage at –80°C[21]. In the BBOP study, the first sample collected would have been stored for 4 years before it was assayed, and the last sample collected stored for 2 years before being assayed. While it is possible that the difference in storage duration might have affected the quantitation of certain cytokines, we were unable to discern any apparent differences in results based on the duration of storage, although numbers of samples at the various durations are too small for meaningful analysis. Thus, banking plasma/serum samples at an ultralow temperature (–150°C) may be preferable for preserving cytokine integrity long-term.

A limitation of the present study is the rate of attrition of study participants at the 6-month visit. Further, the BBOP study did not include a matched healthy control cohort; including healthy children would present challenging practical and ethical considerations. However, in an earlier pilot study, we showed that healthy children had significantly lower levels of sLRP1 than did most JIA children[9].

The present analyses indicate that sLRP1 is associated with fewer active joints and more favorable short-term outcomes. On balance, our statistical analyses suggest that sLRP1 likely exerts a net beneficial influence in JIA. The clinical correlates reported here, together with our previous work, suggest sLRP1 at first presentation might be useful as a predictive biomarker. Although our study was not a mechanistic one, this first report, to our knowledge, of sLRP1 in JIA and its clinical and biologic correlates should inspire and propel future research aimed at clarifying the pathogenic role of sLRP1 in JIA. sLRP1 has the potential to be both a consequence of inflammation (that is, it is shed from the cell-bound LRP1 as a response to inflammation) and an agent for promoting inflammation. Thus, given its putative pathologic roles, sLRP1 at once can be elevated in the contexts of both promoting and mitigating inflammation, as we have shown. By showing relationships of sLRP1 with indicators of both antiinflammation (for example, reduced number of active joints and antiinflammatory cytokines) and proinflammation (for example, the elevation of proinflammatory cytokines), our results are consistent with these dual roles for sLRP1.

Our observation that sLRP1 can predict short-term JIA outcomes should be interpreted in a statistical context, and not necessarily in a biologic context. While one might speculate that the statistical relationship we showed relates to a biologic process,

we have not proven that, as we did not undertake a biologically based mechanistic study. However, showing a statistical relationship provides support for future studies that explore biologic mechanisms relating to sLRP1 in JIA. Our results provide the impetus for future mechanistic studies that will elucidate the nuances of how sLRP1's contrasting actions drive the pathogenesis of JIA.

We are unaware of evidence to explain higher levels of sLRP1 levels in younger children. Future studies could investigate if there is a loss of LRP1 expression with aging in the pediatric and adolescent populations as occurs in adults[22] or if there are physiologic factors such as sex hormones that influence LRP1 and sLRP1 expression.

When the entire cohort was considered, we showed a relationship between higher levels of sLRP1 with fewer active joints and with younger age. As children with oligoarticular JIA tend to be younger and have fewer joints involved, it is conceivable that higher sLRP1 levels are not independently correlated with active joints and with age—that is, active joint count might be a surrogate for age or age a surrogate for the number of active joints. However, our analyses showed no significant differences in the relationships of sLRP1 with active joint count between younger and older children, suggesting that higher levels of sLRP1 are independently correlated with fewer active joints and younger age. A larger sample size is required to determine definitively if active joint count and age are each independently associated with higher sLRP1 levels.

Future prospective studies that interpret sLRP1 levels in the context of treatment interventions and predictive utility for longer-term outcomes would be informative (including control groups of healthy children and those with other inflammatory conditions). sLRP1 might prove to be a valuable component of a biomarker panel to help guide personalized approaches to assessing and managing JIA. Further, as an upstream mediator of the inflammatory cascade, studying sLRP1 in JIA might provide additional insight into disease pathogenesis.

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