Circulation Research

RESEARCH LETTER

Distinct Urinary Protein Signatures in Adolescent Blood Pressure Phenotypes

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The increasing prevalence of childhood hypertension constitutes an important risk factor for cardiovascular disease (CVD), contributing to long-term morbidity and mortality and imposing substantial healthcare costs.[1] Among potential mechanisms, early-life immune activation, characterized by low-grade inflammatory and immune-cell responses, may contribute to endothelial dysfunction and vascular remodeling, initiating processes that predispose individuals to long-term cardiovascular sequelae. We recently reported transcriptional alterations indicative of cytotoxic (*PRF1*, *GZMB*, *IL2RB*) and inflammatory responses (*IL1B*, *FOS*) in blood from adolescents with elevated blood pressure (BP).[2] This reflects circulating immune cell activation, but does not permit tissue-specific attribution. Urinary proteins, in contrast, provide a complementary downstream biological readout that may capture inflammatory signals at the circulation-vascular/renal interface, and thus may serve as noninvasive indicators of early target-organ-related processes.

Given the distinct pathophysiological characteristics and CVD risk profiles of the BP subphenotypes[3,4]—isolated diastolic hypertension (IDH), isolated systolic hypertension, and combined systolic and diastolic hypertension (SDH)—we hypothesize that early-life BP-related immune activation is associated with endothelial dysfunction, and subphenotype-specific subclinical inflammation before the formal diagnosis of hypertension.

IDH, recently defined as a distinct entity in the 2023 European Society of Hypertension guideline, remains insufficiently characterized in early life.[3] It is more prevalent in adolescents than in adults and often underdiagnosed. Although its contribution to CVD sequelae remains inconclusive,[3] IDH has been shown to predict chronic kidney disease risk,[4] supporting the investigation of IDH during adolescence.

We quantified 10 inflammatory protein markers (LEGENDplex Human Vascular Inflammation Panel 1-U, BioLegend) in spot urine samples from 15-year-old adolescents of the GINIplus birth cohort study (n=3199; ethical approvals: Bavarian Board of Physicians: 10090; Board of Physicians of North-Rhine-Westphalia: 2010424/2015491; Figure [A]). Participants reporting physician-diagnosed hypertension at the 20-year follow-up or a urinary tract infection within 4 weeks before sampling were excluded. After restricting analyses to individuals with complete BP, protein, and confounder data, 1502 participants remained (Figure [A] and [B]). BP was measured twice on the right arm, and the mean of the 2 valid measurements was used.[2] Elevated BP subphenotypes, isolated systolic hypertension, IDH, SDH (≥P95th), and normotension (<P90th), were classified according to the 2023 European Society of Hypertension guideline. As BP was assessed at a single visit, classifications represent elevated BP phenotypes rather than confirmed hypertension. To account for urine dilution, immune protein concentrations were creatinine-normalized (Urinary Creatinine Assay Kit, Cell Biolabs) following NHANES recommendations, and dichotomized at the 90th percentile (≥P90th versus <P90th), or as detectable versus nondetectable where applicable (Figure [B]). Associations between BP phenotypes and high (≥P90th) protein concentrations were examined by

Key Words: adult ■ blood pressure ■ humans ■ phenotype ■ urine

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Circulation Research is available at www.ahajournals.org/journal/res

Nonstandard Abbreviations and Acronyms

BP	blood pressure
CRP	C-reactive protein
CVD	cardiovascular disease
IDH	isolated diastolic hypertension
SAA	serum amyloid A
SDH	systolic and diastolic hypertension

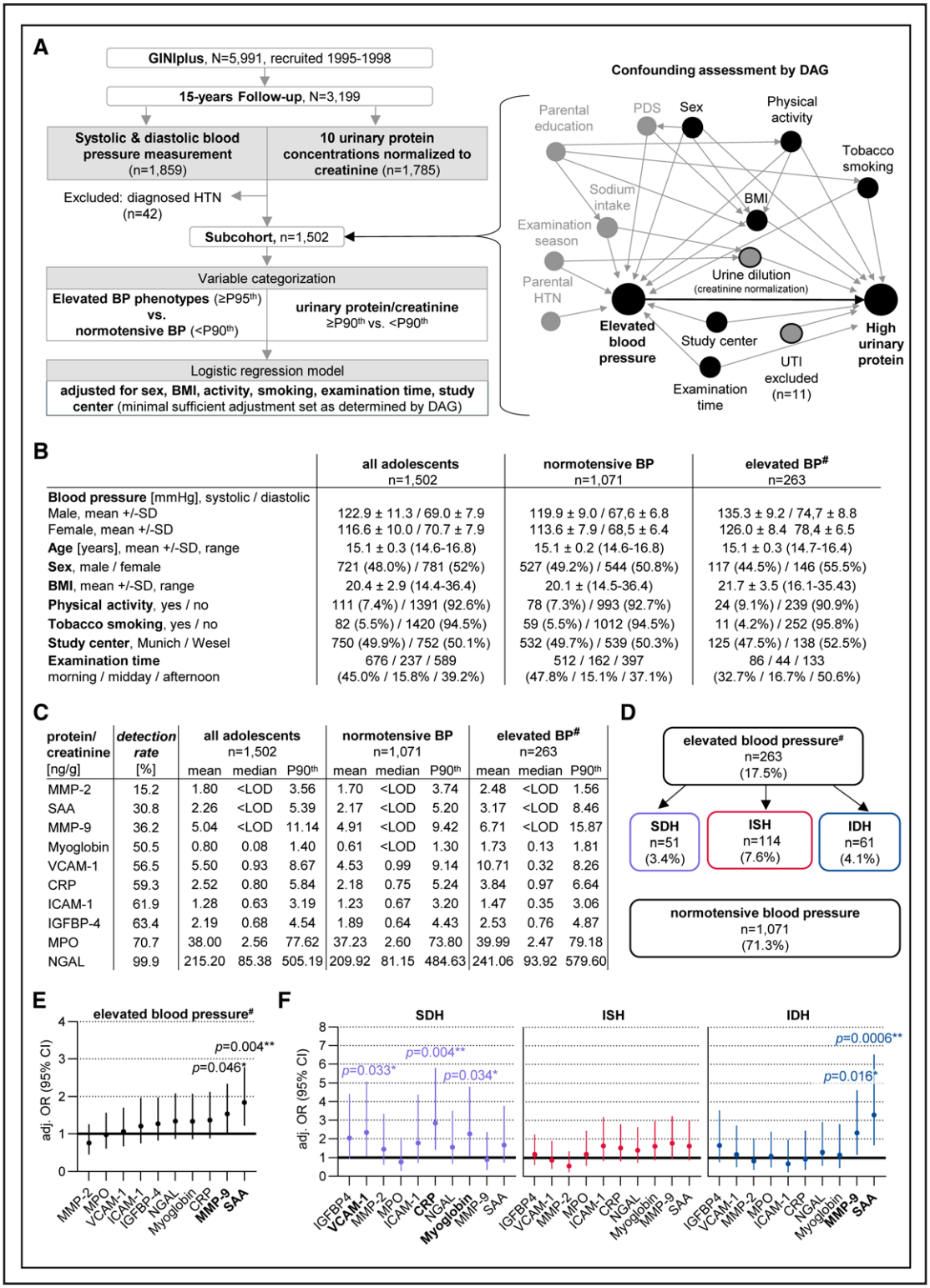
multivariate logistic regression using a minimal sufficient adjustment set derived from a directed acyclic graph assessing previously known confounding factors (DAGitty; Figure [A]). Bonferroni correction was applied. Analyses were restricted to complete cases, and the study adhered to the STROBE guideline.

Adolescents with elevated systolic and diastolic BP showed higher mean urinary concentrations of all inflammatory markers compared with normotensive participants (Figure [B]). After adjustment, elevated systolic and diastolic BP was associated with an increased risk of high (≥P90th) urinary concentrations of serum amyloid A (SAA) and, to a lesser extent, MMP-9 (matrix metalloproteinase-9), both involved in hypertension and vascular remodeling (Figure [D]). Notably, the SAA profile was largely driven by IDH (high SAA frequency [≥P90th]: normotensive=9.2%, SDH=15.7%, isolated systolic hypertension=14.0%, IDH=23.0%; Figure [E]). The association of IDH with SAA persisted when using the detection limit as the cutoff (adjusted OR, 2.25, [95% CI, 1.31–3.88]; *P*=0.003). In contrast, only SDH was associated with elevated CRP (C-reactive protein; Figure [E]; high CRP frequency: normotensive=8.8%, SDH=25.5%, isolated systolic hypertension=14.0%, IDH=8.2%). CRP analyzed as a continuous creatinine-normalized variable was consistent with the dichotomized analysis, although values below the detection limit carry uncertainty (adjusted multiple regression: *b*[SE]=1.63[0.74]; *P*=0.028). Although both SAA and CRP are markers of chronic inflammation, they differ in biological function and relevance to CVD. The higher proportion of adolescents with elevated urinary CRP in SDH compared with IDH (Fisher exact test, *P*=0.019) supports distinct inflammatory activity across BP subphenotypes. Although not significant following multiple-testing correction, we observed distinct associations of vascular cell adhesion molecule-1 (VCAM-1; raw *P*=0.033) and myoglobin with SDH (raw *P*=0.034), and of MMP-9 with IDH (raw *P*=0.016) and elevated systolic and diastolic BP (raw *P*=0.046; Figure [E]).

Taken together, our findings extend previous suggestions of distinct pathophysiological features among pediatric BP subphenotypes.[3] By focusing on urinary inflammatory proteins, this study provides evidence that subclinical inflammatory activity may accompany elevated BP already during adolescence. As this was observed in adolescents with elevated BP without a formal hypertension diagnosis, vascular dysfunction may begin earlier than assumed—even without clinical symptoms.

This study has limitations. BP measurements and urine sampling were performed at a single visit and subgroup sizes were modest. Accordingly, classifications reflect elevated BP rather than confirmed hypertension. Nevertheless, associations with SAA and CRP were observed even among individuals without a clinical diagnosis of hypertension, consistent with subclinical inflammatory activity. Data on kidney or vascular function were unavailable. Given the established relationship between elevated BP and proteinuria, increases in urinary CRP and SAA could reflect increased urinary protein excretion associated with higher BP. To address this, proteinuria (>0.2 mg total urinary protein/mg creatinine) was assessed in a nested case-control analysis including all participants with SDH (n=51), IDH (n=61), and randomly selected normotensive controls (n=127). Proteinuria was significantly associated with CRP (OR, 6.76 [95% CI, 2.29–19.98], *P*=0.0005; n=239) and SAA (OR, 3.17 [95% CI, 1.02–9.84], *P*=0.045; n=239). However, adjustment for proteinuria did not materially change the associations between BP subphenotypes and inflammatory proteins (IDH-SAA [n=188]: OR, 3.14 [95% CI, 1.32–7.46]; *P*=0.009 versus proteinuria-adjusted OR, 3.17 [95% CI, 1.33–7.55]; *P*=0.009; SDH-CRP [n=178]: OR, 2.70 [95% CI, 1.13–6.44]; *P*=0.024 versus proteinuria-adjusted OR, 2.63 [95% CI, 1.06–6.49]; *P*=0.035). Thus, proteinuria is unlikely to be the primary explanation. However, given the use of spot urine samples, low-grade proteinuria may not have been fully captured. Further, whether elevated CRP/SAA reflects systemic or local inflammation cannot be determined. However, associations of IDH with SAA and of SDH with CRP remained robust after excluding participants with serum hs-CRP>10 mg/L or self-reported chronic diseases at age 15 (SDH-CRP: adjusted OR, 2.73 [95% CI, 1.18–6.34], n=903/38; *P*=0.019; IDH-SAA: adjusted OR, 2.87 [95% CI, 1.35–6.13], n=903/51; *P*=0.006). While undiagnosed conditions cannot be fully excluded, these results suggest that elevated SAA or CRP is unlikely attributable to acute infections or underlying chronic inflammatory disorders. Although observed effect sizes were large, BP subgroup sizes were modest, and replication in an independent cohort is warranted. As this is an observational study, causal mechanisms cannot be inferred.

Like serum CRP[2] urinary CRP was associated with the systolic-dominant phenotype, whereas SAA, elevated in IDH, was associated with the diastolic phenotype, suggesting distinct underlying biological mechanisms. Future mechanistic studies should investigate how BP-phenotype-specific inflammatory pathways activated



	all adolescents n=1,502	normotensive BP n=1,071	elevated BP# n=263
Blood pressure [mmHg], systolic / diastolic			
Male, mean +/-SD	122.9 ± 11.3 / 69.0 ± 7.9	119.9 ± 9.0 / 67,6 ± 6.8	135.3 ± 9.2 / 74,7 ± 8.8
Female, mean +/-SD	116.6 ± 10.0 / 70.7 ± 7.9	113.6 ± 7.9 / 68,5 ± 6.4	126.0 ± 8.4 78,4 ± 6.5
Age [years], mean +/-SD, range	15.1 ± 0.3 (14.6-16.8)	15.1 ± 0.2 (14.6-16.8)	15.1 ± 0.3 (14.7-16.4)
Sex, male / female	721 (48.0%) / 781 (52%)	527 (49.2%) / 544 (50.8%)	117 (44.5%) / 146 (55.5%)
BMI, mean +/-SD, range	20.4 ± 2.9 (14.4-36.4)	20.1 ± (14.5-36.4)	21.7 ± 3.5 (16.1-35.43)
Physical activity, yes / no	111 (7.4%) / 1391 (92.6%)	78 (7.3%) / 993 (92.7%)	24 (9.1%) / 239 (90.9%)
Tobacco smoking, yes / no	82 (5.5%) / 1420 (94.5%)	59 (5.5%) / 1012 (94.5%)	11 (4.2%) / 252 (95.8%)
Study center, Munich / Wesel	750 (49.9%) / 752 (50.1%)	532 (49.7%) / 539 (50.3%)	125 (47.5%) / 138 (52.5%)
Examination time morning / midday / afternoon	676 / 237 / 589 (45.0% / 15.8% / 39.2%)	512 / 162 / 397 (47.8% / 15.1% / 37.1%)	86 / 44 / 133 (32.7% / 16.7% / 50.6%)

protein/ creatinine [ng/g]	*detection rate* [%]	all adolescents n=1,502 mean	median	P90th	normotensive BP n=1,071 mean	median	P90th	elevated BP# n=263 mean	median	P90th
MMP-2	15.2	1.80	<LOD	3.56	1.70	<LOD	3.74	2.48	<LOD	1.56
SAA	30.8	2.26	<LOD	5.39	2.17	<LOD	5.20	3.17	<LOD	8.46
MMP-9	36.2	5.04	<LOD	11.14	4.91	<LOD	9.42	6.71	<LOD	15.87
Myoglobin	50.5	0.80	0.08	1.40	0.61	<LOD	1.30	1.73	0.13	1.81
VCAM-1	56.5	5.50	0.93	8.67	4.53	0.99	9.14	10.71	0.32	8.26
CRP	59.3	2.52	0.80	5.84	2.18	0.75	5.24	3.84	0.97	6.64
ICAM-1	61.9	1.28	0.63	3.19	1.23	0.67	3.20	1.47	0.35	3.06
IGFBP-4	63.4	2.19	0.68	4.54	1.89	0.64	4.43	2.53	0.76	4.87
MPO	70.7	38.00	2.56	77.62	37.23	2.60	73.80	39.99	2.47	79.18
NGAL	99.9	215.20	85.38	505.19	209.92	81.15	484.63	241.06	93.92	579.60

Figure. Inflammation-associated urinary proteins in 15-year-old adolescents with elevated blood pressure (BP) phenotypes. **A**, Study design, analysis workflow, DIRECTED ACYCLIC GRAPH (DAG), and statistical model. The subcohort (n=1502) included participants with complete data on protein concentrations, BP measurements, and confounders. Initially assessed confounders included sex, body mass index (BMI), pubertal development score, tobacco use, recent physical activity (with sweating within 12 hours), sodium intake (food frequency questionnaire), parental education, parental hypertension, study center, examination season, day time of examination, urine dilution, and urinary tract infection in the previous 4 weeks. Urinary protein concentrations were normalized to creatinine. Protein-to-creatinine ratios were dichotomized at the 90th percentile (≥P90th vs <P90th). **B**, Basic characteristics of the study cohort. **C**, Urinary protein/creatinine concentrations stratified by BP status. **D**, Prevalence of BP phenotypes according to the 2023 European Society of Hypertension Guideline. **E** and **F**, Forest plots showing associations between elevated BP phenotypes and high protein/creatinine ratios. Odds ratios from adjusted (*Continued*)

Figure Continued. logistic regression are shown with exact *P* values. Asterisks indicate associations significant before correction (*raw *P*<0.05) or remaining significance after Bonferroni correction (**adjusted *P*<0.05). All statistical analyses were conducted in R, version 4.3.2 and Statistica (Tibco Software, Inc), version 14.0.0.15. #Elevated BP (systolic and diastolic) includes more adolescents than the sum of individual BP phenotypes, as BP values between ≥P90th and <P95th are excluded from isolated systolic hypertension (ISH) and IDH definitions. ISH, IDH, and systolic and diastolic hypertension (SDH) represent elevated BP phenotypes, not confirmed hypertension diagnoses. CRP indicates C-reactive protein; ICAM-1, intercellular adhesion molecule-1; ID(S)H, isolated diastolic (systolic) hypertension; IGFBP-4, insulin-like growth factor-binding protein 4; MMP-2(9), matrix metallopeptidase 2(9); MPO, myeloperoxidase; NGAL, neutrophil gelatinase associated lipocalin; PDS, pubertal development score; SAA, serum amyloid A; UTI, urinary tract infection; and VCAM-1, vascular cell adhesion molecule-1.

early in life contribute to vascular and renal dysfunction. In line with this, a population-based study of 1 492 291 adults demonstrated distinct BP-dependent chronic kidney disease risk profiles, with diastolic BP emerging as the strongest predictor.[4] Since SAA, particularly when elevated in IDH, has been shown to stimulate vascular endothelial and renal dysfunction in vivo,[5] its increase during adolescence may indicate an elevated risk for later CVD or chronic kidney disease.

This study is embedded in the well-characterized, population-based GINIplus cohort, providing novel insight into early, phenotype-specific inflammation, particularly in IDH. Being more common in youth than in adults and linked to adverse outcomes,[3–5] adolescent elevated diastolic BP constitutes a clinically relevant phenotype. Noninvasive biospecimens with protein markers in well-child visits may support future early risk stratification to prevent long-term cardiovascular and renal complications.

ARTICLE INFORMATION

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Acknowledgments

The authors thank all families for their participation in the GINIplus study and theGINIplus Study Group members for their excellent work.

Author Contributions

S. Trump, M. Standl, and I. Lehmann provided project leadership. M. Standl, A. von Berg, D. Berdel, S. Koletzko, J. Heinrich, and T. Schikowski performed cohort recruitment and sample collection. S. Trump, L. Thürmann, and A. Seegebarth coordinated or performed protein measurements. L. Thürmann performed data processing and analyses. L. Thürmann prepared figure and initial manuscript. All authors contributed to the final manuscript.

Sources of Funding

The GINIplus study was supported for the first 3 years by the Federal Ministry for Education, Science, Research and Technology (BMBF, 01EG9705/2) and the involved study institutions. The 4- to 20-year follow-up examinations were covered by intramural funding of the study centers (HMGU, Research Institute at Marien-Hospital Wesel/EVK Düsseldorf, LMU Munich, TU Munich) and by a grant from the Federal Ministry for Environment (IUF, FKZ 20462296). The15-year follow-up examination was supported by the Commission of the European Communities, the 7th Framework Program: MeDALL project. The 15-year and 20-year follow-up examinations were supported by the companies Mead Johnson and Nestle.

Disclosures

The data sets are available to interested researchers from the corresponding author on reasonable request (eg, reproducibility), provided the release is consistent with the consent given by the GINIplus study participants.

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