Hypertension

ORIGINAL ARTICLE

Undiagnosed Pediatric Elevated Blood Pressure Is Characterized by Induction of Proinflammatory and Cytotoxic Mediators

Loreen Thürmann, Mario Bauer, Maike Ferland, Marey Messingschlager, Tamara Schikowski, Andrea von Berg, Joachim Heinrich, Gunda Herberth, Irina Lehmann, Marie Standl, Saskia Trump

BACKGROUND: Inflammatory processes have been suggested as a culprit of vascular damage in pediatric hypertension. We aimed to investigate transcriptional changes of immune modulators and determine their association with office blood pressure in adolescents who were not diagnosed with hypertension at the time of the study visit.

METHODS: Office blood pressure measurements and blood samples were taken from adolescents of 2 German birth cohorts, GINIplus (The German Infant Study on the Influence of Nutrition Intervention Plus Air Pollution and Genetics on Allergy Development; discovery cohort, n=1219) and LISA (Influences of Lifestyle-related factors on the Immune System and the Development of Allergies in Childhood; validation cohort, n=809), during the 15-year follow-up visit and categorized based on the European Society of Hypertension Guideline. Hs-CRP (high-sensitivity C-reactive protein) and expression of 51 genes encoding cytokines/receptors and transcription factors were analyzed.

RESULTS: The prevalence of elevated systolic blood pressure (overweight/obese) was 14.0% (5.1%) and 16.4% (5.2%) in the discovery and validation cohorts, respectively. An enhanced cytotoxic (*GZMB, PRF1, IL2RB*) and proinflammatory (*FOS, IL1B*, hs-CRP) immune profile was observed in association with the hypertension class in both cohorts. Expression of hs-CRP and *IL1B* was driven by overweight with *IL1B* being identified as a mediator between body mass index and elevated systolic blood pressure (adj.β/95% CI, 0.01/0.0002–0.02). The association of *GZMB* (adjusted odds ratio/95% CI, 1.67/1.26–2.21; *P*=0.0004) and *PRF1* (adjusted odds ratio/95% CI, 1.70/1.26–2.29; *P*=0.0005) in the hypertension class remained significant in normal-weight individuals without parental predisposition. These effects were confirmed in LISA.

CONCLUSIONS: Adolescent hypertension is not limited to known risk groups. As adolescents in the hypertension class show an inflammatory profile similar to that of established hypertension in adults, blood pressure monitoring at a young age is critical to ensure early intervention and prevention of adverse sequelae. ***(Hypertension.* 2023;80:2425–2436. DOI: 10.1161/HYPERTENSIONAHA.123.21489.)** • **Supplement Material**.

Key Words: child ■ cytokines ■ gene expression ■ hypertension ■ immune system ■ overweight ■ risk factor

Hypertension is a prolonged pathological elevation of blood pressure (BP) that can lead to long-term sequelae affecting various organs and is a major cause of cardiovascular disease and all-cause mortality.[1] Hypertension is not only a disease burden in adults but has now been recognized to also affect the pediatric population. Although early hypertension poses little immediate risk to most children, an elevated BP in childhood can progress into adulthood and promote end organ damage.[2] As such, early intervention is crucial for

Correspondence to: Saskia Trump, Molecular Epidemiology Unit, Berlin Institute of Health at Charité -Universitätsmedizin Berlin, Kapelle-Ufer 2, 10117 Berlin. Email saskia.trump@bih-charite.de
*L. Thürmann, M. Bauer, M. Ferland, I. Lehmann, M. Standl, and S. Trump contributed equally.
Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.123.21489.
For Sources of Funding and Disclosures, see page 2435.

Hypertension is available at www.ahajournals.org/journal/hyp

ORIGINAL ARTICLE

Novelty and Relevance

What Is New?

In adolescents without a clinical hypertension diagnosis increased blood pressure was associated with elevated expression of cytotoxic (*GZMB/PRF1*) and proinflammatory mediators (*FOS, IL1B, CRP*). The cytotoxic activation was retained in adolescents without hypertensive risk factors (overweight, parental predisposition) underscoring the importance to consider hypertension as a common health concern not limited to known risk groups.

What Is Relevant?

Implementing blood pressure monitoring in well-child visits should be reinforced in countries not yet following current guideline recommendations.

Clinical/Pathophysiological Implications?

Stratification of pediatric patients to potential immune modulatory therapies is warranted, as proinflammatory *IL1B* (interleukin 1B)/hs-CRP (high-sensitivity C-reactive protein)-induction was driven by overweight, while in normal-weight adolescents' hypertension was associated with an elevated cytotoxic profile.

Nonstandard Abbreviations and Acronyms

BMI	body mass index
BP	blood pressure
DBP	diastolic blood pressure
eSBP	elevated systolic blood pressure
FOS	Fos proto-oncogene
GINIplus	The German Infant Study on the Influence of Nutrition Intervention Plus Air Pollution and Genetics on Allergy Development
GZMB	granzyme B
hs-CRP	high-sensitivity C-reactive protein
IL-1B	interleukin 1B
LISA	Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood
PRF1	perforin 1
SBP	systolic blood pressure

the prevention of hypertension-related adverse health effects. However, pediatric hypertension is frequently underdiagnosed.[3,4] Additionally, a considerable proportion of asymptomatic cases contribute to underdiagnosis.[5]

Hypertension often occurs together with risk factors such as overweight/obesity, unhealthy diet, and physical inactivity.[6,7] In recent years, the concerning global rise of pediatric hypertension is mainly attributed to the concomitant increase in body mass index (BMI), in particular, in Western style countries.[8] However, increasing rates of obesity in children and adolescents of the Chinese National Survey were accompanied with a fluctuating but stable prevalence of elevated BP over the same period, suggesting that also other factors contribute to pediatric hypertension. One leading factor that is involved in the origin and progression of hypertension, and of overweight, is an activated immune system. A large number of animal models and human studies have shown that activation of the innate or adaptive immune response plays a central role in hypertension. A landmark study in recombination-activating 1 -deficient mice lacking B and T cells showed that while these animals were protected from deoxycorticosterone acetate or salt-induced hypertension, adoptive transfer of T cells restored sensitivity to hypertension induction.[9] Patients with hypertension have been shown to have increased numbers of circulating T cells compared with normotensive control subjects[10,11] establishing the role of the adaptive immune system in hypertension development. But also cells of the innate immune system have been implicated in hypertension pathophysiology. For example, an increase of monocytes/macrophages and their infiltration in vasculature and other BP-regulating organs has been described in different rodent models of hypertension.[12,13]

The contribution of these immune-modulatory processes in pediatric hypertension is less well investigated and has predominantly focused on smaller studies in clinically confirmed cases. These studies, in line with what has been seen in adults, showed an increase in inflammatory mediators and a shift in T cell subsets in children with hypertension diagnosis compared with normotensive peers.[14,15]

The current study aimed to investigate the association between BP/hypertension and immune modulation in 2 German population-based birth cohorts, GINIplus (The German Infant Study on the Influence of Nutrition Intervention Plus Air Pollution and Genetics on Allergy Development) and LISA (Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood). Office blood pressure measurements were taken at the 15-year study follow-up and transformed to standardized *Z* scores that subsequently were categorized according to the European Society of Hypertension (ESH) guideline.[16] BP and hypertension

categories were analyzed in conjunction with whole blood gene expression changes assed by multiplex-quantitative polymerase chain reaction. The investigated gene set was comprised of immune modulators, such as alarmins, cytokines, cytokine receptors, and transcription factors regulating various immune processes, known to be involved in the pathophysiology of adult hypertension.[17–22]

Gene expression was examined for its association with BP and BP classes. In addition, risk factors for hypertension, including a predisposition by parental hypertension and overweight, were independently studied for their relationship with transcriptional changes in both cohorts. This is one of the first studies comprehensively assessing the immune modulation at the transcriptional level in a population-based sample of adolescents (n=2128) in relation to BP/hypertension.

METHODS

Data Availability

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

Study Design

The present study is based on the 15-year follow-up of the ongoing prospective population-based birth cohorts GINIplus and LISA, both with very similar study design and questionnaires. During the 15-year follow-up, blood was drawn and BP was measured. Details on the study design can be found in the Supplemental Material and have been described elsewhere.[23]

Subcohorts were selected based on the availability of 2 BP measurements on the day of the 15-year follow-up study visit, relevant confounder information, and gene expression data (Figure 1). For the initial analysis and subsequent validation, n=1219 participants of GINIplus (discovery cohort) and n=809 participants of LISA (validation cohort) were included.

Participation in the studies was voluntary. Written informed consent was obtained from both parents and adolescents. Approval was obtained by local Institutional Review Boards (Bavarian Board of Physicians: 10090, Board of Physicians of North-Rhine-Westphalia: 2010424 and 2015491).

This study is reported in line with the Strengthening the Reporting of Observational Studies in Epidemiology statement.

BP Measurement, *Z* Score Transformation and Hypertension Definition

Arterial systolic and diastolic BP (SBP/DBP) were measured twice at the 15-year follow-up using a standardized protocol according to the procedure used in the population-based German Health Interview and Examination Survey for Children and Adolescents (Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland, 2003–2006) and as previously described.[24] Only participants with 2 measurements for DBP and SBP were included in this study. The average of the 2 BP measurements was used for further analyses irrespective of the difference between both measurements.

BP measurements were standardized for height, sex, and age by *Z* score transformation based on reference percentiles of Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland and conversion formulas given in Neuhauser et al.[25] The standardized *Z* scores of BP measurements were used for further categorization according to the ESH Guideline.[16] For details on BP measurements and BP class categorization, see Supplemental Material.

Physician Diagnosis of Hypertension

A 20-year follow-up for GINIplus and LISA was conducted, of which questionnaire information was available while no BP measurements or blood samples were taken. Information on physician diagnosis of hypertension was only available from this 20-years follow-up questionnaire in which also the age of diagnosis was inquired. A confirmative answer to the question "Have you ever been diagnosed with hypertension by a physician?" was considered as physician diagnosed hypertension.

Consideration of Body Weight

BMI in kg/m^2 was calculated from body weight and height derived from the 15-year examination protocol. As BMI depends on age and sex, percentile curves of the Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland 2003 to 2006

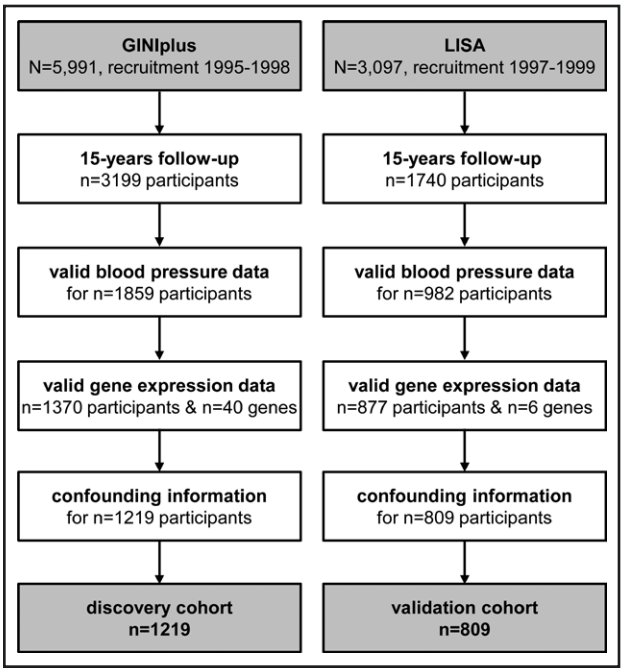

Figure 1. Study design.
Evaluation of immune gene expression in relation to systolic and diastolic blood pressure was performed in the 15-years follow-up of the GINIplus study (The German Infant Study on the Influence of Nutrition Intervention plus Air pollution and Genetics on Allergy Development; discovery cohort). Identified candidate genes, which were associated to BP, were subsequently analyzed in the 15-years follow-up of the LISA study (Influences of Lifestyle-related factors on the Immune System and the Development of Allergies in Childhood; validation cohort).

reference data set were used for *Z* score transformation.[26] For details, see Supplemental Material. In regression analyses examining BMI as a risk factor for hypertension, all participants with a BMI *Z* score above ≥90th percentile were treated as one group. In all other analyses, nonstandardized BMI was considered as a metric variable.

Gene Expression Analysis

The quantitative polymerase chain reaction was performed independently in 2 different laboratories for the discovery and the validation cohorts on the Biomark HD system (Fluidigm, San Francisco, CA) using BioMark 96.96 Dynamic Array chips according to the manufactures recommendations and as previously described.[27] Only genes that could be analyzed with a PCR efficiency between 90% and 110%,[28] and a linear standard curve with a Pearson correlation coefficient $r^2 \geq 0.96$ were included in down-stream analysis (Table S1). For details, see Supplemental Material.[29]

hs-CRP Concentration

Serum concentrations of hs-CRP (high-sensitivity C-reactive protein; mg/L) were determined in 15-year-old participants of the GINIplus or LISA studies using the Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitive assay according to the manufacturer's instructions. Values above 10 mg/L were excluded as those values are commonly considered as a result of acute phase response to infections.[30]

Confounding Factors

Potential confounding factors were tested by univariate logistic regression with either elevated SBP (eSBP) or elevated DBP (eDBP) as the dependent variable and the respective influencing factor as the independent variable. This analysis was restricted to participants of the GINIplus and LISA subcohorts for whom 2 BP measurements and gene expression data were available. The initial selection of potential confounding factors was based on a priori knowledge of known factors contributing to hypertension in adolescence,[31] including genetic-lifestyle factors,[32,33] and influential factors at the time of BP examination (season of BP measurement, study center). See Supplemental Material for the investigated confounders.

Whenever a significant association with either eSBP or eDBP in both cohorts of LISA and GINIplus was observed, the respective factor was retained in the final adjusted model (Table S2). To this end, the adolescent's sex, BMI, parental history of hypertension, parental educational level, season of BP measurement and study center were included in the final model.

Since *Z* score BP values already account for adolescent's sex and height, in models with *Z* score values as the dependent variable these 2 factors were excluded as covariates, instead weight was introduced as a confounding factor. Likewise, in logistic regression models with BP/hypertension as the dependent variable, BMI and the adolescent's sex were included in addition to the aforementioned predictor variables.

Statistics

χ^2 and Mann-Whitney *U* test were used to test for equal parameter distribution between the analyzed subcohort of GINIplus or LISA and the entire 15-year follow-up cohort, and between the discovery and validation subcohorts.

Multiple regression models were applied to study the association of gene expression with *Z* score transformed SBP or DBP (adjusted for: weight at the age of 15, parental hypertension, parental education, study center, season of BP measurement). The association of gene expression with BP classes (adjusted for sex, BMI at the age of 15, parental hypertension, parental education, study center, season of BP measurement) was assessed by adjusted logistic regression. Hs-CRP values were naturally log-transformed and considered as independent variable in all adjusted regression analyses except mediation analysis.

Adjusted mediation analyses were performed with the *PROCESS* macro version 4.2[34] for SPSS version 29. Statistical significance of the indirect effect was determined by bootstrapping as implemented in *PROCESS*. Bias-corrected 95% CIs were derived from the distribution of bootstrap estimates of the indirect effect from random resampling of 5000 samples. Note that standardized effect size is not available for a dichotomous dependent variable.

Subgroup analyses were performed in adolescents without the 2 hypertension risk factors overweight/obesity and a parental history of hypertension (low-risk group). Therefore, the adjusted logistic regression models were repeated excluding adolescents with either one of these risk factors. The same analysis was then repeated in this low-risk group, excluding those with a physician diagnosis of hypertension up to age 20.

Accounting for missing data was not necessary as the discovery and validation cohorts were selected based on complete outcome and confounder information. Whenever applicable, we performed multiple test corrections by Bonferroni. $P \leq 0.05$ were considered significant. See Supplemental Material for software used.

RESULTS

Prevalence of Elevated BP in Adolescents

The discovery cohort comprised 1219 participants of GINIplus and 809 participants of LISA who were included in the subsequent validation analyses (validation cohort, Figure 1). There was no general selection bias regarding the basic study characteristics (Table S3A). In the validation cohort, the participation rate of adolescents with higher educated parents and BP measurement in the summer season was slightly higher compared with the discovery cohort (Table 1). To account for this bias potentially affecting the influence of gene expression on BP, both factors were included in downstream analyses as confounding factors in addition to the participant's sex, height and weight (where applicable), the history of parental hypertension and study center.

The vast majority of adolescents were of normal weight. Approximately, 5% were overweight or obese, while 11.2% and 13.5% of the 15-year-olds in the discovery and validation cohort, respectively, were underweight (Table 1).

Table 1. Study Characteristics of the Discovery and Validation Cohort

	Discovery cohort, N=1219 (GINIplus)	Validation cohort, N=809 (LISA)	
Basic characteristics	**n (%)**	**n (%)**	***P* value***
Study center			
Munich	465 (38.1)	348 (43.0)	NA
Wesel	754 (61.9)	92 (11.4)	
Leipzig	NA	255 (31.5)	
Bad Honnef	NA	114 (14.1)	
Adolescents's sex			
Female	612 (50.2)	381 (47.1)	0.170
Male	607 (49.8)	428 (52.9)	
Weight classification†			
Underweight	137 (11.2)	109 (13.5)	0.380
Normal weight	1019 (83.6)	658 (81.3)	
Overweight	48 (3.9)	35 (4.3)	
Obese	15 (1.2)	7 (0.9)	
Parental education‡			
Low	90 (7.4)	25 (3.1)	<0.001
Middle	356 (29.2)	216 (26.7)	
High	773 (63.4)	568 (70.2)	
Season of BP measurement			
Spring	354 (29.0)	231 (28.6)	<0.001
Summer	387 (31.7)	291 (36.0)	
Autumn	263 (21.6)	199 (24.6)	
Winter	215 (17.6)	88 (10.9)	
Parental history of hypertension			
Parental	324 (26.6)	228 (28.2)	0.427
No history	895 (73.4)	581 (71.8)	
BP classes			
Diastolic eBP	98 (8.0)	88 (10.9)	0.016
Systolic eBP	171 (14.0)	132 (16.3)	
HTN	225 (18.5)	179 (22.1)	
Isolated systolic HTN	112 (9.2)	77 (9.5)	
Isolated diastolic HTN	36 (3.0)	26 (3.2)	
Normotensive BP	877 (71.9)	529 (65.4)	
BP, mm Hg	Median, 25%–75% quartiles	Median, 25%–75% quartiles	*P* value§
	(min/max)	(min/max)	
Females			
Diastolic BP	70.0, 64.5–75.0	70.0, 65.0–76.0	0.575
	(48.5/96.5)	(51.5/97.0)	
Systolic BP	116.5, 110.0–123.5	117.0, 110.0–125.5	0.528
	(87.5/149.0)	(87.0/151.0)	
Males			
Diastolic BP	68.0, 63.0–73.5	70.0, 65.0–75.5	<0.001
	(41.0/98.0)	(46.5/96.5)	
Systolic BP	122.5, 115.5–131.5	123.5, 116.5–132.5	0.181
	(81.5/167.5)	(95.5/177)	

BP indicates blood pressure; eBP, elevated BP; GINIplus, The German Infant Study on the Influence of Nutrition Intervention Plus Air Pollution and Genetics on Allergy Development; HTN, hypertension; KiGGS, Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland; and LISA, Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood.

**P* value from χ^2 test for cross-relationship between discovery- and validation cohort.

§*P* value from Mann-Whitney *U* test *U* test comparing discovery- and validation cohort.

†Classification based on the German Health Interview and Examination Survey for Children and Adolescents (KiGGS) provided by the Robert Koch Institute.

‡Based on the highest number of years of school education of either parent: low <10 y, medium=10 y, high >10 y.

Based on the ESH guideline[16] to classify BP 18.5% (n=225) of the adolescents in the discovery and 22.1% (n=179) of those in the validation cohort were in the hypertension class. Isolated systolic hypertension, that is, normotensive DBP, but eSBP, was observed in 9.2% (n=112) of the individuals in the discovery cohort and 9.5% (n=77) of the validation cohort. Isolated diastolic hypertension, that is, normotensive SBP but eDBP was observed in 3.0% (n=36) participants of the discovery and in 3.2% (n=26) of the validation cohort.

Note, none of the adolescents in our study reported the use of antihypertensive medication or a physician diagnosis of hypertension at the age of 15 years. Less than 5% of those in the hypertension class at the age of 15 years reported hypertension diagnosis by a physician until the age of 20 years (discovery cohort: n=10 diagnosed until age of 20 years of n=225 in the hypertension class at age 15 (4.4%), validation cohort: n=9 diagnosed until age of 20 years of n=179 in the hypertension class at age 15 (5.0%). Table S3B shows the comparison of study characteristics between normotensive participants and those in the hypertension class. We observed a significantly higher SBP in female and male study participants from the Eastern part of Germany compared with the Western part (Mann-Whitney *U* test *U* test [Leipzig versus Wesel/Bad Honnef]: girls, *P*=0.003/boys, *P*<0.0001) or compared with South Germany (Mann-Whitney *U* test *U* test (Leipzig versus Munich): girls, *P*=0.0002/ boys, *P*<0.0001, Table S4A). No regional differences for the BMI were observed in GINIplus or in LISA (Table S4B).

Transcriptional Immune Profile in Adolescents With High BP

Initially, the expression of 51 immune modulatory genes was measured in the discovery cohort (for gene selection

see Supplemental Material; Figure S1; Table S1). Eleven genes did not pass quantitative polymerase chain reaction quality control thresholds (Figure S1) such as *IL17A* or *IFNG*, which showed generally low expression values across all samples. As such 40 genes remained (Table S1) that were analyzed in the discovery cohort for their relationship with *Z* score transformed DBP and SBP.

Expression of *FOS*, *IL1B*, *GZMB*, and *PRF1, CXCR2*, *IFNGR2*, and *TIPARP* was directly associated with both SBP and DBP, whereas *IL2RB*, *IL33*, and *PPARG* were solely linked to SBP and *IL18R1*, *IL18RAP*, and *IFNGR1* to DBP, respectively (Table 2; Table S5). Although most genes had a positive association with BP, elevated *IL33* and *PPARG* related to lower BP (Table 2).

To determine whether expression of these 13 BP-related genes was associated with the different BP classes, adjusted logistic regression models were applied. Expression of *IL1B* and the AP-1 transcription factor subunit *FOS*, *PRF1,* and *GZMB* showed a positive relationship with eSBP and a trend towards higher *IL2RB* expression. *GZMB* and *PRF1* increase was associated with all BP classes, although only in trend for *PRF1* in isolated systolic hypertension. Although enhanced *IL33* expression was associated with decreased risk for eSBP, and hypertension, isolated systolic hypertension, respectively (Figure 2A; Figure S2A; Table S6).

Findings were then evaluated in the validation cohort (Table S7).

In the validation cohort, elevated *GZMB*, *PRF1,* and *IL2RB* levels were associated with higher risk for all BP classes, except isolated systolic hypertension that only associated with higher *IL2RB*, confirming the results for *PRF1*, *GZMB* in eSBP, eDPB, hypertension, and isolated diastolic hypertension. Although induction of *IL1B* was only associated with an increased risk for eSBP (Figure 2B; Table S6). The negative association of *IL33* with BP classes could not be confirmed in the validation cohort.

Influence of Body Weight and Parental Predisposition on the Transcriptional Immune profile

We next sought to determine whether the hypertension-related expression changes could be explained—at least in part—by body weight and a parental history of hypertension. Both, an increased BMI or the presence of parental history of hypertension were predictors of hypertension (Figure 2C).

Regression analyses revealed that parental hypertension was not consistently associated with the expression of any of the genes evaluated in the discovery and the validation cohorts (Table S8). However, a positive relationship of adolescent BMI and increased *IL1B* was found for both cohorts (β/SE [SE; β] discovery cohort: 0.15/0.03, n=1219, $P<0.0001$; validation cohort: 0.11/0.03, n=809, $P=0.001$, Table S9; Figure 3A).

A similar trend was observed for *FOS* (Fos proto-oncogene), albeit only in the discovery cohort (β/SE [β] discovery cohort: 0.08/0.03, n=1219, $P=0.005$; validation cohort: 0.04/0.03, n=809, $P=0.299$). *IL2RB* was negatively associated with the BMI solely in the validation cohort (Table S9; Figure 3A).

Weight-Related Effects on Adolescent Elevated BP

Since increased *IL1B* was positively associated with body weight, we investigated whether the relationship between *IL1B* and hypertension was related to a systemic inflammatory response driven by overweight.

Table 2. Association Between Immune-Inflammatory Gene Expression and Systolic or Diastolic BP *Z* Scores in the Discovery Cohort (n=1219)

	Systolic BP (*Z* score)			Diastolic BP (*Z* score)			
Gene	β value*	95% CI	Raw *P* value	Gene	β value*	95% CI	Raw *P* value
FOS	0.32	0.20/0.43	<0.0001	*FOS*	0.29	0.18/0.40	<0.0001
GZMB	0.19	0.10/0.27	<0.0001	*IL1B*	0.20	0.10/0.30	<0.0001
IL1B	0.21	0.11/0.30	<0.0001	*GZMB*	0.16	0.07/0.24	0.0004
PRF1	0.17	0.08/0.26	0.0003	*IFNGR2*	0.20	0.08/0.32	0.0010
CXCR2	0.16	0.05/0.27	0.0047	*PRF1*	0.13	0.04/0.22	0.0053
IL2RB	0.13	0.03/0.23	0.0098	*TIPARP*	0.23	0.07/0.38	0.0057
TIPARP	0.18	0.02/0.34	0.0240	*IL18R1*	0.17	0.05/0.29	0.0066
IL33	−0.08	−0.16/−0.01	0.0271	*CXCR2*	0.14	0.03/0.25	0.0136
PPARG	−0.08	−0.16/−0.01	0.0271	*IL18RAP*	0.12	0.02/0.21	0.0146
IFNGR2	0.12	0.00/0.24	0.0416	*IFNGR1*	0.17	0.03/0.30	0.0199

Given are multiple regression models adjusted for weight, parental HTN, parental education, season of BP measurement and study center. Shown are all significant ($P<0.05$) associations, Bonferroni correction threshold $P<0.0013$. Table S7 shows list for all investigated genes. BP indicates blood pressure; and HTN, hypertension.

*Nonstandardized regression coefficient β.

Table 3. Association Between Immune-Inflammatory Gene Expression and HTN Excluding Adolescents With Overweight or a Parental History of HTN

	Discovery cohort				Validation cohort			
	adj.OR*	95% CI	*P* value	n, HTN class (yes/no)	adj.OR*	95% CI	*P* value	n, HTN class (yes/no)
Cytotoxic mediators								
IL2RB	1.32	0.97/1.80	0.076	127/655	1.81	1.21/2.71	0.004	93/398
GZMB	1.67	1.26/2.21	0.0004	127/655	1.70	1.24/2.33	0.001	93/398
PRF1	1.70	1.26/2.29	0.0005	127/655	1.75	1.22/2.49	0.002	94/398
Inflammatory immune response								
FOS	1.78	1.23/2.57	0.002	127/655	1.75	1.12/2.73	0.01	93/398
IL1B	1.29	0.96/1.73	0.088	127/655	1.25	0.84/1.84	0.272	93/398
hs-CRP	1.03	0.89/1.20	0.669	126/648	0.86	0.63/1.16	0.323	91/393

Given are adjusted logistic regression models with HTN as the dependent variable. adj.OR indicates adjusted odds ratio; BP, blood pressure; hs-CRP, high-sensitivity C-reactive protein; and HTN, hypertension.

*Adjusted for sex, parental education, season of BP measurement, and study center.

The systemic inflammation marker hs-CRP that has been suggested as an independent risk factor for hypertension is known to be induced by IL-1B (interleukin 1B).[35] In line, we observed an association of hs-CRP with *IL1B* (β/SE [β] discovery cohort: 0.39/0.04, *P*<0.0001, n=1200; validation cohort: 0.24/0.05, *P*<0.0001, n=793), and an association of hs-CRP with SBP *Z* scores (β/SE(β) discovery cohort: 0.10/0.02, *P*<0.0001, n=1205; validation cohort: 0.07/0.03, *P*<0.0001, n=793) as determined by univariate multiple regression.

However, when BMI was added as a confounding factor to the models, only the association of hs-CRP with *IL1B* persisted (β/SE [β] discovery cohort: 0.33/0.04, *P*<0.0001, n=1200; validation cohort: 0.19/0.05, *P*=0.0001, n=793), while the association between hs-CRP and SBP *Z* scores disappeared (β/SE(β) discovery cohort: −0.00/0.02, *P*=0.881, n=1205; validation cohort: 0.00/0.03, *P*=0.989, n=793). Note that BMI was still significantly associated with hs-CRP even when *IL1B* or SBP were included in the model.

Confounder adjusted mediator analysis was then performed to examine the relationship between BMI, *IL1B* expression, and eSBP. (Figure 3B). *IL1B* was identified as a mediator between increased BMI and eSBP in the discovery cohort (indirect mediating effect adj.β, 0.01 [95% CI, 0.0002–0.02]), which was confirmed in the validation cohort (adj.β, 0.02 [95% CI, 0.0003–0.02]). A significant direct effect and a significant indirect effect of increased BMI on eSBP—via *IL1B* transcription—was observed (discovery cohort: β=0.23 [95% CI, 0.18–0.29] *P*<0.0001; validation cohort: β=0.20 [95% CI, 0.13–0.27] *P*<0.0001, Figure 3B).

In line with the multiple regression results, hs-CRP did not mediate the effect between BMI and eSBP (indirect mediating effect adj.β, 95% CI for discovery cohort: 0.003, −0.02/0.02, n=1037; validation cohort: −0.01, −0.04/0.01, n=651, Figure S3).

Immune Profile in Nonrisk Adolescents

More than half of the adolescents, who were classified as hypertensive (systolic or diastolic) were neither overweight nor had a parental history of hypertension (discovery cohort: n/N=127/225, 56.4%, validation cohort: n/N=94/179, 52.5%).

To investigate the cytotoxic (*GZMB*, *PRF1, IL2RB*) and proinflammatory immune profile (*IL1B, FOS,* and CRP protein) in this subgroup of adolescents, being hypertensive despite absence of known risk factors for hypertension, we repeated the adjusted logistic regression analyses for both cohorts by excluding participants with a BMI *Z* score above ≥90th percentile or those with a parental history of hypertension (Table 3).

Although *PRF1*, *GZMB,* and *FOS* were still significantly associated with an increased risk for hypertension even when only nonrisk participants were included, the effect of *IL1B* on hypertension was no longer significant (discovery cohort: *P*=0.088, validation cohort: *P*=0.200, Table 3). Again, hs-CRP protein concentration was not linked to hypertension (discovery cohort: *P*=0.669, validation cohort: *P*=0.332, Table 3).

In both cohorts, no physician-diagnosed hypertension was reported at the time of the 15-year follow-up visit, however in the 20-years questionnaire, n=10 participants in GINIplus and n=9 in LISA reported a hypertension diagnosis after age of 15. Further refining our risk group by restricting the analysis to adolescents without risk factors for hypertension and individuals with hypertension diagnosis, retained the positive association of *GZMB*, *PRF1,* and *FOS* with hypertension (Table 4). For both, *IL1B* and hs-CRP no significant relationship to hypertension was observed neither in the discovery nor in the validation cohort.

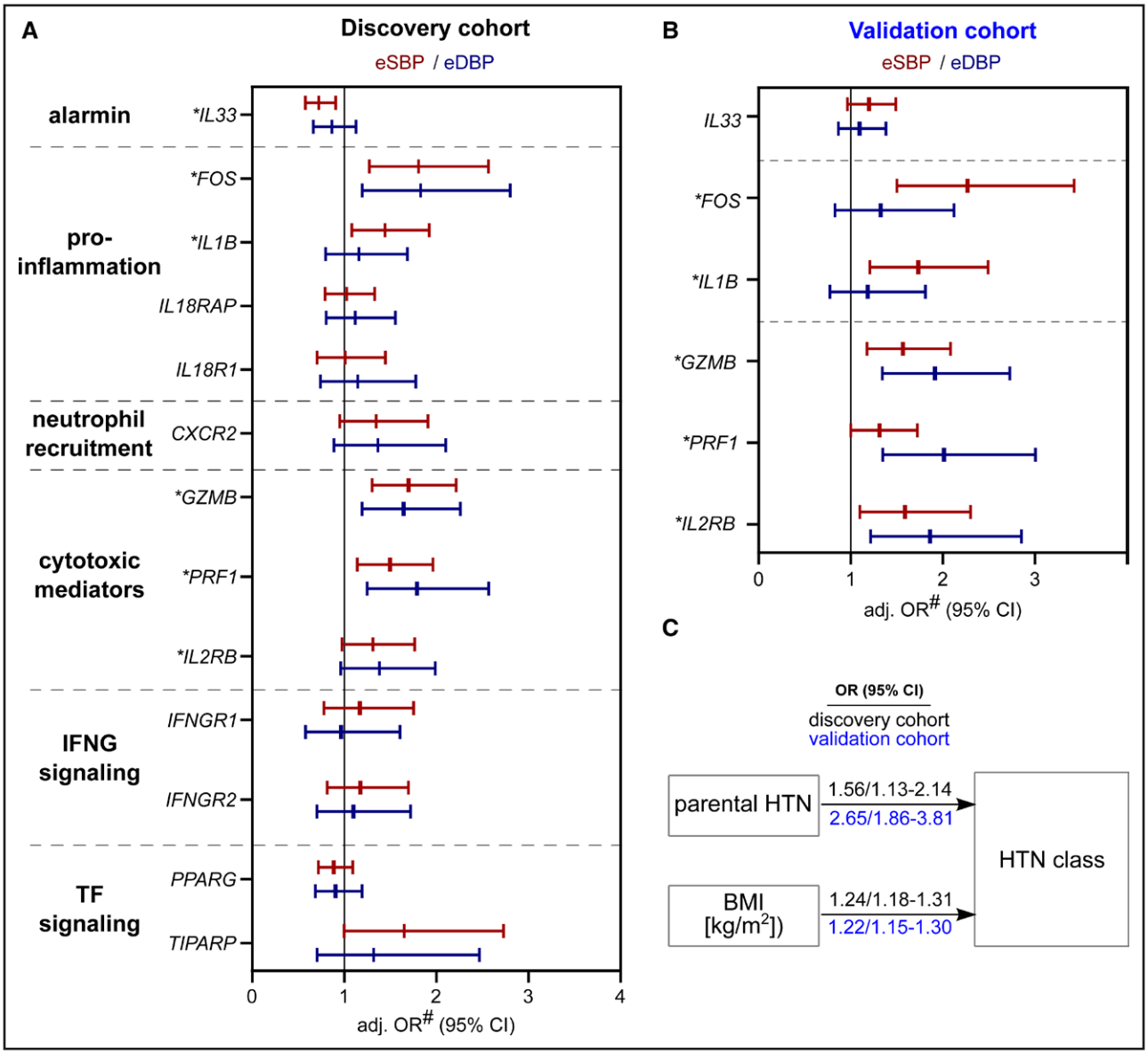

Figure 2. Immune gene expression in adolescents with elevated blood pressure (BP).
A, Forest plots indicate associated risk for elevated systolic BP (eSBP) and elevated diastolic BP (eDBP) of those immune genes, which were significantly associated with BP in the discovery cohort (n=877 controls, n=98 eDBP, n=171 eSBP) as determined by adjusted logistic regression. **B**, Risk increase for elevated BP of significantly associated genes identified in the discovery cohort was assessed in the validation cohort (n=529 controls, n=88 eDBP, n=133 eSBP). Odds ratio (#OR) from logistic regression adjusted for sex, body mass index (BMI), parental hypertension (HTN), parental education, season of BP measurement and study center. *Significant association with $P<0.05$. **C**, Risk factors associated with HTN in adolescents. Shown are ORs from univariate logistic regression for the discovery cohort in black (n=877 controls, n=225 HTN class), and in blue for the validation cohort (n=529 controls, n=179 HTN class). IFNG indicates interferon gamma; and TF, transcription factor.

DISCUSSION

Based on the 15-year follow-up of 2 large birth cohort studies including >2000 participants, we characterized the transcriptional immune profile of adolescents in association with OBP measurements.

In adolescents whose OBP measurements on the day of the 15-year study visit met the criteria for categorization into the hypertension class, we observed a transcriptional activation of cytotoxic *(GZMB, PRF1)* and proinflammatory (*IL1B, FOS*) mediators. We found that the expression of very similar genes was associated with either SBP or DBP.

GZMB (granzyme B) and PRF1 (perforin 1) are cytolytic mediators that are secreted by cytotoxic lymphocytes including natural killer cells, natural killer T cells and cytotoxic or senescent T cells.[36,37] Although all these cell types have been implicated in the pathophysiology of hypertension in animal models, in humans particularly cytotoxic or senescent CD8+ T cells have been implicated in promoting hypertension-associated end-organ damage.[11,38] In

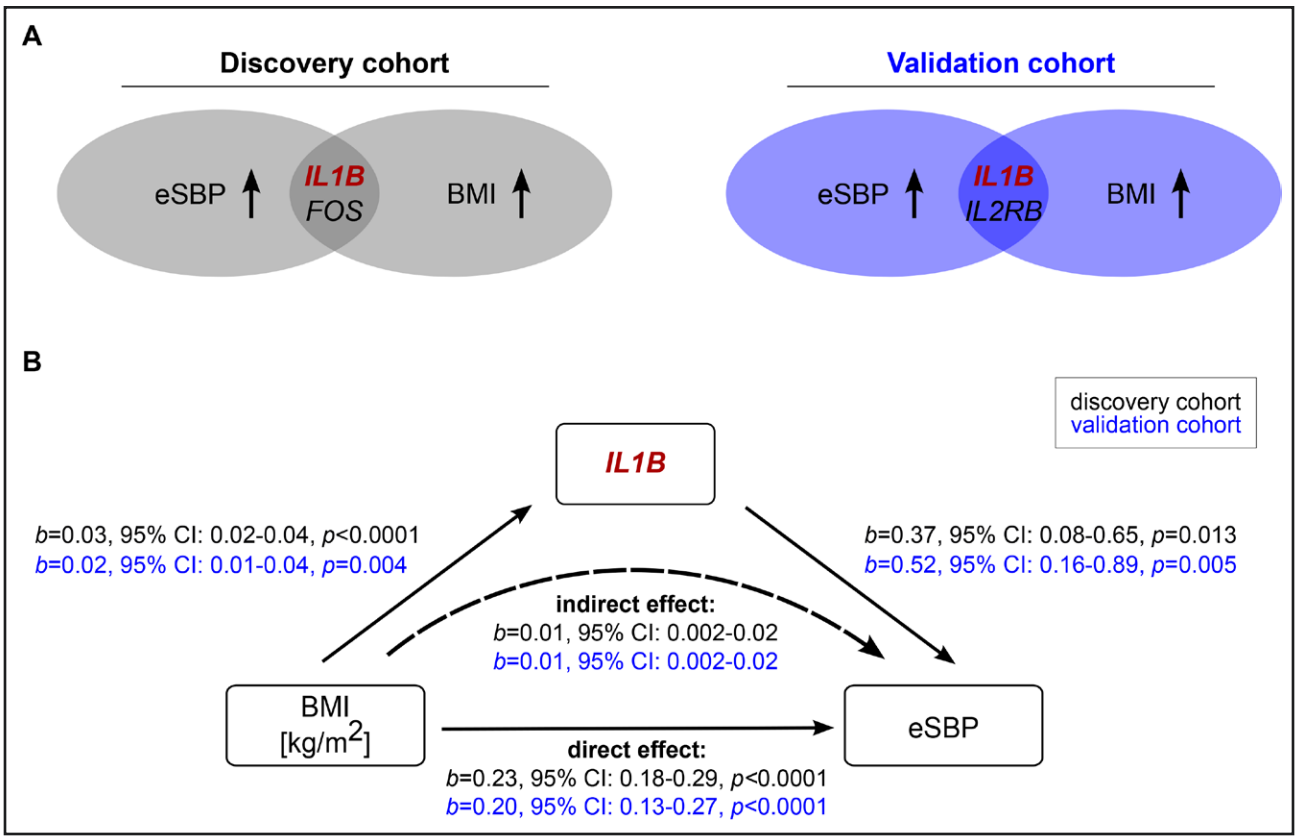

Figure 3. Influence of body mass index (BMI) on *IL1B* (interleukin 1B) expression and elevated systolic blood pressure (eSBP) in adolescents.

A, Overlap of genes significantly associated to eSBP (refer to Figure 2) and with BMI as determined by multiple regression analysis (see Table S9 for multiple regression results). **B**, Mediation analysis for the relationship of BMI, *IL1B* transcription, and eSBP in the GINIplus (The German Infant Study on the Influence of Nutrition Intervention Plus Air Pollution and Genetics on Allergy Development) discovery cohort (n=1044, black font) and the LISA (Influences of Lifestyle-related factors on the Immune System and the Development of Allergies in Childhood) validation cohort (n=661, blue font), respectively. Model was adjusted for sex, parental hypertension (HTN), parental education, season of BP measurement and study center. Effect sizes for the indirect paths are given as β values with +/−95% CIs. Significance determined by bias-corrected bootstrapping. *FOS* indicates Fos proto-oncogene; and and IL2RB, interleukin 2 receptor beta.

line, increased numbers of circulating cytotoxic CD8+ T cells were found in humans and in murine models of hypertension.[10,11]

An explorative study suggested changes in CD8+ T cell subpopulations in pediatric hypertension. By comparing clinically confirmed cases of hypertension

Table 4. Association Between Immune-Inflammatory Gene Expression and HTN Excluding Adolescents With Overweight or a Parental History of HTN, or With HTN Diagnosis

	Discovery cohort				Validation cohort			
	adj.OR*	95% CI	*P* value	n, HTN class (yes/no)	adj.OR*	95% CI	*P* value	n, HTN class (yes/no)
Cytotoxic mediators								
IL2RB	1.31	0.96/1.79	0.091	125/651	1.71	1.14/2.58	0.009	89/398
GZMB	1.63	1.22/2.17	0.001	125/651	1.64	1.19/2.26	0.003	89/398
PRF1	1.65	1.22/2.23	0.001	125/651	1.69	1.18/2.40	0.004	89/398
Inflammatory immune response								
FOS	1.80	1.24/2.61	0.002	125/651	1.79	1.13/2.81	0.012	89/398
IL1B	1.30	0.97/1.75	0.081	125/651	1.22	0.82/1.83	0.324	89/398
hs-CRP	1.04	0.90/1.21	0.588	124/644	0.87	0.64/1.17	0.365	88/393

Given are adjusted logistic regression models with HTN as the dependent variable. adj.OR indicates adjusted odds ratio; BP, blood pressure; hs-CRP, high-sensitivity C-reactive protein; and HTN, hypertension.

*Adjusted for sex, parental education, season of BP measurement and study center

(n=34, 85% male) to normotensive controls (n=35, 69% male) Gackowska et al observed an overall increase in CD8+ T cells, with a higher proportion of naïve and memory CD8+ T cells. However, these differences were diminished after correcting for BMI, age and sex.[15]

In our study, we were not able to distinguish between specific immune cell populations since no cell count data were available. Although we observe a cytotoxic immune profile (*PRF1*, *GZMB*) that could be associated to senescent or cytotoxic CD8+ T cells also other cytotoxic lymphocytes might be involved. Adolescents with OBP measurements fulfilling hypertension criteria had higher expression levels of *PRF1*, *GZMB,* and *IL2RB* than their normotensive peers. In our case, the relationship between *GZMB* and *PRF1* and BP/hypertension was not affected by BMI, suggesting that this cytotoxic phenotype is rather related to increased BP/hypertension and not promoted by overweight. This hypothesis is further supported by the fact that *GZMB* and *PRF1* were still increased in hypertensive adolescents when excluding overweight individuals. It is known that already modest increases in BP can lead to ROS production and neoantigen formation promoting subsequent activation of T cells. As such the cytotoxic activation, we observed in our study is a likely consequence of tissue damage caused by the elevated BP.[39]

However, we cannot exclude the possibility that also other factors contributed to the cytotoxic phenotype that we were not able to further delineate.

Next to the hypertension-associated induction of *GZMB* and *PRF1,* we also observed an increase in IL2 receptor subunit beta (*IL2RB*) transcription. IL-2RB was already described in the context of adult DBP and has been functionally linked to fine-tuning of CD8+ T cells.[40] As this receptor mediates IL-2-triggered proliferative response in CD8+ T cells, an increased *IL2RB* expression might counteract the previously described accumulation of senescent CD8+ T cells in hypertension by promoting expansion.[41]

In children with clinically confirmed hypertension increased chemokine concentrations including Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES; *CCL5*) have been suggested as a consequence of monocyte-endothelial cell adhesive interaction, which supposedly plays a role in early vascular inflammation.[14] In our study, *CCL5* transcription was not associated with BP. Also, *CXCR2* a receptor shown to be important for monocyte infiltration in hypertension was not affected by BP changes, probably because the increase in BP was not yet prevalent to the same extent and for a shorter period in the adolescents compared with adults. However, we found *IL1B*, which is mainly produced by activated monocytes or macrophages, to be associated with higher BP. IL-1B has been described to induce Fos proto-oncogene, which likely contributes to the significantly enhanced *FOS* expression related to hypertension observed in this study.[42] Of note, overexpression of c-Fos was shown to be essential for IL-1 mediated CRP induction in vitro.[43] IL-1B and CRP are both markers of systemic inflammation that have been associated with cardiovascular diseases.[44] In the recent clinical trial, CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) the anti-IL-1B antibody canakinumab was investigated for its efficacy to lower BP and hypertension incidence.[45] Canakinumab reduced hs-CRP and the number of recurrent cardiovascular events, but had no significant effect on BP.[45] Indicating that the beneficial anti-inflammatory effect of IL-1B reduction on cardiovascular events might not be directly linked to changes in BP. In line, also our results suggest that the increased levels of *IL1B* and hs-CRP are related to BMI rather than BP, suggesting inflamed adipose tissue as a source of these mediators.[46]

Overall, our results point to an activation of cytotoxic lymphocytes already at this early stage of elevated BP. However, we do not observe an increase in chemokines or adhesion molecules that might yet have promoted tissue infiltration and damage. Suggesting that interventions at this disease stage might be particularly valuable to prevent endothelial dysfunction and end-organ damage.

Our results have to be interpreted in light of some study limitations. Although standardized BP measurements were performed twice on the day of the study visit, it was not possible to follow-up with additional BP measurements on subsequent occasions or with ambulatory BP monitoring. These one-time measurements do not adhere to the criteria necessary for a clinical diagnosis of hypertension and likely overestimated the proportion of adolescents classified as hypertensive. However, the prevalence of hypertension in our study is in line with recent observations in Germany and global reports showing a peak in prevalence of hypertension around the age of 15.[47] Furthermore, the observed positive relationship between *TIPARP* expression and BP, as repeatedly described in studies in adults, supports the validity of our BP measurements.[48] Although we assessed the influence of BMI, a known risk factor for hypertension, our study did not include information on visceral obesity and waist circumference, which may contribute to BP elevation despite normal BMI.

We are aware that the targeted gene expression approach, we applied limited the overall number of genes investigated. For some of the genes, quality control thresholds for further analysis were not reached. As such, we could not follow-up on some of the immune-inflammatory mediators previously implicated in adult hypertension. How the transcriptional changes observed in this study contribute to change on the protein level or to a cell population shift needs to be further pursued in future studies.

Perspective

In this population-based sample of German 15-year-olds, we showed that elevated BP is associated with enhanced expression of cytotoxic and proinflammatory immune mediators already in the pediatric population. As none of the participants in this study had been diagnosed with hypertension by age 15 and only a few had received a physician diagnosis by the age of 20, the prevalence of underdiagnosed hypertension is alarmingly high and represents a major obstacle for prevention strategies. The majority of adolescents classified as hypertensive in our study were neither overweight nor predisposed by parental hypertension. These children may currently escape regular BP monitoring as they are thought to have a low risk for hypertension. However, these individuals already show an elevated transcription of cytotoxic mediators that may increase their risk for long-term sequelae. This underscores the need to closely monitor BP in all children and to reinforce implementation in well-child visits, also in those countries that do not yet follow current guideline recommendations, to prevent adverse long-term cardiovascular events.

ARTICLE INFORMATION

Received May 11, 2023; accepted August 23, 2023.

Affiliations

Molecular Epidemiology Unit, Center of Digital Health, Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, associated partner of the German Center for Lung Research (DZL) (L.T., M.M., I.L., S.T.). Department of Environmental Immunology, Helmholtz Centre for Environmental Research-UFZ, Leipzig, Germany (M.B., G.H.). Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany (M.F., J.H., M.S.). IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany (T.S.). Research Institute, Department of Pediatrics, Marien-Hospital-Wesel, Germany (A.v.B.). Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, Ludwig Maximilians University of Munich, Germany (J.H.). Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics, School of Population & Global Health, The University of Melbourne, Australia (J.H.).

Acknowledgments

The authors thank all families for their participation in the *GINIplus* and the *LISA* studies. The authors thank all *GINIplus* study group and *LISA* study group members for their excellent work. Please refer to the Supplemental Material for their members. The authors thank Anke Seegebarth and Beate Fink for their excellent technical assistance. Graphical abstract was created with BioRender.com.

Author Contributions

S. Trump, I. Lehmann, and M. Standl provided project leadership. J. Heinrich, M. Standl, A. von Berg, T. Schikowski, I. Lehmann, G. Herberth performed cohort recruitment and sample collection. M. Ferland performed cohort study data curation. S. Trump, L. Thürmann, M. Messingschlager, and M. Bauer coordinated or performed experimental work. L. Thürmann and S. Trump performed formal analysis. L. Thürmann prepared figures and tables. L. Thürmann, S. Trump, and I. Lehmann wrote the initial article. All authors revised or commented on the final article.

Sources of Funding

This work was supported by institutional funding from the BIH at Charité—Universitätsmedizin Berlin. The GINIplus (The German Infant Study on the Influence of Nutrition Intervention Plus Air Pollution and Genetics on Allergy Development) and the LISA (Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood) study were supported for the first 3 years by the Federal Ministry for Education, Science, Research and Technology (01EG9705/2) and the involved study institutions. The 4-to 20-year follow-up examinations were covered by intramural funding of the study centers (Helmholtz Zentrum München - Deutsches Forschungszentrum für Gesundheit und Umwelt, Research Institute at Marien-Hospital Wesel/Evangelisches Krankenhaus Düsseldorf, LMU Munich, Technische Universität Munich, Helmholtz-Zentrum für Umweltforschung, Pediatric Practice, Bad Honnef for the first 2 years) and in addition by a grant from the Federal Ministry for Environment (IUF, FKZ 20462296). Further, the 15-year follow-up examination was supported by the Commission of the European Communities, the seventh Framework Program: MeDALL project. The 15-year and 20-year follow-up examinations of the GINIplus study were additionally supported by the companies Mead Johnson and Nestle.

Disclosures

None.

Supplemental Material

Abbreviations
Supplementary Methods
Tables S1–S9
Figures S1–S3

REFERENCES

1. Wilson PW. Established risk factors and coronary artery disease: the Framingham Study. *Am J Hypertens*. 1994;7:7S–12S. doi: 10.1093/ajh/7.7.7s
2. Tirosh A, Afek A, Rudich A, Percik R, Gordon B, Ayalon N, Derazne E, Tzur D, Gershnabel D, Grossman E, et al. Progression of normotensive adolescents to hypertensive adults: a study of 26,980 teenagers. *Hypertension*. 2010;56:203–209. doi: 10.1161/HYPERTENSIONAHA.109.146415
3. Moin A, Mohanty N, Tedla YG, Carroll AJ, Padilla R, Langman CB, Smith JD. Under-recognition of pediatric hypertension diagnosis: examination of 1 year of visits to community health centers. *J Clin Hypertens (Greenwich)*. 2021;23:257–264. doi: 10.1111/jch.14148
4. Kaelber DC, Liu W, Ross M, Localio AR, Leon JB, Pace WD, Wasserman RC, Fiks AG; Comparative Effectiveness Research Through Collaborative Electronic Reporting (CER2) Consortium. Diagnosis and medication treatment of pediatric hypertension: a retrospective cohort study. *Pediatrics*. 2016;138:e20162195. doi: 10.1542/peds.2016-2195
5. Bouhanick B, Sosner P, Brochard K, Mounier-Vehier C, Plu-Bureau G, Hascoet S, Ranchin B, Pietrement C, Martinerie L, Boivin JM, et al. Hypertension in children and adolescents: a position statement from a panel of multidisciplinary experts coordinated by the French Society of Hypertension. *Front Pediatr*. 2021;9:680803. doi: 10.3389/fped.2021.680803
6. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: a Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension*. 2018;71:e13–e115. doi: 10.1161/HYP.0000000000000065
7. Falaschetti E, Hingorani AD, Jones A, Charakida M, Finer N, Whincup P, Lawlor DA, Davey Smith G, Sattar N, Deanfield JE. Adiposity and cardiovascular risk factors in a large contemporary population of pre-pubertal children. *Eur Heart J*. 2010;31:3063–3072. doi: 10.1093/eurheartj/ehq355
8. Song P, Zhang Y, Yu J, Zha M, Zhu Y, Rahimi K, Rudan I. Global prevalence of hypertension in children: a systematic review and meta-analysis. *JAMA Pediatr*. 2019;173:1154–1110. doi: 10.1001/jamapediatrics.2019.3310
9. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med*. 2007;204:2449–2460. doi: 10.1084/jem.20070657
10. Youn JC, Yu HT, Lim BJ, Koh MJ, Lee J, Chang DY, Choi YS, Lee SH, Kang SM, Jang Y, et al. Immunosenescent CD8+ T cells and C-X-C chemokine receptor type 3 chemokines are increased in human hypertension. *Hypertension*. 2013;62:126–133. doi: 10.1161/HYPERTENSIONAHA.113.00689
11. Itani HA, McMaster WG Jr, Saleh MA, Nazarewicz RR, Mikolajczyk TP, Kaszuba AM, Konior A, Prejbisz A, Januszewicz A, Norlander AE, et al. Activation of human T cells in hypertension: studies of humanized mice and hypertensive humans. *Hypertension*. 2016;68:123–132. doi: 10.1161/HYPERTENSIONAHA.116.07237
12. Bush E, Maeda N, Kuziel WA, Dawson TC, Wilcox JN, DeLeon H, Taylor WR. CC chemokine receptor 2 is required for macrophage infiltration and

vascular hypertrophy in angiotensin II-induced hypertension. *Hypertension*. 2000;36:360–363. doi: 10.1161/01.hyp.36.3.360
13. Mervaala EM, Müller DN, Park JK, Schmidt F, Löhn M, Breu V, Dragun D, Ganten D, Haller H, Luft FC. Monocyte infiltration and adhesion molecules in a rat model of high human renin hypertension. *Hypertension*. 1999;33:389–395. doi: 10.1161/01.hyp.33.1.389
14. Litwin M, Michalkiewicz J, Niemirska A, Gackowska L, Kubiszewska I, Wierzbicka A, Wawer ZT, Janas R. Inflammatory activation in children with primary hypertension. *Pediatr Nephrol*. 2010;25:1711–1718. doi: 10.1007/s00467-010-1548-4
15. Gackowska L, Michalkiewicz J, Niemirska A, Helmin-Basa A, Klosowski M, Kubiszewska I, Obrycki L, Szalecki M, Wierzbicka A, Kulaga Z, et al. Loss of CD31 receptor in CD4+ and CD8+ T-cell subsets in children with primary hypertension is associated with hypertension severity and hypertensive target organ damage. *J Hypertens*. 2018;36:2148–2156. doi: 10.1097/HJH.0000000000001811
16. Mancia G, Kreutz R, Brunström M, Burnier M, Grassi G, Januszewicz A, Muiesan ML, Tsioufis K, Agabiti-Rosei E, Algharably EAE, et al. 2023 ESH Guidelines for the management of arterial hypertension [published online June 21, 2023]. *J Hypertens*. 2023; doi: 10.1097/hjh.0000000000003480
17. Linke M, Fritsch SD, Sukhbaatar N, Hengstschlager M, Weichhart T. mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett*. 2017;591:3089–3103. doi: 10.1002/1873-3468.12711
18. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med*. 2018;215:21–33. doi: 10.1084/jem.20171773
19. Cai G, Zhang X, Weng W, Shi G, Xue S, Zhang B. Associations between PPARG polymorphisms and the risk of essential hypertension. *PLoS One*. 2017;12:e0181644. doi: 10.1371/journal.pone.0181644
20. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. *Nat Rev Endocrinol*. 2017;13:710–730. doi: 10.1038/nrendo.2017.91
21. Norton M, Screaton RA. SGK1: master and commander of the fate of helper T cells. *Nat Immunol*. 2014;15:411–413. doi: 10.1038/ni.2875
22. Yang YH, Istomine R, Alvarez F, Al-Aubodah TA, Shi XQ, Takano T, Thornton AM, Shevach EM, Zhang J, Piccirillo CA. Salt sensing by Serum/Glucocorticoid-regulated kinase 1 promotes Th17-like inflammatory adaptation of Foxp3(+) regulatory T cells. *Cell Rep*. 2020;30:1515–1529.e4. doi: 10.1016/j.celrep.2020.01.002
23. Heinrich J, Bruske I, Cramer C, Hoffmann U, Schnappinger M, Schaaf B, von Berg A, Berdel D, Kramer U, Lehmann I, et al. GINIplus and LISAplus - Design and selected results of two German birth cohorts about natural course of atopic diseases and their determinants. *Allergol Select*. 2017;1:85–95. doi: 10.5414/ALX01455E
24. Markevych I, Thiering E, Fuertes E, Sugiri D, Berdel D, Koletzko S, von Berg A, Bauer CP, Heinrich J. A cross-sectional analysis of the effects of residential greenness on blood pressure in 10-year old children: results from the GINIplus and LISAplus studies. *BMC Public Health*. 2014;14:477. doi: 10.1186/1471-2458-14-477
25. Neuhauser HK, Thamm M, Ellert U, Hense HW, Rosario AS. Blood pressure percentiles by age and height from nonoverweight children and adolescents in Germany. *Pediatrics*. 2011;127:e978–e988. doi: 10.1542/peds.2010-1290
26. Neuhauser H SA, Schaffrath AR, Dortschy R, Kurth BM. *Referenzperzentile für anthropometrische Maßzahlen und Blutdruck aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS). 2. erweiterte Auflage*. Robert Koch Institut, Beiträge zur Gesundheitsberichterstattung des Bundes. 2013.
27. Bauer T, Trump S, Ishaque N, Thurmann L, Gu L, Bauer M, Bieg M, Gu Z, Weichenhan D, Mallm JP, et al. Environment-induced epigenetic reprogramming in genomic regulatory elements in smoking mothers and their children. *Mol Syst Biol*. 2016;12:861. doi: 10.15252/msb.20156520
28. Taylor SC, Nadeau K, Abbasi M, Lachance C, Nguyen M, Fenrich J. The ultimate qPCR experiment: producing publication quality, reproducible data the first time. *Trends Biotechnol*. 2019;37:761–774. doi: 10.1016/j.tibtech.2018.12.002
29. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009;55:611–622. doi: 10.1373/clinchem.2008.112797
30. Ridker PM. A test in context: high-sensitivity C-reactive protein. *J Am Coll Cardiol*. 2016;67:712–723. doi: 10.1016/j.jacc.2015.11.037
31. Ewald DR, Haldeman PhD LA. Risk factors in adolescent hypertension. *Glob Pediatr Health*. 2016;3:2333794X–15625159. doi: 10.1177/2333794X15625159
32. Janssen I. Physical activity guidelines for children and youth. *Can J Public Health*. 2007;98:S109–S121.
33. Harris C, Flexeder C, Thiering E, Buyken A, Berdel D, Koletzko S, Bauer C-P, Brüske I, Koletzko B, Standl M. Changes in dietary intake during puberty and their determinants: results from the GINIplus birth cohort study. *BMC Public Health*. 2015;15:841. doi: 10.1186/s12889-015-2189-0
34. Hayes AF. *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York, NY, US: Guilford Press; 2013.
35. Ganter U, Arcone R, Toniatti C, Morrone G, Ciliberto G. Dual control of C-reactive protein gene expression by interleukin-1 and interleukin-6. *EMBO J*. 1989;8:3773–3779. doi: 10.1002/j.1460-2075.1989.tb08554.x
36. Madhur MS, Harrison DG. Senescent T cells and hypertension: new ideas about old cells. *Hypertension*. 2013;62:13–15. doi: 10.1161/HYPERTENSIONAHA.113.01410
37. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. *Nat Rev Immunol*. 2015;15:388–400. doi: 10.1038/nri3839
38. Trott DW, Thabet SR, Kirabo A, Saleh MA, Itani H, Norlander AE, Wu J, Goldstein A, Arendshorst WJ, Madhur MS, et al. Oligoclonal CD8+ T cells play a critical role in the development of hypertension. *Hypertension*. 2014;64:1108–1115. doi: 10.1161/HYPERTENSIONAHA.114.04147
39. Barrows IR, Ramezani A, Raj DS. Inflammation, immunity, and oxidative stress in hypertension-partners in crime? *Adv Chronic Kidney Dis*. 2019;26:122–130. doi: 10.1053/j.ackd.2019.03.001
40. Smith GA, Taunton J, Weiss A. IL-2Rbeta abundance differentially tunes IL-2 signaling dynamics in CD4(+) and CD8(+) T cells. *Sci Signal*. 2017;10:eaan4931. doi: 10.1126/scisignal.aan4931
41. Cho JH, Kim HO, Kim KS, Yang DH, Surh CD, Sprent J. Unique features of naive CD8+ T cell activation by IL-2. *J Immunol*. 2013;191:5559–5573. doi: 10.4049/jimmunol.1302293
42. Nadjar A, Bluthe RM, May MJ, Dantzer R, Parnet P. Inactivation of the cerebral NFkappaB pathway inhibits interleukin-1beta-induced sickness behavior and c-Fos expression in various brain nuclei. *Neuropsychopharmacology*. 2005;30:1492–1499. doi: 10.1038/sj.npp.1300755
43. Nishikawa T, Hagihara K, Serada S, Isobe T, Matsumura A, Song J, Tanaka T, Kawase I, Naka T, Yoshizaki K. Transcriptional complex formation of c-Fos, STAT3, and hepatocyte NF-1 alpha is essential for cytokine-driven C-reactive protein gene expression. *J Immunol*. 2008;180:3492–3501. doi: 10.4049/jimmunol.180.5.3492
44. Braunwald E. Biomarkers in heart failure. *N Engl J Med*. 2008;358:2148–2159. doi: 10.1056/NEJMra0800239
45. Rothman AM, MacFadyen J, Thuren T, Webb A, Harrison DG, Guzik TJ, Libby P, Glynn RJ, Ridker PM. Effects of Interleukin-1beta inhibition on blood pressure, incident hypertension, and residual inflammatory risk: a secondary analysis of CANTOS. *Hypertension*. 2020;75:477–482. doi: 10.1161/HYPERTENSIONAHA.119.13642
46. Unamuno X, Gomez-Ambrosi J, Ramirez B, Rodriguez A, Becerril S, Valenti V, Moncada R, Silva C, Salvador J, Fruhbeck G, et al. NLRP3 inflammasome blockade reduces adipose tissue inflammation and extracellular matrix remodeling. *Cell Mol Immunol*. 2021;18:1045–1057. doi: 10.1038/s41423-019-0296-z
47. Sarganas G, Schaffrath Rosario A, Niessner C, Woll A, Neuhauser HK. Tracking of blood pressure in children and adolescents in germany in the context of risk factors for hypertension. *Int J Hypertens*. 2018;2018:8429891. doi: 10.1155/2018/8429891
48. Huan T, Esko T, Peters MJ, Pilling LC, Schramm K, Schurmann C, Chen BH, Liu C, Joehanes R, Johnson AD, et al; International Consortium for Blood Pressure GWAS (ICBP). A meta-analysis of gene expression signatures of blood pressure and hypertension. *PLoS Genet*. 2015;11:e1005035. doi: 10.1371/journal.pgen.1005035