Research Square

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Vasorin as a multi-omic biomarker of cardiometabolic health in paediatric obesity

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Abstract

Background

Effective early stratification of cardiometabolic risk in children with obesity remains a major clinical challenge. Existing classification approaches rely largely on indirect indicators, limiting their utility for timely personalized preventive strategies.

Methods

We conducted a multi-omic study integrating leukocyte genomic and epigenomic (DNA methylation and genotype arrays), whole-blood transcriptomic (bulk RNA-seq), and circulating protein data from three independent paediatric populations aged 3–18 years. The study included a prospective discovery population of 138 individuals with and without obesity, and a cross-sectional validation population of 237 children with overweight or obesity. Additionally, gene expression was profiled in visceral adipose tissue (VAT) samples from a third independent population of 13 children.

Results

We identified vasorin (VASN) as a consistent biomarker of metabolic health across multiple molecular layers, populations and tissues. Pubertal DNA methylation at the *VASN* locus (cg00041083) was positively associated with several cardiometabolic risk factors consistently in both the discovery and validation populations, including BMI z-score ($\beta = 0.40$, FDR < 0.001; $\beta = 0.20$, $p < 0.05$) and blood pressure z-score ($\beta = 0.36$, FDR < 0.05; $\beta = 0.19$, $p < 0.05$), and functioned as a cis-eQTM for *VASN* expression in whole-blood ($\beta = -0.71$, $p < 0.05$). Circulating VASN protein levels during puberty were inversely associated with fat mass index ($\beta = -0.30$, $p < 0.05$) in the discovery population and positively associated with HDL-c in the validation population ($\beta = 0.23$, $p < 0.05$). In pooled analyses, pubertal VASN protein levels showed inverse associations with HOMA-IR ($\beta = -0.11$, $p < 0.05$) and FMI ($\beta = -0.22$, $p < 0.05$), mainly driven by males ($\beta = -0.30$, FDR < 0.05) and but not in females ($\beta = -0.10$, $p > 0.05$). In VAT samples from the third population, *VASN* expression was significantly downregulated in prepubertal children with obesity compared to normal-weight controls ($p < 0.05$), supporting its relevance in this metabolically active tissue.

Conclusion

VASN emerges as a novel biomarker of cardiometabolic health in paediatric obesity. Its reproducible associations with cardiometabolic risk factors across molecular layers, populations and tissues support its potential utility for early risk stratification in precision prevention strategies.

Background

Childhood obesity, defined by excessive body fat accumulation, has become a critical public health challenge globally [1–3]. Between 1975 and 2022, the prevalence of overweight and obesity among

children aged 5–19 increased dramatically from 4% to over 20% [4, 5]. This alarming trend is particularly concerning as childhood obesity is difficult to reverse and triggers a cascade of metabolic disturbances, substantially increasing the risk of type 2 diabetes (T2D) and cardiovascular disease (CVD) in adulthood [6–8]. Excess visceral adipose tissue (VAT) is a major driver of these metabolic disturbances, promoting insulin resistance (IR), hypertension, and dyslipidaemia as key mediators of elevated CVD risk [9–12].

Interestingly, not all individuals with obesity develop metabolic complications. A subset (6–22%) [13–15] maintains metabolic health despite excess adiposity, a phenotype referred to as metabolically healthy obesity and has a significantly lower risk of CVD and premature mortality compared to individuals with metabolically unhealthy obesity [16]. The biological mechanisms that distinguish these metabolic phenotypes are not fully understood but are believed to involve complex molecular interactions between metabolically active tissues, including adipose tissue, liver, and skeletal muscle [17–19].

The emergence of high-throughput multi-omics technologies has transformed our understanding of obesity-related metabolic dysfunction [20, 21]. These approaches have revealed promising multi-omics signatures, spanning genomics, transcriptomics, epigenomics, proteomics, and metabolomics, that mediate inter-tissue communication and metabolic disturbances. Of particular clinical interest are alterations in the proteomic and metabolomic layers, as they involve molecules measurable in liquid biopsies such as blood, enabling non-invasive monitoring of risk, disease progression, and targeted interventions [22–26]. Circulating proteins such as S100A4 and ISM-1 have recently emerged as novel non-invasive biomarkers of metabolic dysfunction in childhood obesity, holding potential for future translational applications [27, 28].

Despite these advances, substantial knowledge gaps remain in this field [29]. Most omic studies on obesity have focused on adults, investigated isolated omic layers, and primarily relied on body weight as a proxy for obesity, often neglecting key metabolic phenotypes such as fat distribution and IR [30, 31]. To address these limitations, we conducted an integrated blood multi-omic analysis using data from two deeply phenotyped paediatric obesity populations recruited across three Spanish regions. The study included a prospective discovery population of 138 individuals, with and without obesity, exhibiting heterogeneous cardiometabolic trajectories from childhood to adolescence [32, 33]. In this population, a previous study identified a set of novel DNA methylation alterations with promising potential as biomarkers of metabolic risk during the metabolically critical period of puberty [34, 35]. Building on these findings, we here added a cross-sectional validation population from the same geographical areas, composed of 237 children and adolescents with overweight and obesity–half of whom exhibiting IR [36]. Additionally, we incorporated a third independent population of 13 children with normal-weight and obesity for which gene expression of VAT was analysed [37]. This study therefore integrates multiple cross-tissue molecular layers with comprehensive assessments of metabolic health, including precise measures of fat distribution and insulin sensitivity, in three complementary clinical cohorts. We hypothesize that blood molecular alterations identified through multi-omic profiling may reflect key drivers or consequences of obesity-associated metabolic dysfunction in children. By uncovering these

multi-omic signatures, we seek to advance precision prevention strategies and ultimately improve long-term health outcomes for children with a higher cardiometabolic risk [38–40].

Methods

Study Populations

This study used data from the PUBMEP, IBEROMICS and GENOBOX projects, hospital cohorts established in three Spanish cities (Córdoba, Santiago de Compostela, and Zaragoza). The PUBMEP project is a prospective study (discovery population) that recruits children with normal weight, overweight, or obesity at the prepubertal stage (baseline) and re-examined them at follow-up once puberty had started [32, 33]. Accordingly, two well-defined time points are available for the discovery population: prepubertal (baseline) and pubertal (follow-up). The mean interval between the baseline and follow-up visits was 5.84 years (median: 6.19 years). At each time point (i.e., baseline and follow-up), additional children not included in the longitudinal design were added. Consequently, the total sample size at each stage differs from that of the longitudinal subgroup with data in both baseline and follow-up. The IBEROMICS project is a cross-sectional study (validation population I) composed of prepubertal and pubertal children with overweight or obesity recruited at a single time point. Additionally, a small cross-sectional sub-cohort from the GENOBOX project (validation population II), including prepubertal children with normal weight or obesity, was used to assess gene expression in VAT [30, 33]. Participants were classified as prepubertal or pubertal at recruitment based on Tanner staging [41]. Data collection was performed by trained staff according to standardized protocols. All clinical assessments, cardiometabolic outcomes and biospecimens were collected at each visit and are aligned with the pubertal status at the time of sampling.

All participating families and children over 12 years provided written informed consent and younger children gave their assent. All the cohorts received approval from their respective local ethical committees at each of the participating centres (code IDs: Córdoba 01/2017, 260/3408; Santiago 2011/198, 2016/522, 2020/501; Zaragoza 12/2010, 22/2016, 24/2020; Granada 01/2017, 01/2021) and were conducted following the principles of the Declaration of Helsinki.

Physical Exploration, Anthropometry and Metabolic Health Outcomes

At each clinical examination, we measured height, weight, and waist circumference (WC) and calculated age- and sex-standardized body mass index (BMI) and WC z-scores based on national reference values [42, 43]. Overweight and obesity were defined using the International Obesity Task Force reference [44, 45]. Total fat mass was assessed by dual-energy X-ray absorptiometry (DXA) (General Electric, Lunar EnCore and QDR-Explorer TM 4500, Hologic Inc, Bedford, MA), and the fat mass index (FMI) was subsequently derived. Blood pressure (BP) was measured three times per participant by the same

trained examiner using an electronic manometer following international guidelines [46]. In both studies, the pubertal stage was evaluated using Tanner staging [47]. Blood samples were collected under overnight fasting conditions, centrifuged, and processed into plasma and serum, with plasma obtained in EDTA tubes, and all samples stored at – 80°C under standardized conditions. Fasting levels of insulin, glucose, HDL-cholesterol (HDL-c), and triacylglycerols (TAG) were determined at the clinical laboratories of each participating hospital following the manufacturer's protocols.

Rather than applying dichotomous clinical thresholds, cardiometabolic health outcomes were analyzed as continuous traits. We constructed age- and sex-specific z-scores for classical cardiometabolic risk factors, including systolic and diastolic BP (SBP and DBP), TAG, HDL-c, glucose, insulin, and homeostatic model assessment for IR (HOMA-IR). These z-scores were calculated using the reference values proposed by Stravbnso et al. [48, 49]. To ensure transparency, harmonization, and reproducibility across cohorts, z-scores were calculated using the ObMetrics tool [36], which was specifically designed to automatically compute cardiometabolic components using published Metabolic Syndrome (MetS) definitions and corresponding paediatric reference values. Further information about physical exploration, anthropometry and biochemical parameters of these studies could be found elsewhere [32, 33].

Blood Multi-omics Assessment

DNA was extracted from peripheral white blood cells using two automated kits: the QIAamp DNA Investigator Kit for coagulated samples and the QIAamp DNA Mini & Blood Mini Kit for non-coagulated samples (QIAGEN Systems, Inc., Valencia, CA, USA). All DNA extracts were further purified using the DNA Clean & Concentrator kit (Zymo Research, Irvine, CA, USA). High-quality DNA samples (≥ 500 ng) were subjected to bisulfite conversion using the EZ-96 DNA Methylation Kit (Zymo Research Corporation, Irvine, CA).

RNA was extracted from peripheral blood using the PAXgene® Blood RNA Kit (PreAnalytiX/QIACUBE). The concentration and integrity of the extracted RNA were assessed using the Qubit 4 Fluorometer (Thermo Fisher Scientific, MA, USA) and the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). mRNA libraries were prepared from 1 µg of total RNA using the TruSeq Stranded mRNA Library Prep Kit (Illumina, CA, USA) according to the manufacturer's instructions. This protocol selectively captures polyadenylated RNA using oligo-dT primers, followed by RNA fragmentation, first- and second-strand cDNA synthesis, 3'-end adenylation, adapter and index ligation, and PCR-based library enrichment. Indexed libraries were pooled and sequenced on the NextSeq 500 system (Illumina, CA, USA) using paired-end 75 bp reads in high-output mode, targeting a sequencing depth of approximately 20 million reads per sample. To achieve this coverage, two sequencing runs were performed, each comprising four lanes.

We assessed DNA methylation using the Illumina 850K EPIC Methylation array, genotyped single nucleotide polymorphisms (SNPs) using the Infinium Global Screening Array-24 v3.0 and whole-blood

profiling by RNA-sequecing (RNA-seq) on the NextSeq 500 system. RNA-seq was performed on a subset of 44 adolescents from the discovery cohort. Standard quality control procedures were performed on genomic data from both studies, which included: (1) excluding SNPs and individuals with a missing data rate ≥ 10%, and (2) excluding SNPs with a minor allele frequency (MAF) < 1% or a Hardy-Weinberg equilibrium (HWE) p-value < 1×10^−5 in controls. Quality control for raw genomic files (.ped, .map, .bed, .bim, and .fam) was conducted in PLINK 1.9 [50]. White blood cell composition was estimated using the FlowSorted.Blood.EPIC R package.

Regarding the RNA-seq pipeline, each step was implemented separately for each run, following standardized recommendations. These analyses involved processing raw sequencing reads, aligning them to the reference genome, and quantifying expression levels. Alignment was performed using HISAT 2.2.1, and the generated SAM files were sorted and converted to BAM files with SAMtools [51]. We then used the featureCounts R package to generate count matrices from aligned reads [52]. The reference genome was the hg38 build from Ensembl, which was transformed into a transcript database using the makeTxDbFromGFF function in the GenomicFeatures R package [53].

Vasorin (VASN) protein levels were determined in serum using an enzyme-linked immune absorbent assay kit (MyBioSource, San Diego, CA, USA; catalog no. MBS451381). Assay performance was evaluated prior to analysis by testing linearity with serial serum dilutions, and assay variability was within the manufacturer-reported range (coefficient of variation < 12%). Further details about preprocessing of omic data of these studies could be found elsewhere [38, 54].

Bioinformatic Analyses

All epigenetic analyses were conducted using linear regression models. Associations between DNA methylation M-values and cardiometabolic outcomes were conducted separately within the discovery population and validation population I, stratified by pubertal status. In the discovery population, analyses were performed independently at the prepubertal visit (baseline) and pubertal visit (follow-up). In the validation population I, analyses were stratified by pubertal stage at recruitment. Models were adjusted by sex, age, hospital. Pooled analyses combining discovery population and validation population I were conducted to identify potential sex- and pubertal-specific effects. To validate an identified subset of 193 CpG sites previously linked to obesity-associated IR in the discovery population [34, 35], we examined whether these CpG sites were also associated with cardiometabolic alterations beyond IR. Assessed outcomes included all key cardiometabolic health parameters included in the definition of MetS, as well as measures of adiposity such as FMI.

Methylation quantitative trait loci (mQTL) and expression quantitative trait methylation (eQTM) analyses were restricted to a targeted set of 193 validated CpG sites that were identified through an agnostic epigenome-wide analysis in the discovery population [34, 35]. mQTL analyses were conducted separately in the discovery population and validation population I, stratified by pubertal stage. In contrast, eQTM analyses were performed exclusively in a subset of 44 pubertal participants from the follow-up (puberty

stage) of the discovery population I which matched DNA methylation and RNA-seq data. In mQTL models, DNA methylation levels were treated as outcomes and SNPs as predictors, whereas in eQTM models, gene expression levels were modelled as outcomes with DNA methylation at CpG sites as the predictor. Both mQTL and eQTM analyses were performed using the MatrixEQTL R package [55].

Associations between circulating VASN protein levels and cardiometabolic outcomes were evaluated using linear regression models, following the same analytical framework applied in the epigenetic analyses. Analyses were conducted separately within discovery population and validation population I, stratifying by pubertal stage. The cardiometabolic outcomes assessed in these models were identical to those used in the epigenetic analyses. In all models, cardiometabolic traits were specified as outcomes and circulating VASN protein levels as the predictor. In addition, pooled analyses combining discovery population and validation population I were performed to explore potential sex- and pubertal-specific effects.

Across all analyses, molecular predictors and cardiometabolic outcomes were aligned cross-sectionally within the same visit and pubertal stage; omic biomarkers were modelled against outcomes measured at the same time point. All models were adjusted for sex, age, and recruitment centre. Where appropriate, additional adjustment was applied for white blood cell composition (epigenetic analyses) and study cohort (only for pooled epigenetic and protein analyses).

VASN gene expression in VAT of children with obesity

VASN gene expression was determined in the VAT of 6 normal-weight children and 7 children with obesity (validation population II). Total RNA was obtained as described previously [37]. After DNase I treatment (Ambion, Paisley, UK), 1 µg of total RNA was reverse transcribed into cDNA using High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). qPCR was performed using an ABI Prism 7900 instrument (Applied Biosystems, Foster City, CA, USA) using SYBR green PCR Master Mix (Applied Biosystems). The target gene expression was normalized to hypoxanthine-guanine phosphoribosyltransferase-1 (*HPRT1*) expression, used as a reference gene, as previously used in previous studies [27]. Differential expression levels were calculated via the delta-delta ct method. The primer sequences are shown in **Table S1**.

Statistical Analyses

Participant characteristics are reported descriptively. Categorical variables are reported as total numbers and percentages, while continuous variables are reported as means and standard deviations. Normality of continuous variables was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using Pearson's Chi-square test for categorical variables. For continuous variables, Welch two-sample t-test was applied with normally distributed variables, whereas Wilcoxon rank-sum test was used for normally distributed variables.

Due to the number of comparisons analysed, FDR correction was applied using the Benjamin Hochberg procedure to adjust for multiple hypothesis testing. Statistical significance for hypothesis-free DNA methylation analyses was defined as FDR < 0.05. For confirmatory analyses, an unadjusted p-value < 0.05 was considered statistically significant.

Results

Study populations

The study involved three paediatric clinical populations: a prospective discovery study and two validation populations. The discovery population included two samples, a total of 102 subjects at prepuberty (baseline) and 138 at puberty (follow-up), with 90 of them available at both stages. While the validation population I (N = 237) included prepuberal and pubertal children under a cross-sectional design. In the discovery population (Table 1), the mean (standard deviation [SD]) age was 8.20 (1.59) years in pre-puberty and 14.09 (1.97) years in puberty. The sex distribution was balanced. The sample was predominantly composed of children with overweight or obesity. The mean (SD) BMI z-score was 1.77 (1.86) in pre-puberty and 1.41 (1.66) in puberty. FMI was slightly higher during puberty compared to pre-puberty. Overall, the population exhibited a cardiometabolic profile consistent with predominantly populations with obesity. Additionally, a subgroup from the discovery population, including 44 pubertal children with and without obesity, was subjected to in-depth molecular investigation using blood RNA-seq analysis (**Table S2**). The validation population I consisted of 237 individuals, evenly distributed across pre-pubertal and pubertal stages, with a balanced sex ratio (**Table S3**). Its larger sample size, exclusively comprising children with overweight or obesity, offered enhanced statistical power to investigate the molecular alterations underlying the metabolic phenotypes of obesity and explore potential mechanisms of sexual dimorphism. A validation population II of 13 pre-pubertal children with normal-weight and obesity was recruited for the collection of VAT samples (**Table S4**).

Table 1
General descriptive of the discovery population.

Characteristic	Prepubertal children (baseline, N = 102[1])	Pubertal children (follow-up, N = 138[1])	p-value[2]
Sex			**0.82**
Male	**48 (47.06%)**	**67 (48.55%)**	
Female	**54 (52.94%)**	**71 (51.45%)**	
Age (years)	**8.20 (1.59)**	**14.09 (1.97)**	**< 0.001**
Recruitment Center			**0.25**
Córdoba	**11 (10.78%)**	**25 (18.12%)**	
Santiago de Compostela	**50 (49.02%)**	**66 (47.83%)**	
Zaragoza	**41 (40.20%)**	**47 (34.06%)**	
Tanner scale			**< 0.001**
1	**102 (100.00%)**	**0 (0.00%)**	
2	**0 (0.00%)**	**20 (14.49%)**	
3	**0 (0.00%)**	**22 (15.94%)**	
4	**0 (0.00%)**	**19 (13.77%)**	
5	**0 (0.00%)**	**77 (55.80%)**	
Obesity status			
Normal-weight	**25 (24.51%)**	**51 (36.96%)**	**0.12**
Overweight	**21 (20.59%)**	**25 (18.12%)**	
Obesity	**56 (54.90%)**	**62 (44.93%)**	
BMI (kg/m^2)	**21.82 (4.88)**	**25.29 (6.02)**	**< 0.001**
BMI z-score	**1.77 (1.86)**	**1.41 (1.66)**	**0.16**
WC (cm)	**72.16 (13.59)**	**83.13 (15.79)**	**< 0.001**
WC z-score	**2.10 (2.30)**	**1.09 (2.02)**	**< 0.001**
FMI (kg/m^2)	**7.84 (3.82)**	**10.12 (4.60)**	**0.017**
Height (m)	**1.32 (0.10)**	**1.61 (0.12)**	**< 0.001**
Height z-score	**0.61 (1.25)**	**0.16 (1.15)**	**< 0.001**

Characteristic	Prepubertal children (baseline, N = 102[1])	Pubertal children (follow-up, N = 138[1])	p-value[2]
Sex			0.82
Male	48 (47.06%)	67 (48.55%)	
Glucose (mg/mL)	82.81 (8.09)	87.30 (19.78)	0.003
Glucose z-score	-0.64 (1.14)	-0.57 (2.76)	0.59
Insulin (µU/mL)	9.26 (6.86)	14.31 (9.47)	< 0.001
Insulin z-score	0.83 (1.35)	0.57 (1.22)	0.22
HOMA-IR	1.92 (1.48)	3.09 (2.18)	< 0.001
HOMA-IR z-score	0.69 (1.32)	0.41 (1.18)	0.15
DBP (mmHg)	62.57 (8.83)	68.66 (10.68)	< 0.001
DBP z-score	0.29 (0.78)	0.38 (0.93)	0.30
SBP (mmHg)	104.43 (12.92)	111.47 (14.27)	< 0.001
SBP z-score	0.41 (1.17)	0.07 (1.31)	0.026
BP z-score	0.35 (0.86)	0.22 (0.88)	0.45
TAG (mg/mLl)	59.08 (27.13)	73.99 (35.08	< 0.001
TAG z-score	-0.16 (0.94)	0.10 (0.97)	0.012
HDL-c (mg/mL)	56.37 (14.66)	51.15 (14.14)	0.005
HDL-c z-score	-0.22 (1.16)	-0.37 (1.13)	0.074
LDL-c (mg/mL)	97.20 (26.53)	91.58 (23.31)	0.10
LDL-c z-score	0.06 (1.10)	0.14 (0.95)	0.23

[1] N (%), Mean (Standard deviation)

[2] Pearson's Chi-squared test; Wilcoxon signed rank test; Welch Two Sample t-test

Epigenetic marks of metabolic health in children with obesity

In the discovery population, we previously conducted an epigenome-wide analysis to identify DNA methylation marks associated with IR [34, 35]. This approach identified a subset of 193 candidate CpG sites, annotated to 89 genes, which were associated with obesity-related IR [34, 35]. In the current work,

these CpG sites were evaluated in a targeted manner in an independent validation population I to confirm their associations with IR and to explore their relationships with additional cardiometabolic phenotypes.

A total of 24 CpG sites, mapping to 20 loci, showed DNA methylation patterns associated with at least one cardiometabolic health outcome (raw p-value < 0.05), with consistent findings across the discovery and validation population I (Fig. 1 **and Table S5**). To our knowledge, this subset of CpG sites included well-known and validated T2D epigenetic biomarkers such as *ABCG1*, *HDAC4*, and *PTPRN2*, which were associated here with central adiposity, IR and BP z-scores. To note, we replicated the association between DNA methylation in *ABCG1* and BMI, WC, HOMA-IR and HDL-c z-scores in both study populations. Also, we replicated the association for *DNM3*, *TINAGL1*, *PRKCQ* and *VASN* with adiposity traits and key cardiometabolic health outcomes in the same way. Then, we pooled data from the discovery and validation population I to stratify analysis by pubertal status and sex (**Figure S1 and Table S5**). The resulting lists included new promising loci that had not been reported as epigenetic marks of obesity-related cardiometabolic alterations (e.g. *ESR1* and *VASN*). Among results, DNA methylation at the cg0041083 site mapping to the *VASN* locus emerged as top association, showing a consistent positive associations with BMI (β = 0.40, FDR < 0.001; β = 0.20, p < 0.05) and BP z-scores (β = 0.36, FDR < 0.05; β = 0.19, p < 0.05) in both populations (FDR < 0.05 in discovery population and raw p-value < 0.05 in validation population I) (Fig. 1).

Cross-omics effects of DNA methylation patterns – A eQTM and mQTL analysis

To investigate the mechanistic relevance of identified obesity-related cardiometabolic epigenetic marks, an RNA-seq analysis was performed on a subset of the discovery population including 44 adolescents with and without obesity. In this subpopulation, we searched for cis-eQTMs within ± 10,000 bp of each transcript and trans-eQTMs for CpG sites located beyond this distance. These analyses were conducted using a candidate list of 193 CpG sites derived from epigenome-wide discovery analysis, and 60,058 high-quality transcripts spanning the whole genome.

The cis-eQTM analysis identified 13 CpG-transcript pairs (raw p-value < 0.05) (Table 2). Notably, the most significant associations corresponded to previously validated obesity-related cardiometabolic epigenetic marks (i.e. *FGD4, VIPR2, CEMIP, VASN, ABCG1, ESR1* and *KIAA0513)*. DNA methylation levels were positively correlated with *ESR1* expression and negatively correlated with *VASN* expression. Furthermore, the cis-eQTM CpGs identified for *VASN* were annotated within enhancer regions, based on the 450k enhancer-predicted element list, suggesting a potential transcriptional regulatory role. The trans-eQTM analysis identified 318 FDR-significant CpG-transcript pairs (**Table S6**). All trans-eQTM associations were subjected to only 5 CpG sites (cg10560689-CEMIP, cg27147114-RASGRF1, cg16370441-CDC42BPB, cg24354819-LIN7A, and cg23391907), and affected genes participating in metabolic pathways, G protein-coupled receptor activity and signalling by Rho GTPases [56].

In parallel, a methylation quantitative trait loci (mQTL) analysis was conducted in discovery population and validation population I to investigate if observed eQTM effects are driven by inherited genetic variations (**Table S7**). The mQTL mapping revealed largely distinct sets of SNP–CpG associations, with *HMCN1* emerging as the only common locus with eQTM analysis (FDR < 0.05). This lack of concordance demonstrates that the transcriptional impact of CpG methylation arises independently of genetic determinants for most of the discovered loci.

Table 2
Cis CpG-transcript pairs (distance of 10,000 bp) identified in the eQTM analysis

CpG site	Transcripts	P-value[1]	FDR	Beta
cg03418231_FGD4	ENSG00000139132_FGD4	< 0.001	0.003	-0.85
cg10956605_VIPR2	ENSG00000106018_VIPR2	< 0.001	0.076	1.75
cg25069618_CEMIP	ENSG00000103888_CEMIP	0.001	0.124	1.97
cg00041083_VASN	ENSG00000262246_CORO7	0.007	0.539	-0.88
cg03565996_ABCG1	ENSG00000160179_ABCG1	0.009	0.539	-0.82
cg21608605_ESR1	ENSG00000091831_ESR1	0.012	0.539	0.73
cg02213678_KIAA0513	ENSG00000135709_KIAA0513	0.014	0.539	-0.79
cg09050582_LIPJ	ENSG00000204022_LIPJ	0.014	0.539	-0.82
cg20463298_HMCN1	ENSG00000143341_HMCN1	0.019	0.660	0.59
cg11078674_RGS6	ENSG00000182732_RGS6	0.022	0.701	-1.41
cg00041083_VASN	ENSG00000168140_VASN	0.036	0.938	-0.71
cg12363898_IL17RB	ENSG00000271976	0.038	0.938	-0.40
cg21891499_CLPTM1L	ENSG00000286388	0.039	0.938	-0.69
1 T-test linear model				

As a measure of eQTMs effect size, we reported the beta regressors estimated by the linear model. The p-values from the linear regression analysis were adjusted for multiple comparisons using the Benjamini-Hochberg FDR procedure. These analyses focused on 193 CpG sites and 60,058 high-quality transcripts as input.

VASN as a potential novel biomarker of obesity-associated cardiometabolic dysfunction

To further investigate the clinical role of *VASN* as one of the most promising targets revealed in our study, we measured the serum levels of this protein in our discovery population and validation population I. Descriptive statistics are presented in **Figures S2, S3 and Table S8**. As expected, serum VASN levels

were comparable between the discovery population and validation population I. Notably, a slight increase in VASN levels was observed in the discovery population from pre-puberty to puberty, which may reflect the physical and hormonal changes associated with pubertal development (**Table S8**). In the validation population I, prepubertal children with obesity showed lower serum VASN levels than those with overweight (p-value = 0.05) (**Figure S2**). We then conducted a comprehensive outcome-wide association analysis to assess the potential utility of VASN protein serum levels as a new biomarker of obesity-related cardiometabolic dysfunction. Analyses were performed separately in the discovery population and validation population I, stratified by pubertal stage and sex (Fig. 2A **and Table S9**). Interestingly, we found significant associations in pubertal individuals; higher VASN serum levels linked to a healthier FMI and HDL-c profile in the discovery and validation population I (raw p-value < 0.05; Fig. 2A), respectively.

We also conducted a pooled analysis across both populations, allowing us to further explore sex- and pubertal-specific associations. Consistent with the previous findings, we observed significant associations between higher VASN levels and lower FMI, and a new inverse association with HOMA-IR z-score arose (β = -0.22, raw p-value < 0.05). Sex-stratified analyses showed a stronger association between VASN and FMI in males than in females (β = -0.30, FDR < 0.05; β = -0.10, raw p-value > 0.05; **Fig. 2A** and **Table S9**).

Figure 2. VASN as a potential novel biomarker of obesity-associated cardiometabolic dysfunction. (A) Forest plot displaying the standardized associations between Vasorin (VASN) protein levels and a range of adiposity and cardiometabolic outcomes, stratified by pubertal status (prepubertal vs. pubertal) and study population. Standardized coefficients are plotted on the x-axis, and the various outcomes (e.g., z-scores for body mass index [BMI], waist circumference [WC], fat mass index [FMI], Homeostatic Model Assessment of Insulin Resistance [HOMA-IR], blood pressure [BP], High-Density Lipoprotein Cholesterol [HDL-c]) are listed on the y-axis. Red markers denote statistically significant associations based on a raw p-value < 0.05. No association surpassed the FDR correction in these analyses. The “Pool” panel combines data from both study populations (discovery population and validation population I) to evaluate sex-specific effects, with circles, triangles, and squares corresponding to both sexes, females, and males, respectively. Bars represent 95% confidence intervals, and the vertical dashed line at zero indicates no effect. Results are fully detailed in **Table S9**. (B) Bar plot illustrating the relative mRNA abundance of Vasorin (*VASN)* in visceral adipose tissue (VAT) from children with normal-weight (blue, *n* = 6) and obesity (red, *n* = 7) of validation population II, normalized to the housekeeping gene *HPRT1*. Dots represent individual subjects; bars indicate mean ± SEM. *p* < 0.05 versus the normal-weight group (unpaired two-tailed *t*-test).

VASN gene expression is downregulated in visceral adipose tissue of children with obesity

To further support the relevance of VASN in adipose tissue biology, we assessed its gene expression levels in VAT, a metabolically active tissue with key endocrine functions. This was evaluated in the validation population II, which included 13 prepubertal participants with and without obesity. Consistent

with our DNA methylation findings (FDR < 0.05 and raw p-value < 0.05 in discovery and validation population I, respectively) (Fig. 1), eQTM findings (raw p-value < 0.05; Table 2) and with the direction of serum protein associations (raw p-value < 0.05; **Fig. 2A**), we observed that *VASN* gene expression was significantly down-regulated in the VAT of prepubertal children with obesity compared to normal-weight children (p-value < 0.05) (Fig. 2B).

Discussion

Despite an urgent clinical need, robust molecular biomarkers capable of stratifying metabolic risk among children with obesity remain scarce. Current classification systems rely predominantly on indirect or late-stage indicators of cardiometabolic dysfunction, limiting opportunities for early detection and targeted intervention. In this study, we leveraged two complementary tissue sources (blood and VAT) to generate a multi-omics landscape encompassing genomic, epigenomic, transcriptomic, and protein data in paediatric obesity. Through a multi-omic and cross-tissue design, we identified *VASN* as a novel and consistent biomarker of metabolic health with associations spanning IR, central adiposity, lipid profiles and BP across multiple paediatric populations. Specifically, blood DNA methylation at *VASN* loci was linked to obesity-associated cardiometabolic traits in two independent populations (discovery population and validation population I) and acted as a cis-eQTM modulating *VASN* expression. Notably, higher circulating VASN protein levels were also associated with a more favourable fat distribution and improved insulin sensitivity, particularly in pubertal males, highlighting its potential role as a non-invasive biomarker. Finally, we demonstrated that *VASN* expression is significantly downregulated in VAT in an independent group of prepubertal children with obesity (validation population II), underscoring its relevance in this metabolically active tissue. The convergence of these findings across molecular layers, tissue types, and independent clinical cohorts supports the potential of *VASN* as a biologically meaningful biomarker of paediatric obesity-related metabolic dysfunction.

VASN is a type I transmembrane glycoprotein highly expressed in vascular smooth muscle cells and involved in key signalling pathways such as TGF-β and Notch [57]. Functionally, VASN acts as a decoy receptor for TGF-β1, modulating its signalling activity and contributing to vascular remodelling and angiogenesis [58]. It is also implicated in Notch1 stabilization under hypoxic conditions, a context relevant for adipose tissue expansion [59]. While initially described as a biomarker in hepatic and vascular pathologies, its role in metabolic tissues remains largely unexplored [60–62]. Our data suggest that VASN may exert protective metabolic functions during adipose tissue remodelling in childhood obesity. We hypothesize that *VASN* expression in adipose tissue facilitates a healthier expansion of adipose tissue, favoring hyperplasia over hypertrophy, by promoting angiogenesis and mitigating TGF-β–driven inflammation and fibrosis. Supporting this, we observed downregulation of *VASN* expression in VAT of children with obesity. This aligns with a model in which lower VASN activity contributes to dysfunctional adipose tissue expansion and systemic metabolic risk [63]. Furthermore, our sex-stratified analyses reveal stronger serum VASN–metabolic trait associations in pubertal males, raising the possibility of hormone-sensitive regulation or sex-specific compensatory mechanisms during puberty.

While these findings underscore the potential of VASN as a modulator of adipose tissue biology, the cellular source of circulating VASN in our study remains to be fully clarified. The observed transcriptional downregulation of *VASN* in VAT of children with obesity suggests that this depot may contribute to systemic levels; however, our study design does not allow for a definitive attribution of the origin of blood levels. Despite this limitation, VASN is a secreted protein detectable in serum, highlighting its translational potential [64]. Taken together, our findings nominate VASN as a biologically plausible modulator of metabolic health in paediatric obesity, potentially acting at the intersection of vascular function, inflammatory tone, and adipose tissue architecture [65].

The strongest associations between circulating VASN protein and metabolic traits in our studies were observed during puberty, a critical developmental window characterized by profound hormonal and physiological remodelling across the cardiovascular system. This period is also marked by the emergence of sex-specific differences driven by the distinct effects of sex hormones on vascular and metabolic physiology [66]. The associations between VASN and metabolic traits in pubertal children suggest that VASN may be linked to sex hormone–dependent mechanisms underlying obesity-related vascular adaptation to metabolic stress. In line with this, we observed a sex-specific association between circulating VASN protein levels and FMI, which remained FDR-significant only in males. This male-specific pattern may reflect the more pronounced anabolic effect of testosterone in the cardiovascular remodelling during puberty [66]. This hypothesis is further supported by findings from in-vivo studies with mices that evidenced the role of VASN in cardiac remodelling and function [67, 68]. These findings reinforce the relevance of VASN as a biomarker precisely during this sensitive developmental window, when early identification of at-risk individuals may yield the greatest long-term benefit. Beyond its biomarker potential, our findings also contribute to a deeper understanding of the molecular mechanisms underlying paediatric obesity and its cardiometabolic complications [69].

Interestingly, emerging evidence suggests that VASN may play a broader role extending beyond paediatric obesity-related cardiometabolic dysfunction and into the maintenance of cardiometabolic health during aging. In a multi-omics study of a female supercentenarian, hypomethylation at a distinct *VASN* CpG site was associated with higher *VASN* expression, suggesting its candidacy as an epigenetic biomarker of healthy aging [70]. In parallel, a large multi-cohort study developing a proteomic frailty score reported that low circulating VASN levels showed strong predictive performance for 199 incident diseases across 13 disease categories [71]. Together, these findings raise the possibility that VASN may represent a molecular link between cardiometabolic resilience, vascular homeostasis and healthy aging across the life course. Further studies are warranted to clarify the mechanistic and clinical relevance of VASN in longevity and age-related cardiometabolic health.

This study has some limitations. First, the modest sample size, especially for the validation population II, which included gene expression in VAT in paediatric participants. Nonetheless, the inclusion of multiple independent cohorts with blood and VAT enhanced resolution for detecting metabolic subphenotypes within this high-risk group. Second, all participants were of Caucasian ancestry, which limits the generalizability of our findings to other ethnicities. However, this genetic homogeneity may have

contributed to reducing population and phenotypic variability. Third, the observational nature of our study and the lack of accompanying mechanistic *in vitro* experiments preclude conclusions about any causal role of VASN in metabolic alterations. Fourth, we were unable to explore whether hypermethylation of *VASN* triggers lower gene expression in VAT because DNA methylation was not measured in that tissue. Similarly, we identified consistent associations between serum VASN and cardiometabolic health traits, but we could not determine the tissue of origin of circulating VASN levels. Despite these limitations, our findings support the biological plausibility of VASN consistently across multiple molecular layers (DNA methylation, gene expression, and protein levels), across tissues (blood and VAT), and across independent cohorts (three populations). This convergence of evidence across complementary biological dimensions reduces the likelihood that the observed signals represent isolated statistical artifacts. Our integration of blood multi-omic layers (i.e. genomics, epigenomic and transcriptomic) and serum protein levels offers valuable biological insight and testable hypotheses for future analyses. VASN signalling has been proposed as a potential therapeutic target to counteract aging-related cardiovascular remodelling and reduce CVD risk [63]. Our blood multi-omics analysis highlights the serum VASN levels as a novel biomarker of cardiometabolic health in paediatric obesity. Future randomized controlled trials and mechanistic investigations, to elucidate the tissue-specific regulation, are warranted to clarify the causal and potential clinical implications of our findings.

Conclusion

Our study identifies circulating VASN levels as a novel and biologically plausible biomarker of metabolic health, with consistent multi-omic associations across independent populations and strongest effects emerging during puberty. By uncovering links between VASN and key cardiometabolic risk factors (i.e. IR, central adiposity and lipid profiles), we not only highlight its potential as a non-invasive biomarker, but also shed light on underexplored molecular pathways involved in adipose tissue remodelling and systemic metabolic regulation. Its reproducible associations with cardiometabolic risk factors across molecular layers, populations and tissues support its potential utility for early risk stratification in precision prevention strategies.

Declarations

Author Contribution

A.T.M, A.A.R, FJ.R.O and M.B.A: Data curation; formal analysis; investigation; methodology; software; visualisation; roles/writing—original draft; and writing—review and editing. A.G, M.G.C, G.B, R.L, J.A.F and CM. A: Conceptualisation; funding acquisition; methodology; project administration; resources; supervision; validation; and writing—review and editing. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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Data Availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author (augusto.anguita@isglobal.org) upon reasonable request. Data is located in controlled access data storage at the University of Granada.

Code availability

To promote open access and reproducibility, the code is publicly available on Github: https://github.com/AlvaroTorresMartos/Multiomics_VASN.

References

1. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol. 2019;15:288–98. https://doi.org/10.1038/s41574-019-0176-8.
2. Jebeile H, Kelly AS, O’Malley G, Baur LA. Obesity in children and adolescents: epidemiology, causes, assessment, and management. Lancet Diabetes Endocrinol. 2022;10:351–65. https://doi.org/10.1016/S2213-8587(22)00047-X.
3. NCD Risk Factor Collaboration (NCD-RisC), Zhou B, Phelps NH, Galeazzi A, O’Driscoll ON, Bennett JE, et al. Obesity rise plateaus in developed nations and accelerates in developing nations. Nature. 2026;653:510–8. https://doi.org/10.1038/s41586-026-10383-0.
4. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet. 2017;390:2627–42. https://doi.org/10.1016/s0140-6736(17)32129-3.
5. Ng M, Gakidou E, Lo J, Abate YH, Abbafati C, Abbas N, et al. Global, regional, and national prevalence of adult overweight and obesity, 1990–2021, with forecasts to 2050: a forecasting study for the

Global Burden of Disease Study 2021. Lancet. 2025;405:813–38. https://doi.org/10.1016/s0140-6736(25)00355-1.

6. Jacobs DR, Woo JG, Sinaiko AR, Daniels SR, Ikonen J, Juonala M, et al. Childhood Cardiovascular Risk Factors and Adult Cardiovascular Events. N Engl J Med. 2022;386:1877–88. https://doi.org/10.1056/nejmoa2109191.
7. Luk A, Wild SH, Jones S, Anjana RM, Hivert M-F, McCaffrey J, et al. Early-onset type 2 diabetes: the next major diabetes transition. Lancet. 2025;405:2313–26. https://doi.org/10.1016/S0140-6736(25)00830-X.
8. Misra S, Khunti K, Goyal A, Gable D, Armocida B, Tandon N, et al. Managing early-onset type 2 diabetes in the individual and at the population level. Lancet. 2025;405:2341–54. https://doi.org/10.1016/S0140-6736(25)01067-0.
9. Magge SN, Goodman E, Armstrong SC, COMMITTEE ON NUTRITION, SECTION ON ENDOCRINOLOGY, SECTION ON OBESITY, et al. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. Pediatrics. 2017;140:e20171603. https://doi.org/10.1542/peds.2017-1603.
10. Powell-Wiley TM, Poirier P, Burke LE, Després J-P, Gordon-Larsen P, Lavie CJ, et al. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 2021;143:e984–1010. https://doi.org/10.1161/cir.0000000000000973.
11. Noubiap JJ, Nansseu JR, Lontchi-Yimagou E, Nkeck JR, Nyaga UF, Ngouo AT, et al. Global, regional, and country estimates of metabolic syndrome burden in children and adolescents in 2020: a systematic review and modelling analysis. Lancet Child Adolesc Health. 2022;6:158–70. https://doi.org/10.1016/s2352-4642(21)00374-6.
12. Lim L-L, Jones S, Cikomola JC, Hivert M-F, Misra S. Understanding the drivers and consequences of early-onset type 2 diabetes. Lancet. 2025;405:2327–40. https://doi.org/10.1016/S0140-6736(25)01012-8.
13. Prince RL, Kuk JL, Ambler KA, Dhaliwal J, Ball GDC. Predictors of Metabolically Healthy Obesity in Children. Diabetes Care. 2014;37:1462–8. https://doi.org/10.2337/dc13-1697.
14. Bervoets L, Massa G. Classification and clinical characterization of metabolically healthy obese children and adolescents. J Pediatr Endocrinol Metab 2016;29. https://doi.org/10.1515/jpem-2015-0395
15. Plaza-Florido A, Altmäe S, Esteban FJ, Cadenas-Sanchez C, Aguilera CM, Einarsdottir E, et al. Distinct whole-blood transcriptome profile of children with metabolic healthy overweight/obesity compared to metabolic unhealthy overweight/obesity. Pediatr Res. 2021;89:1687–94. https://doi.org/10.1038/s41390-020-01276-7.
16. Stefan N, Häring H-U, Schulze MB. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? Lancet Diabetes Endocrinol. 2018;6:249–58. https://doi.org/10.1016/s2213-8587(17)30292-9.

17. Blüher M. Metabolically Healthy Obesity. Endocr Rev. 2020;41:bnaa004. https://doi.org/10.1210/endrev/bnaa004.
18. Iacobini C, Pugliese G, Blasetti Fantauzzi C, Federici M, Menini S. Metabolically healthy versus metabolically unhealthy obesity. Metabolism. 2019;92:51–60. https://doi.org/10.1016/j.metabol.2018.11.009.
19. Christiansen C, Tomlinson M, Eliot M, Nilsson E, Costeira R, Xia Y, et al. Adipose methylome integrative-omic analyses reveal genetic and dietary metabolic health drivers and insulin resistance classifiers. Genome Med. 2022;14:75. https://doi.org/10.1186/s13073-022-01077-z.
20. Aleksandrova K, Egea Rodrigues C, Floegel A, Ahrens W. Omics Biomarkers in Obesity: Novel Etiological Insights and Targets for Precision Prevention. Curr Obes Rep. 2020;9:219–30. https://doi.org/10.1007/s13679-020-00393-y.
21. Paczkowska-Abdulsalam M, Kretowski A. Obesity, metabolic health and omics: Current status and future directions. WJD. 2021;12:420–36. https://doi.org/10.4239/wjd.v12.i4.420.
22. Buergel T, Steinfeldt J, Ruyoga G, Pietzner M, Bizzarri D, Vojinovic D, et al. Metabolomic profiles predict individual multidisease outcomes. Nat Med. 2022;28:2309–20. https://doi.org/10.1038/s41591-022-01980-3.
23. Elhadad MA, Jonasson C, Huth C, Wilson R, Gieger C, Matias P, et al. Deciphering the Plasma Proteome of Type 2 Diabetes. Diabetes. 2020;69:2766–78. https://doi.org/10.2337/db20-0296.
24. Doumatey AP, Shriner D, Zhou J, Lei L, Chen G, Oluwasola-Taiwo O, et al. Untargeted metabolomic profiling reveals molecular signatures associated with type 2 diabetes in Nigerians. Genome Med. 2024;16:38. https://doi.org/10.1186/s13073-024-01308-5.
25. Miras-Moreno S, Torres-Martos Á, Ruiz JR, Carter J, Abreu De Carvalho C, Aguilera CM et al. Metabolomic and Proteomic Signatures of Cardiorespiratory Fitness for Predicting All-Cause Mortality and Non-Communicable Disease Risk: A Prospective Study in the UK Biobank. Circ: Genomic and Precision Medicine. 2026:e005736. https://doi.org/10.1161/CIRCGEN.125.005736
26. Zhang X, Yan W, Fan X, Zhang P, Gao Y, Wang Y, et al. Proteomic health archetypes identified in disease-free adults enable risk assessment for diverse chronic diseases. Genome Med. 2026. https://doi.org/10.1186/s13073-026-01696-w.
27. Anguita-Ruiz A, Mendez-Gutierrez A, Ruperez AI, Leis R, Bueno G, Gil-Campos M, et al. The protein S100A4 as a novel marker of insulin resistance in prepubertal and pubertal children with obesity. Metabolism. 2020;105:154187. https://doi.org/10.1016/j.metabol.2020.154187.
28. Ruiz-Ojeda FJ, Anguita-Ruiz A, Rico MC, Leis R, Bueno G, Moreno LA, et al. Serum levels of the novel adipokine isthmin-1 are associated with obesity in pubertal boys. World J Pediatr. 2023;19:864–72. https://doi.org/10.1007/s12519-022-00665-8.
29. Drouard G, Hagenbeek FA, Whipp AM, Pool R, Hottenga JJ, Jansen R, et al. Longitudinal multi-omics study reveals common etiology underlying association between plasma proteome and BMI trajectories in adolescent and young adult twins. BMC Med. 2023;21:508. https://doi.org/10.1186/s12916-023-03198-7.

30. Rubino F, Cummings DE, Eckel RH, Cohen RV, Wilding JPH, Brown WA, et al. Definition and diagnostic criteria of clinical obesity. Lancet Diabetes Endocrinol. 2025;13:221–62. https://doi.org/10.1016/s2213-8587(24)00316-4.
31. Guglielmi G. New obesity definition sidelines BMI to focus on health. Nature. 2025;637:773–4. https://doi.org/10.1038/d41586-025-00123-1.
32. De Lamas C, Kalén A, Anguita-Ruiz A, Pérez-Ferreirós A, Picáns-Leis R, Flores K, et al. Progression of metabolic syndrome and associated cardiometabolic risk factors from prepuberty to puberty in children: The PUBMEP study. Front Endocrinol. 2022;13. https://doi.org/10.3389/fendo.2022.1082684.
33. González-Gil EM, Anguita-Ruiz A, Kalén A, De Las Lamas Perez C, Rupérez AI, Vázquez-Cobela R, et al. Longitudinal associations between cardiovascular biomarkers and metabolic syndrome during puberty: the PUBMEP study. Eur J Pediatr. 2022;182:419–29. https://doi.org/10.1007/s00431-022-04702-6.
34. Anguita-Ruiz A, Torres-Martos A, Ruiz-Ojeda F, Alcalá-Fdez J, Bueno G, Gil-Campos M et al. Integrative analysis of blood cells DNA methylation, transcriptomics and genomics identifies novel epigenetic regulatory mechanisms of insulin resistance during puberty in children with obesity. medRxiv 2022:2022.12.13.22283415. https://doi.org/10.1101/2022.12.13.22283415
35. Anguita-Ruiz A, Torres-Martos Á, Bustos-Aibar M, Setó-Llorens A, Ruiz-Ojeda FJ, Moreno LA, et al. Novel epigenetic marks of insulin resistance trajectories in a longitudinal study of childhood obesity. Cardiovasc Diabetol. 2026;25:105. https://doi.org/10.1186/s12933-026-03101-7.
36. Torres-Martos Á, Requena F, López-Rodríguez G, Hernández-Cabrera J, Galván M, Solís-Pérez E, et al. ObMetric: A Shiny app to assist in metabolic syndrome assessment in paediatric obesity. Pediatr Obes. 2025;20:e70016. https://doi.org/10.1111/ijpo.70016.
37. Aguilera C, Gomez-Llorente C, Tofe I, Gil-Campos M, Cañete R, Gil Á. Genome-Wide Expression in Visceral Adipose Tissue from Obese Prepubertal Children. Int J Mol Sci. 2015;16:7723–37. https://doi.org/10.3390/ijms16047723.
38. Torres-Martos Á, Anguita-Ruiz A, Bustos-Aibar M, Ramírez-Mena A, Arteaga M, Bueno G, et al. Multiomics and eXplainable artificial intelligence for decision support in insulin resistance early diagnosis: A pediatric population-based longitudinal study. Artif Intell Med. 2024;156. https://doi.org/10.1016/j.artmed.2024.102962.
39. Stratakis N, Anguita-Ruiz A, Fabbri L, Maitre L, González JR, Andrusaityte S, et al. Multi-omics architecture of childhood obesity and metabolic dysfunction uncovers biological pathways and prenatal determinants. Nat Commun. 2025;16:654. https://doi.org/10.1038/s41467-025-56013-7.
40. Wang J, Zenere A, Wang X, Bergström G, Edfors F, Uhlén M, et al. Longitudinal analysis of genetic and environmental interplay in human metabolic profiles and the implication for metabolic health. Genome Med. 2025;17:68. https://doi.org/10.1186/s13073-025-01492-y.
41. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51:170–9.

https://doi.org/10.1136/adc.51.3.170.

42. Sobradillo B, Aguirre A, Aresti U, Bilbao A, Fernández-Ramos C, Lizárraga A, et al. Curvas y Tablas de Crecimiento (Estudios Longitudinal y Transversal). Instituto de Investigación sobre Crecimiento y Desarrollo. Fundación Faustino Orbegozo Eizaguirre; 2004.
43. Ferrández A. Curvas y Tablas de Crecimiento (Estudios Longitudinal y Transversal). Fundación Andrea Prader; 2005.
44. Cole TJ, Bellizzi M, Flegal K, Dietz W. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320:1240–3. https://doi.org/10.1136/bmj.320.7244.1240.
45. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012;7:284–94. https://doi.org/10.1111/j.2047-6310.2012.00064.x.
46. Muntner P, Shimbo D, Carey RM, Charleston JB, Gaillard T, Misra S, et al. Measurement of Blood Pressure in Humans: A Scientific Statement From the American Heart Association. Hypertension. 2019;73:e35–66. https://doi.org/10.1161/HYP.0000000000000087.
47. Growth and Development at Adolescence. Endokrinologie der Entwicklung und Reifung. Berlin, Heidelberg: Springer Berlin Heidelberg; 1970. pp. 117–30. https://doi.org/10.1007/978-3-642-80591-2_14.
48. Stavnsbo M, Resaland GK, Anderssen SA, Steene-Johannessen J, Domazet SL, Skrede T, et al. Reference values for cardiometabolic risk scores in children and adolescents: Suggesting a common standard. Atherosclerosis. 2018;278:299–306. https://doi.org/10.1016/j.atherosclerosis.2018.10.003.
49. Ahrens W, Moreno LA, Mårild S, Molnár D, Siani A, De Henauw S, et al. Metabolic syndrome in young children: definitions and results of the IDEFICS study. Int J Obes. 2014;38:S4–14. https://doi.org/10.1038/ijo.2014.130.
50. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am J Hum Genet. 2007;81:559–75. https://doi.org/10.1086/519795.
51. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO et al. Twelve years of SAMtools and BCFtools. GigaScience. 2021;10:giab008. https://doi.org/10.1093/gigascience/giab008
52. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014;30:923–30. https://doi.org/10.1093/bioinformatics/btt656.
53. Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, et al. Software for Computing and Annotating Genomic Ranges. PLoS Comput Biol. 2013;9:e1003118. https://doi.org/10.1371/journal.pcbi.1003118.
54. Torres-Martos Á, Bustos-Aibar M, Ramírez-Mena A, Cámara-Sánchez S, Anguita-Ruiz A, Alcalá R, et al. Omics Data Preprocessing for Machine Learning: A Case Study in Childhood Obesity. Genes. 2023;14:248. https://doi.org/10.3390/genes14020248.

55. Shabalin AA. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. Bioinformatics. 2012;28:1353–8. https://doi.org/10.1093/bioinformatics/bts163.
56. Garcia-Moreno A, López-Domínguez R, Villatoro-García JA, Ramirez-Mena A, Aparicio-Puerta E, Hackenberg M, et al. Funct Enrich Anal Regul Elem Biomedicines. 2022;10:590. https://doi.org/10.3390/biomedicines10030590.
57. Ikeda Y, Imai Y, Kumagai H, Nosaka T, Morikawa Y, Hisaoka T, et al. Vasorin, a transforming growth factor β-binding protein expressed in vascular smooth muscle cells, modulates the arterial response to injury *in vivo*. Proc Natl Acad Sci USA. 2004;101:10732–7. https://doi.org/10.1073/pnas.0404117101.
58. Murugesan S, Addis DR, Hussey H, Powell MF, Saravanakumar L, Sturdivant AB et al. Decreased Extracellular Vesicle Vasorin in Severe Preeclampsia Plasma Mediates Endothelial Dysfunction. JAHA 2025;14:e037242. https://doi.org/10.1161/JAHA.124.037242
59. Man J, Yu X, Huang H, Zhou W, Xiang C, Huang H, et al. Hypoxic Induction of Vasorin Regulates Notch1 Turnover to Maintain Glioma Stem-like Cells. Cell Stem Cell. 2018;22:104–e1186. https://doi.org/10.1016/j.stem.2017.10.005.
60. Li S, Li H, Yang X, Wang W, Huang A, Li J, et al. Vasorin is a potential serum biomarker and drug target of hepatocarcinoma screened by subtractive-EMSA-SELEX to clinic patient serum. Oncotarget. 2015;6:10045–59. https://doi.org/10.18632/oncotarget.3541.
61. Wan F, Li H, Huang S, Sun J, Li J, Li Y, et al. Vasorin promotes proliferation and migration via STAT3 signaling and acts as a promising therapeutic target of hepatocellular carcinoma. Cell Signal. 2023;110:110809. https://doi.org/10.1016/j.cellsig.2023.110809.
62. Keyif B, Yurtçu E, Başbuğ A, Yavuzcan A, Goynumer FG. An Exploratory Study of Serum Vasorin Levels in Polycystic Ovary Syndrome: A Novel Potential Biomarker for Diagnosis and Pathogenesis. Metabolites. 2025;15:182. https://doi.org/10.3390/metabo15030182.
63. Wang M, McGraw KR, Monticone RE, Giordo R, Eid AH, Pintus G. Enhanced vasorin signaling mitigates adverse cardiovascular remodeling. Aging Med. 2024;7:414–23. https://doi.org/10.1002/agm2.12332.
64. Pischon T, Nimptsch K. Blood-based obesity biomarkers and their relevance for disease risk. Nat Rev Endocrinol. 2026;22:338–55. https://doi.org/10.1038/s41574-025-01229-2.
65. Cartwright BR, Scherer PE. Adipose Tissue as a Target for Precision Medicine Approaches in Childhood Obesity. Diabetes. 2025;74:1710–9. https://doi.org/10.2337/dbi24-0044.
66. González Represas A. Development of the Cardiovascular System During Adolescence. In: Xu M, Xiao J, editors. Enhancing Adolescent Health. Volume 1505. Singapore: Springer Nature Singapore; 2026. pp. 47–66. https://doi.org/10.1007/978-981-95-7000-3_3.
67. Sun J, Guo X, Yu P, Liang J, Mo Z, Zhang M, et al. Vasorin deficiency leads to cardiac hypertrophy by targeting MYL7 in young mice. J Cell Mol Medi. 2022;26:88–98. https://doi.org/10.1111/jcmm.17034.

68. Sun J, Yin S, Li Q, Zhang J, Guo X, Yu N, et al. VASN knockout induces myocardial fibrosis in mice by downregulating non-collagen fibers and promoting inflammation. Front Pharmacol. 2025;15:1500617. https://doi.org/10.3389/fphar.2024.1500617.
69. Palacios-Marin I, Serra D, Jiménez-Chillarón JC, Herrero L, Todorčević M. Childhood obesity: Implications on adipose tissue dynamics and metabolic health. Obes Rev. 2023;24:e13627. https://doi.org/10.1111/obr.13627.
70. Santos-Pujol E, Noguera-Castells A, Casado-Pelaez M, García-Prieto CA, Vasallo C, Campillo-Marcos I, et al. The multiomics blueprint of the individual with the most extreme lifespan. Cell Rep Med. 2025;6:102368. https://doi.org/10.1016/j.xcrm.2025.102368.
71. Jia X, Gao W, Hagelin H, Zhao Y, Zhang J, Cao X, et al. Plasma proteomic signature of frailty in 50,506 adults. Cell Metabol. 2026;38:1012–e10286. https://doi.org/10.1016/j.cmet.2026.02.013.

Figures

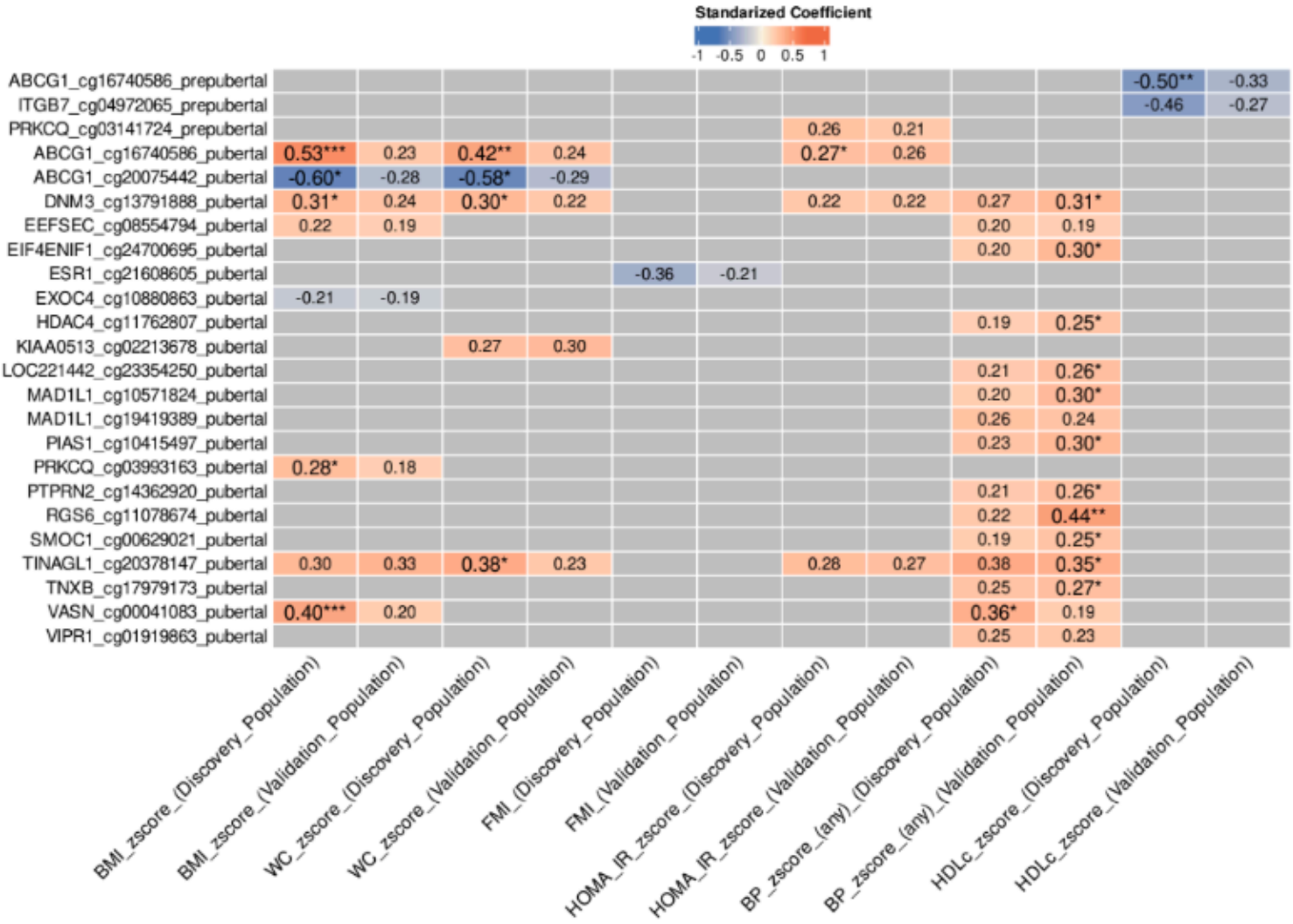


Figure 1

DNA methylation marks as potential epigenetic biomarkers of obesity-associated cardiometabolic dysfunction. Heatmap displays consistent and significant associations between DNA methylation levels at selected CpG sites and cardiometabolic risk outcomes in both discovery populations and validation population I. Only CpG–outcome pairs reaching significance (raw p < 0.05) in both populations are shown. The colour scale represents the standardized regression coefficients, with red indicating positive associations and blue indicating negative associations. CpG–outcome pairs that did not reach significance in both populations are displayed in gray. Significance thresholds are denoted as follows: FDR < 0.05 (*), FDR < 0.01 (**), and FDR < 0.001 (***). Results are fully detailed in **Table S5**.

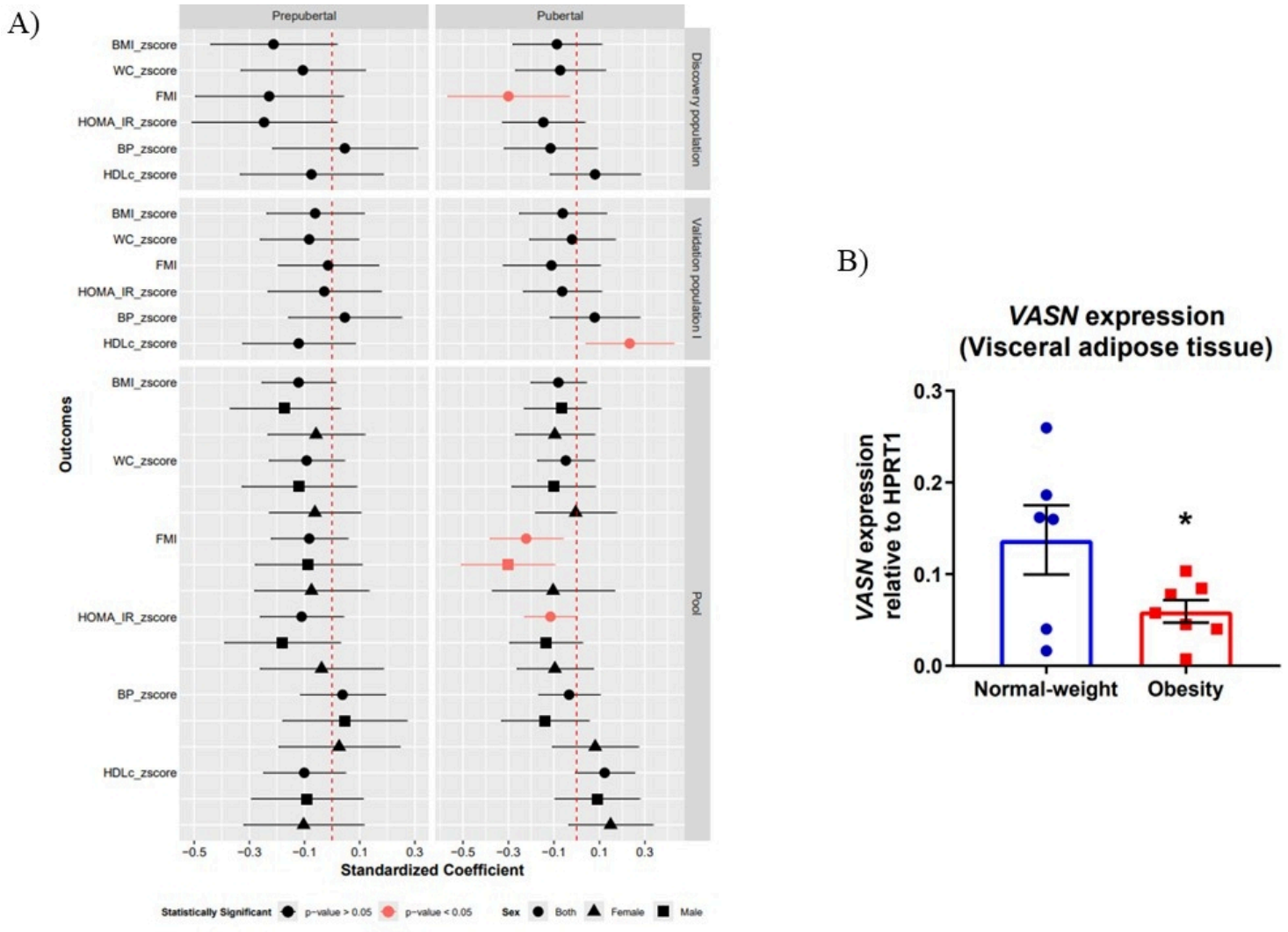


Figure 2

VASN as a potential novel biomarker of obesity-associated cardiometabolic dysfunction. (A) Forest plot displaying the standardized associations between Vasorin (VASN) protein levels and a range of adiposity and cardiometabolic outcomes, stratified by pubertal status (prepubertal vs. pubertal) and study population. Standardized coefficients are plotted on the x-axis, and the various outcomes (e.g., z-scores for body mass index [BMI], waist circumference [WC], fat mass index [FMI], Homeostatic Model Assessment of Insulin Resistance [HOMA-IR], blood pressure [BP], High-Density Lipoprotein Cholesterol

[HDL-c]) are listed on the y-axis. Red markers denote statistically significant associations based on a raw p-value < 0.05. No association surpassed the FDR correction in these analyses. The “Pool” panel combines data from both study populations (discovery population and validation population I) to evaluate sex-specific effects, with circles, triangles, and squares corresponding to both sexes, females, and males, respectively. Bars represent 95% confidence intervals, and the vertical dashed line at zero indicates no effect. Results are fully detailed in **Table S9**. (B) Bar plot illustrating the relative mRNA abundance of Vasorin (*VASN)* in visceral adipose tissue (VAT) from children with normal-weight (blue, n= 6) and obesity (red, n = 7) of validation population II, normalized to the housekeeping gene *HPRT1*. Dots represent individual subjects; bars indicate mean ± SEM. $p < 0.05$ versus the normal-weight group (unpaired two-tailed *t*-test).

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