Received: 8 October 2021 | Accepted: 11 October 2021

DOI: 10.1111/obr.13389

SUPPLEMENT ARTICLE

OBESITY Reviews WILEY

Perspectives and challenges of epigenetic determinants of childhood obesity: A systematic review

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Funding information
Horizon 2020 Framework Programme, Grant/Award Number: 774548; Science and Technology in childhood Obesity Policy; Bijzonder Onderzoeksfonds UGent

Summary

The tremendous increase in childhood obesity prevalence over the last few decades cannot merely be explained by genetics and evolutionary changes in the genome, implying that gene–environment interactions, such as epigenetic modifications, likely play a major role. This systematic review aims to summarize the evidence of the association between epigenetics and childhood obesity. A literature search was performed via PubMed and Scopus engines using a combination of terms related to epigenetics and pediatric obesity. Articles studying the association between epigenetic mechanisms (including DNA methylation and hydroxymethylation, non-coding RNAs, and chromatin and histones modification) and obesity and/or overweight (or any related anthropometric parameters) in children (0–18 years) were included. The risk of bias was assessed with a modified Newcastle–Ottawa scale for non-randomized studies. One hundred twenty-one studies explored epigenetic changes related to childhood obesity. DNA methylation was the most widely investigated mechanism ($N = 101$ studies), followed by non-coding RNAs ($N = 19$ studies) with evidence suggestive of an association with childhood obesity for DNA methylation of specific genes and microRNAs (miRNAs). One study, focusing on histones modification, was identified. Heterogeneity of findings may have hindered more insights into the epigenetic changes related to childhood obesity. Gaps and challenges that future research should face are herein described.

KEYWORDS
epigenetics, pediatric obesity, STOP project, systematic review

1 | INTRODUCTION

An estimated 40 million children under the age of 5 and 340 million children and adolescents aged 5 to 19 years were affected by overweight or obesity in 2016.[1] This global epidemic of childhood overweight and obesity poses serious threats to health from childhood throughout the entire life course.

Childhood overweight and obesity are the direct consequence of sustained positive energy balance, which finds its proximal determinants in the complex interplay between genetic makeup,

Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; DMR, differentially methylated region; EWASs, epigenome-wide association studies; *HDAC4*, histone deacetylase 4; *HIF3A*, hypoxia-inducible factor-3 alpha; *IGF2*, insulin-like growth factor 2; IOTF, International Obesity Task Force; *KLF13*, Kruppel-like factor 13; *LEP*, leptin; *LINE-1*, long interspersed nucleotide element 1; *MEST*, mesoderm-specific transcript homolog protein; miRNA, microRNA; NOS, Newcastle–Ottawa scale; PECO, Population, Exposure, Comparators, and Outcomes; *PRDM16*, PR domain containing 16; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; *PRLHR*, prolactin-releasing hormone receptor; *RPH3AL*, Rabphilin 3A Like; *TNXB*, tenascin XB; WHO, World Health Organization.

lifestyle factors, obesogenic environment, and social determinants.[2] Despite studies documenting childhood obesity heritability, the tremendous increase in the prevalence of obesity in children over the last few decades cannot merely be explained by evolutionary changes in the genome, implying that gene–environment interaction likely drives the childhood obesity epidemic.[2–5] Mitotically inheritable changes in gene function not explained by changes in the DNA sequence, referred to as epigenetics, are one of the primary molecular mechanisms underlying gene–environment interaction. It has been hypothesized that, during development, epigenetics is particularly vulnerable to modifications in response to environmental influences and may program developmental and metabolic pathways towards obesity.[6–11] Epigenetics includes as major mechanisms: DNA modification (DNA methylation and hydroxymethylation), non-coding RNAs gene regulation, and chromatin and histones modification. These mechanisms dynamically interplay, with changes at one epigenetic layer reflecting the others, although it is unclear if they follow a hierarchical order.[12] Moreover, epigenetics may have a role in childhood obesity etiology,[10] but epigenetic modifications may also be a consequence of metabolic changes related to obesity.[13]

Previous reviews summarizing the evidence of epigenetic changes related to childhood obesity were narrative,[14–17] or systematic but focused on one single epigenetic mechanism[12] and body size changes in early life (in children under 12 years of age).[13] No systematic review of multiple epigenetic mechanisms in association with childhood obesity has been performed. Therefore, this systematic review aims to summarize the epigenetic changes related to obesity in children. A greater understanding of the mechanisms underpinning childhood obesity may contribute to informing the development of new interventions and treatments to prevent childhood obesity.

2 | METHODS

This registered systematic review (CRD42020207282) was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[18]

2.1 | Search strategy

The literature search was performed via PubMed and Scopus engines using a combination of words related to children, obesity, and epigenetics as detailed in Table S1. The last search was run on January 4, 2021. Manual search, using the snowballing technique, was performed to retrieve additional relevant references. References of the identified articles were imported and duplicates were removed using Revtool package in R. Two researchers (RA and MP) independently screened titles and abstracts and assessed eligibility based on full text of the articles, with disagreements resolved by the involvement of a third independent researcher (OR).

2.2 | Eligibility criteria

Eligibility criteria were defined, following the Population, Exposure, Comparators, and Outcomes (PECO) statement components, as follows: (P) the study population is made up of human children; (E and O) the association between epigenetics (including DNA methylation and hydroxymethylation, non-coding RNAs, and chromatin and histones modification) measured in children and obesity and/or overweight (and/or anthropometric parameters related to obesity and/or overweight including body mass index [BMI], weight, waist circumference, adiposity, fat mass, waist-to-hip ratio, and weight gain) in childhood is analyzed; and (C) the levels of the epigenetic markers are compared between children with or without obesity and/or overweight (or with continuous and/or categorical variation of anthropometric parameters). Additional eligibility criteria were as follows: (1) the paper's full text is available, (2) the paper is written in English, (3) the paper is an original article published in a peer-reviewed journal, (4) the data described in the paper have not been described by another article included in the systematic review (the most detailed and recent paper is preferred for inclusion), (5) the paper's study design is observational or experimental, and (6) the paper is not an in vitro or in silico study.

2.3 | Data extraction

Two researchers (RA and MP) independently extracted from identified articles the following information: study authors, year, country, study design, study population characteristics and size, details on anthropometric parameters and epigenetic marker, main findings, confounders, and validation or replication of the findings. A summary table has been structured by epigenetic measures, within which studies are ordered by the most recent publication placing the untargeted studies at the beginning, to increase the prominence of the most trustworthy evidence.

2.4 | Risk of bias

As no assessment tool has been validated for molecular epidemiology studies, the risk of bias of included studies was assessed with a modified Newcastle–Ottawa scale (NOS) for non-randomized studies (Table S2), considering as low bias articles awarding six or more stars.

3 | RESULTS

3.1 | DNA methylation

As depicted in Figure 1, the systematic review included 101 studies on DNA methylation, among which 23 investigated DNA methylation using an epigenome-wide approach, 17 studied repetitive elements and imprinted regions, 5 investigated epigenetic age acceleration, and 60 studied specific candidates (Table S3).

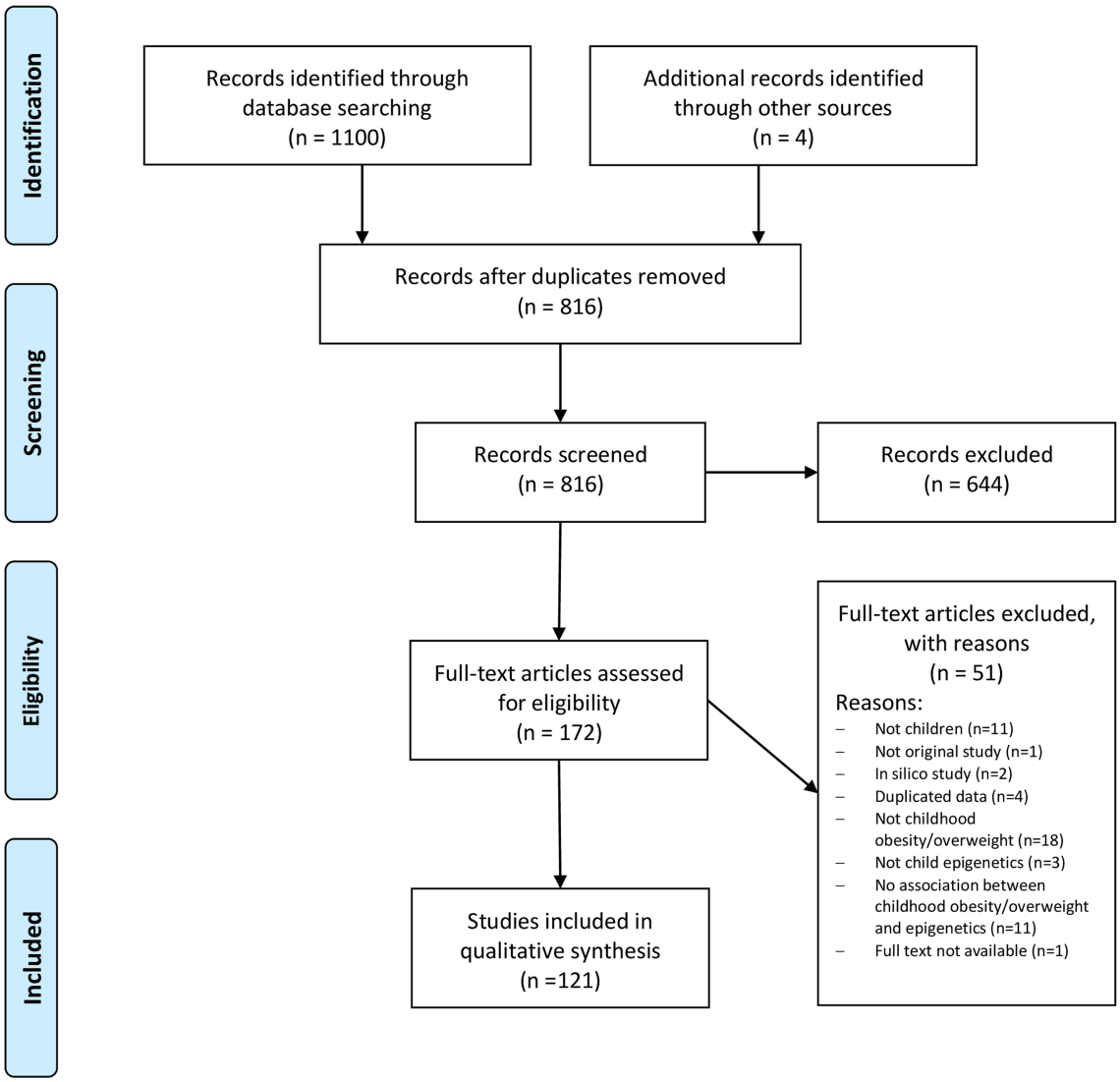


FIGURE 1 Flow diagram of the study selection, following the PRISMA statement guidelines. *N*, number

3.1.1 | Epigenome-wide association studies

Methylation was analyzed with different techniques in the 23 epigenome-wide association studies (EWASs). Illumina arrays were the most widely used technique (*N* studies = 17 out of 23 studies), while a few studies used enzyme digestion-based tagging assay,[19,20] reduced representation bisulfite sequencing,[21] chromatin immunoprecipitation combined with DNA microarray,[22] targeted bisulfite sequencing,[23] and methylated DNA immunoprecipitation sequencing.[24] Peripheral blood was the most analyzed sample type (*N* = 15 studies), while a few studies used cord blood (*N* = 4 studies),[22,25–27] saliva or buccal cells (*N* = 4 studies),[23,28–30] neonatal blood spots samples (*N* = 1 study),[31] and placenta (*N* = 1 study).[32] The sample size of studied populations varied from 7 to 4133 children, which in most cases were European (*N* = 11) or from North America (*N* = 8), with low representation of other countries (*N* = 2 studies took place in Australia and 2 in China). Case–control was the most widely used design (*N* = 10 studies), followed by cross-sectional (*N* = 6 studies) and longitudinal (*N* = 6 studies). More than half of the studies (*N* = 12) considered important confounders (age, sex, and white blood cell composition) in the analyses. Overall, 19 studies out of 23 found an association between childhood anthropometric parameters with at least one methylated CpG site or region (no significant epigenome-wide association at all was found in just four studies[21,30,33,34]). The main outcomes investigated included obesity (*N* = 10), BMI (*N* = 9), skinfold thickness (*N* = 1), and fat mass (*N* = 2) in children over 1 year, while one study examined catch-up growth at 12 months.[26] All the studies, except one,[35] were rated at low risk of bias.

Overall, methylation at only four genes, histone deacetylase 4 (*HDAC4*), prolactin-releasing hormone receptor (*PRLHR*), tenascin XB (*TNXB*) genes, and PR domain containing 16 (*PRDM16*), was associated with obesity-related outcomes by more than one EWAS study.

3.1.2 | Repetitive elements and imprinted regions

Three studies analyzed long interspersed nucleotide element 1 (*LINE-1*). Two prospective studies found an inverse association between *LINE-1* methylation in blood spots at birth with obesity onset in preschool children[36] and *LINE-1* in peripheral blood of adolescents with changes of waist circumference, BMI, and skinfold thickness over 30-month follow-up, in boys but not in girls.[37] While another study did not find any cross-sectional association with *LINE-1* in saliva and obesity or anthropometric parameters in adolescents.[38] Two[36,38] out of the three studies were rated at high risk of bias, and each study considered different sets of confounders.

Sixteen studies analyzed methylation at imprinted regions. The insulin-like growth factor 2 (*IGF2*) and the neighboring *H19* gene region were the most studied ($N = 12$ studies). Out of the ten studies focusing on *IGF2*, only four did find an association with child anthropometrics,[39–42] and out of the nine studies focusing on *H19*, similarly, only three did find an association with child anthropometrics.[41,43,44] Five out of the ten studies investigating *IGF2* methylation and three out of nine studies investigating *H19* methylation were at high risk of bias. Among the other imprinted regions, only one region at the mesoderm-specific transcript homolog protein (*MEST*) promoter was associated with obesity-related measures by two different studies.[45,46] On the contrary, another study did not find an association between cord blood *MEST* methylation and infant fat mass.[47]

3.1.3 | Epigenetic age acceleration

Five studies analyzed epigenetic age acceleration in cord[48,49] and peripheral blood[50–52] in relation to child weight or BMI. Population size was substantial (785–1145 children) in all the studies except one, in which only 94 girls participated. Epigenetic age was measured using the Horvath,[49–52] Hannum[50,51] method, and gestational epigenetic age using the Bohlin methods.[48] Gestational epigenetic age acceleration was associated with higher weight after birth up to 9 months; this association attenuated over time and reversed at the age of 10 years.[48] On the contrary, epigenetic age acceleration at birth was associated with a higher fat mass on average from 7 up to 17 years.[49] In childhood, cross-sectional studies found that age acceleration was positively associated with BMI at 6 years[50] and 17 years.[51] In girls, growth in childhood was not associated with peri-pubertal age acceleration.[52] All the studies were rated at low risk of bias.

3.1.4 | Candidate genes

Sixty studies investigated methylation at candidate genes. Methylation was mainly measured in peripheral blood ($N = 37$ studies) and cord blood or neonatal blood spots ($N = 21$ studies), while a few studies investigated methylation in placenta ($N = 4$ studies) and saliva ($N = 4$ studies). Study population sizes varied from 14 to 1074 children, which were primarily European ($N = 24$ studies), but also American ($N = 14$ from the United States, 2 from Mexico, 3 from Canada, and 1 from Brazil) and Asian ($N = 11$ studies), while just four studies investigated children in Australia and one in Egypt. Most of the candidate genes studies had longitudinal ($N = 33$ studies), fewer case–control ($N = 11$ studies), and cross-sectional ($N = 13$ studies) designs, and only three studies were trials ($N = 3$ studies[53–55]). Main outcomes investigated included BMI ($N = 28$ studies), obesity ($N = 17$ studies), body fat ($N = 5$ studies), waist circumference ($N = 1$ study) in children over 1 year, and infant obesity ($N = 1$ study), weight ($N = 6$ studies, in which rapid growth or weight gain were measured), and fat mass ($N = 1$ study) during the first year of life. Fifteen out of 60 studies considered important confounders (age, sex, and white blood cell composition) in the analyses and almost half (31 out 60 studies) were rated at low risk of bias. Nine studies[56–64] did not find any association between methylation at candidate gene and childhood obesity or anthropometric parameters. Leptin (*LEP*) and hypoxia-inducible factor-3 alpha (*HIF3A*) genes were the most studied ($N = 10$ and 4 studies, respectively).

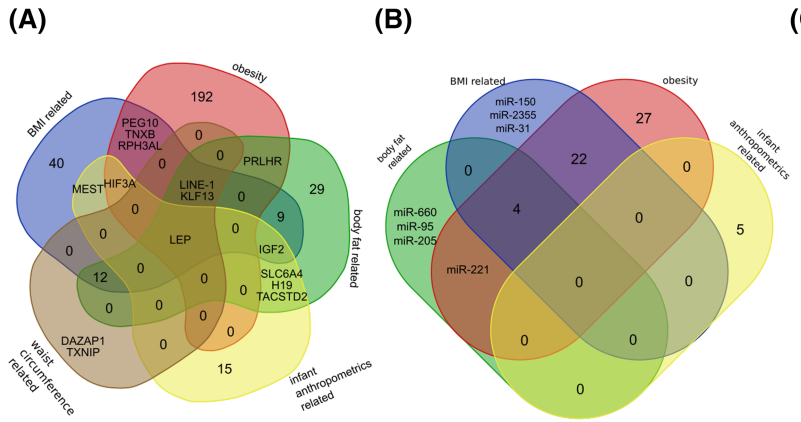
Out of the ten studies focused on *LEP*, eight found an association with childhood anthropometric parameters.[38,53,55,65–69] Four studies focused on the *HIF4A* gene, all finding an association with childhood anthropometric parameters.[70–73]

Of interest, one candidate study found that BMI at 10 years was predictive of changes in DNA methylation at the Rabphilin 3A Like (*RPH3AL*) gene between 10 and 18 years,[74] and this association was later confirmed in an EWAS, which found methylation at this same gene was higher in children with obesity compared with controls by an EWAS.[35]

In total methylation at 314 genes was found by at least one study included in the review to be associated with obesity or anthropometric parameters in infancy and childhood (Figure 2A and Table S4).

3.2 | Non-coding RNA

A summary of the 19 studies on non-coding RNAs included in our systematic review is reported in Table S3. Most of the studies ($N = 11$) analyzed non-coding RNAs using untargeted approaches. Peripheral blood was the most analyzed sample type ($N = 16$ studies), while just a few studies used cord blood,[75] adipose tissue,[76] and placenta.[77] The sample size of the studied populations varied from 8 to 250 children, which in most cases were European ($N = 9$) and Asian ($N = 7$) and with poor representation of other parts of the world ($N = 2$ studies took place in the United States and 1 in Chile). Case–control was the most used design ($N = 15$ studies), a longitudinal setup was applied twice,[75,77] and one study was cross-sectional.[78] One study was a quasi-randomized controlled before-and-after intervention.[79] Few studies ($N = 5$) considered important confounders (age and sex) in the analyses, while the rest did not adjust for any confounder at all. Most of the studies were nevertheless rated at low risk of bias ($N = 12$ out of 19). All the studies analyzed childhood obesity or BMI, two studies analyzed catch-up growth and weight gain, and one subcutaneous abdominal tissue thickness. Two studies did not find any significant



(C)

	Conclusions		
DNA methylation	Methylation at *LEP* and *HIF3A* genes was consistently associated with multiple anthropometrics by multiple candidate studies	Findings of candidate studies were not confirmed by the EWASs and needed careful interpretation	*PRLHR* and *TNXB* were the only genes whose methylation has been associated with multiple outcomes by multiple EWASs
Non-coding RNAs	The most studied non-coding RNAs were the miRNAs, while only one study focused on long non-coding RNAs	Most of the miRNA studies analysed child obesity and BMI, explaining the overlap between miRNAs associated with BMI and obesity	miR-122, miR-222 and miR-423, were found associated with multiple child anthropometrics by at least four different studies (targeted and untargeted)
Histone	Evidence for histone modifications is limited		

Limitations of the available evidence
Evidence is limited to European children
Obesity/overweight definition differs among the studies
Adjustment for covariates is often missing
Size of study population is on average small
Positive publication bias cannot be excluded
Studies are mainly cross-sectional
Analysis of single CpG site may not capture complexity of the association between methylation and childhood obesity
Interplay between the epigenetic mechanisms, or more in general with other biological mechanisms, is poorly known

FIGURE 2 Venn diagram summarizes epigenetics findings ((A) differentially methylated genes and (B) differentially expressed non-coding RNAs) grouped by children—above the age of 1 year—anthropometric measures distinguished in: BMI related, which includes BMI and weight; obesity, which includes overweight, obesity, and extreme obesity; body fat related, which includes body fat percentage and body fat mass, visceral adiposity, subcutaneous adiposity, subscapular skinfold thickness, and triceps skinfold thickness; and waist circumference related, which includes waist circumference, waist-to-hip ratio, and hip circumference; and infant—below the age of 1 year—anthropometrics-related measures, which include BMI, weight, fat mass, obesity, and triceps skinfold thickness. (C) Conclusions and limitations of the available evidence are summarized

association between non-coding RNAs and childhood anthropometric parameters.[80,81] Most of the studies were at low risk of bias ($N = 12$) (Figure S1).

One untargeted study analyzed long non-coding RNA expression profile in adipose tissue finding a large amount of long non-coding RNAs linked to childhood obesity, among which six were further validated using quantitative reverse transcription PCR (qRT-PCR).[76]

Ten untargeted studies profiled microRNA (miRNA) expression and all found child anthropometrics related at least to one miRNA.[75,77,78,82–88] Interestingly, 10 miRNAs were associated with child anthropometrics by more than one untargeted study: miR-191,[84,87] miR-122,[78,82,85] miR-125,[78,85] miR-130,[84,85] miR-197,[78,83] mi-R221,[78,85] miR-222,[83,85,87] miR-423,[82,85] miR-486,[82,85] and miR-532.[82,85] Among the eight studies investigating candidate miRNAs, all, except two,[80,81] found some associations with child anthropometrics. Two candidate studies confirmed associations from untargeted miRNAs studies: Al-Rawaf[89] found 10 miRNAs associated with childhood obesity, five of which were found related to obesity in childhood by untargeted miRNA studies (miR-130,[84,85] miR-142,[85] miR-146,[83] miR-222,[83,85] and mi-R423[78,82,85]); and Thompson et al.[90] found that in children affected by obesity and concurrent non-alcoholic fatty liver disease 16 miRNAs were differentially expressed, four of which were found commonly by untargeted studies (miR-122,[78,82,85] miR-15,[83] miR-191,[84,87] miR-199,[84] and miR-222[83,85]). Interestingly, the association of one miRNA (miR-142) with BMI found by an untargeted analysis,[85] was later confirmed in a candidate study.[91]

In total, 65 non-coding RNAs were found by at least one study included in the review to be associated with obesity or anthropometric parameters in infancy and childhood (Figure 2B and Table S5).

3.3 | Histones modification

The only study on histones modification we identified (Figure 1) found higher levels of H2AX histone phosphorylation, measured via immunofluorescence, in children with overweight and obesity compared with normal weighted prepuberal children (Table S3) and was overall rated at low risk of bias.[92]

3.4 | Risk of bias

Most of the studies were at low risk of bias (64 out of 101 for DNA methylation, 12 out of 19 for non-coding RNAs studies, and the only 1 study for histones modification) (Table S6). The domain with the highest risk of bias in both DNA methylation and non-coding RNAs studies was the one related to comparability (Figure S1).

4 | DISCUSSION

In one decade, more than 100 studies explored epigenetic associations with childhood obesity, which testifies the great interest of the scientific community on this topic.

4.1 | DNA methylation

DNA methylation was the most studied mechanism as an epigenetic determinant of childhood obesity. The candidate-based

approach was the most recurring methodology to investigate DNA methylation. In most cases, there was evidence of an association with childhood obesity; for example, *LEP* and *HIF3A* genes were consistently associated with multiple anthropometric parameters. However, the findings of candidate studies were generally not confirmed by the EWASs and need careful interpretation because the selection of candidates mainly was based on previous literature on adult obesity,[93,94] and positive publication bias cannot be excluded.

On the other side, EWASs did identify hundreds of sites differentially methylated with different child anthropometric parameters, with overlaps between multiple studies at four genes: *HDAC4*,[95,96] *PRLHR*,[24,25] *TNXB*,[31,96] and *PRDM16*.[27,96] These genes warrant further investigation, particularly considering that *PRLHR* and *TNXB* have been associated with multiple outcomes in children (Figure 2) and *HDAC4*, *TNXB*, and *PRDM16* could be involved in obesity in adults.[97–99] Lack of overlap between EWAS findings could be due to the different techniques used to measure methylation or the threshold used to account for statistical significance. Furthermore, population size surpassed 1000 participants in only two studies.[27,100] Consortia, such as pregnancy and childhood epigenetics consortium (PACE), using more than 8000 children found weight at birth is associated with widespread differences in DNA methylation,[101] while only a few epigenetic signatures have been associated with BMI and obesity in childhood.[27] Finally, while most of the studies analyzed methylation at single CpG sites, the findings of several differentially methylated regions (DMRs)[22,23,25,31] related to childhood obesity suggest that regulation of epigenetics underlying obesity in childhood could be a more complex event in which multiple CpG sites act simultaneously.

Evidence for the association of DNA methylation at the global level, at imprinted regions and epigenetic age acceleration with childhood obesity is still scarce. Findings mostly suggest an inverse association of *LINE-1*, a surrogate marker for global methylation levels, with child anthropometrics. As lower global DNA methylation is associated with increased genomic instability and chromosomal rearrangements, future studies should clarify its link with childhood obesity. Studies investigating imprinted regions focused mainly on *IGF2* and *H19*. Few reports did find an association, while most of the evidence pointed towards a lack of significant association with children anthropometrics. However, investigation of other imprinted regions may provide a better understanding of the non-genomic transgenerational inheritance of childhood obesity. Available evidence shows an association between epigenetic gestation age acceleration and obesity-related measures, although the direction of this association is still debated. There is no clear evidence for association with epigenetic age acceleration in childhood. This inconsistency in findings could be attributed to the use of different methods to measure epigenetic acceleration. Further research is needed, using the most appropriate method for the sample type analyzed. Other molecular markers of aging, such as telomeres, have been already associated with childhood obesity and even prenatal maternal factors, such as pre-pregnancy BMI.[102,103]

4.2 | Non-coding RNAs

The most studied non-coding RNAs were the miRNAs, while only one study focused on long non-coding RNAs. Most of the miRNA studies analyzed child obesity and/or BMI, while fewer studies also analyzed other anthropometric parameters, which may explain most of the overlaps between miRNAs associated with BMI and obesity (Figure 2).

Untargeted approaches have been preferred over candidate studies, and both suggested an association with mi-RNAs. Among the findings, miR-122, miR-222, and miR-423 were found associated with child anthropometrics by at least four different studies (two of which being untargeted).[78,82,83,85,89,90] Untargeted studies were based on a small sample size of populations (max *N* participants = 18), but candidate studies with larger sample sizes corroborated these results (max *N* participants = 250),[89,90] indicating that future research should prioritize their investigation. Some of these miRNAs (e.g., miR-122, miR-222, and miR-423) have also been previously found to be associated with obesity in adults.[104,105]

4.3 | Causal relationships: Epigenetics is a cause or a consequence?

Epigenetics has been postulated to represent a bridge between nutrition during pregnancy and early life and childhood obesity.[9,106] Beyond nutrition, many other prenatal and early life factors may influence childhood obesity phenotype via epigenetics, including maternal pre-pregnancy overweight and obesity, gestational diabetes, and even paternal socioeconomic status.[107–109] The effect of these exposures on epigenetics can be direct or mediated via other omic layers (e.g., via the microbiome[110]). However, very few studies formally investigated the exact chain of events leading to childhood obesity in a causal framework. For example, despite that breastfeeding and childhood obesity were associated with LEP methylation by multiple studies,[56,66,69] no indirect effect via methylation was detected when formal mediation was tested.[69] Further, epigenetic changes may fade but still impact anthropometric measures indirectly, as demonstrated by Junge et al. that found that methylation at *MEST* promotor in cord blood did not modify BMI of children at 6 years directly, but indirectly through changes of BMI at 1 year.[45] Finally, the temporal sequence between epigenetic changes and the onset of childhood obesity is uncertain because epigenetics may be altered by a wide range of stimuli, including metabolic changes associated with obesity itself. Most of the studies included in the review used a cross-sectional design, making it impossible to disentangle the temporal sequence of events. Some DNA methylation studies employed longitudinal designs or statistical techniques, such as Mendelian randomization, to disentangle this relationship. In the ALSPAC cohort, childhood BMI was associated with methylation at *HIF3A* gene in adolescence, but childhood methylation was not robustly associated with BMI in adolescence, and based on Mendelian randomization, *HIF3A* methylation did not play a causal role on BMI.[72] In the same cohort, causal relationships

between DNA methylation score and BMI were assessed using cross-lagged and longitudinal models finding that the DNA methylation score was a poor predictor of future BMI. At the same time, Mendelian randomization analysis supported that BMI was predictive of later DNA methylation, although the evidence was weak.[111] Another possible indication of the temporal sequence of BMI–epigenetics association derives from the study of Shah et al., which found that DNA methylation score could predict BMI better in adolescent individuals when the effect sizes used for generating the score were derived from a younger cohort than when they were derived from older individuals.[112] The authors speculated that the more robust effects seen on methylation could be due to reverse causation and the difference could be attributable to the fact that older individuals are exposed to adiposity phenotype for a longer time. A similar pattern of BMI being predictive of later life methylation was also in longitudinal studies; for example, Han et al. found that BMI at 10 years was predictive of changes in *RPH3AL* gene methylation between 10 and 18 years.[74]

Although based on a few observations, the available evidence supports adiposity in childhood leading to changes in methylation, rather than the other way around, consistently with findings in adults.[113,114] This has important implications in the prevention of obesity-related diseases as epigenetics may be an intermediate biomarker between obesity and obesity-related diseases. If obesity causes epigenetic changes, then epigenetics may fall on the causal pathway between obesity and obesity-related outcomes, as already demonstrated in children[111] and adult studies.[115] Future research should further investigate the causality of the biomarkers identified to be associated with obesity in childhood.

4.4 | The interplay between the three epigenetic mechanisms and with other biological layers

We found some indication pointing towards a possible cross-talk between epigenetic markers related to childhood obesity. Two different studies found an association between methylation at two different sites of the *HDAC4* gene, a member of the histone deacetylase family, and obesity in childhood, although with opposite direction of the association, which could be explained by the different position of the identified CpGs in the gene (at 5′UTR in one study and the gene body in the other study).[95,96] Furthermore, one of the two studies also found differential methylation in children with obesity compared with controls at the *HIST1H2BG* gene, encoding for a histone member of the histone H2B family.[96] Whether the identified methylation changes are reflected in differential chromatin regulation is not known. In adults, a study found that after an exercise intervention, *HDAC4* methylation decreased in adipose tissue, which correlated with expression levels.[116] No study has yet investigated histone deacetylation in relation to childhood obesity; however, higher levels of H2AX histone phosphorylation were reported in children with overweight and obesity compared with controls.[92]

A possible interplay between methylation and non-coding RNA targets related to childhood obesity is suggested by the finding from several studies that methylation of genes putatively encoding for long non-coding RNAs (*nc886*,[31] *LOC101929268*,[117] *LOC254559*, *LOC100133991*,[96] *LOC102723493*, and *LOC100049716*[25]) and miRNAs[23,118,119] were associated with children anthropometric parameters. Despite DNA methylation theoretically affecting gene expression, none of these studies provide evidence of such an association through measurement of circulating levels of the corresponding RNAs. However, among the genes identified as differentially methylated, two (one gene putatively associated with miR-27[118] and the *MIRLET7BHG* host gene[119]) encoded miRNAs that were found to be differentially expressed in children with obesity compared with controls.[84,90,120] Further, one study found that miRNA expression levels in the placenta were correlated with methylation levels at an imprinted gene (*C19MC*), which was associated with the offspring's anthropometry at 6 years, supporting the hypothesis of a close cross-talk between methylation and miRNAs.[121] Finally, one study identified BMI in girls of 5 years was associated with lower levels of promoter methylation at *DNMT3B* gene,[122] which encodes a DNA methyltransferase, whose recruitment is regulated by numerous mechanisms, including chromatin modifications and non-coding RNAs.[123]

Finally, epigenetics represents only a tiny part of the many biological molecules interacting in our body. Mechanisms through which environmental exposures in early life shape the risk of obesity in childhood may involve many other biological layers. For example, the microbiome may induce epigenetic changes involved in the development of obesity via metabolic products such as short-chain fatty acids.[110] Multi-omics studies,[124] investigating multiple omic layers, and holo-omics studies,[125] investigating the interactions between the multiple omic layers of the host organisms and their associated microbiome, may provide a better understanding of the developmental programming of childhood obesity. Because there are well-known genetic predispositions to obesity in children, future studies should also incorporate genetic data in their analysis and testing gene–gene interactions.[126,127]

4.5 | Gaps and constraints

Heterogeneity of findings, mainly attributable to lack of comparability between the studies (Figure S1), may have hindered more insights into the epigenetic changes related to childhood obesity, and future research should try to overcome the limitations summarized in Figure 2C.

Childhood obesity prevalence differs by country as a result of both genetic and epigenetic factors.[128] Most of the studies analyzed in the review were conducted in European or American populations, while the representation of Asian and Australian populations was much lower. Despite that North Africa is one of the regions with the highest rate of childhood obesity prevalence, only one African study population was included.[3,53] Only four studies investigated the relationship between epigenetics and childhood obesity through pooling populations (mostly European) from different countries or via

meta-analytical approaches.[27,87,100,117] Future research should assess the generalizability of the findings available in different ethnic groups.

There is no single measure of adiposity that can be used across infancy, childhood, and adolescence periods. In the reviewed studies, the definition of overweight or obesity was based: on the weight in infants below 2 years (apart from one study that used weight for adolescents[59]) and on BMI in children at later ages. A variety of references, including standard references of the International Obesity Task Force (IOTF) or the World Health Organization (WHO), country-specific growth chart references, or setting-specific reference values were used. Using different references may lead to different classifications of children as being affected by overweight and obesity, explaining the heterogeneity of the findings. In addition, it is uncertain if in children BMI reflects increases in lean or fat mass and which is the best measure of adiposity to use.[129,130] To overcome this issue, some studies also investigated other anthropometric parameters (e.g., calculating weight gain across infancy and using fat mass and skinfold thickness or waist and hip circumferences) and found that some epigenetic marks were related to multiple outcomes (Figure 2).

Further, not all the studies adjusted analyses for the same set of confounders. One third of the studies performed their analysis adjusting at least for sex and age (and white blood cells for DNA methylation studies), which are known sources of variation of children's anthropometrics and epigenetics. In contrast, another third did not adjust for any factor at all, although in most cases age- and sex-specific anthropometric measures were calculated. It could be interesting to investigate sexual dysmorphism, as sex-specific associations between DNA methylation and epigenetics have also been reported using longitudinal design.[46,131,132] Further, paternal influences on epigenetics and childhood obesity have been considered in just a few papers, testifying that the father's role is underrepresented in this field of research, although recent evidence suggests it could be of importance.[107,133] Cell composition within a tissue represents an additional source of potential confounding of DNA methylation patterns.[134] Only 30% of the studies adjusted for cell-type composition, mainly using established bioinformatic correction methods. However, multicollinearity between cell estimates themselves may have a tremendous impact on results. Barton et al.[135] demonstrated that, after adjustment for cell types composition, the association of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) methylation with BMI was no longer significant and had inverse direction compared with not adjusted results.[22] Future research should consider assessing possible multicollinearity and applying new strategies to minimize it.

Most of the studies in this review analyzed peripheral and cord blood, while fewer studies analyzed saliva, placenta, and neonatal blood spots. Epigenetics is tissue specific[136,137] and is subjected to dramatic changes during development in childhood.[138] In adults, studies reported a degree of correlation between methylation in blood and adipose tissue,[139] and epigenetic changes associated with obesity in blood were found to mirror those found in adipose tissue,[140,141] but whether it is the same also for children is not yet known. Only one study used adipose tissue to validate the association with childhood obesity obtained in peripheral blood in children. Interestingly, this study found that differential DNA methylation patterns in the Kruppel-like factor 13 (*KLF13*) gene, which have been previously related to adult BMI,[93] were present in blood, pre-adipocytes, and pancreatic islet cells, suggesting that blood samples could be used as a surrogate for representing epigenetic changes in cells related to childhood obesity.[142] Moreover, only circulating non-coding RNAs were studied in relation to childhood obesity. Non-coding RNAs can be released from cells directly in the circulation and packaged in extracellular vesicles. Extracellular vesicles, including exosomes, modulating via their miRNA cargos the metabolic processes, have been reported to be linked to adult obesity, but their role in childhood obesity remains poorly understood.[143]

Heterogeneity of the findings could be attributed to different techniques used to measure the epigenetics (especially for studies of DNA methylation as represented in Figure S1) and to small sample size populations (especially for studies of miRNAs as represented in Figure S1), which may increase the risk of both false positive and negative results. In this regard, although we did not test publication bias formally, results of the present systematic review should be carefully interpreted because most of the included articles reported at least one significant association with obesity/overweight or any other related anthropometrics in childhood, and most of them have a small sample size. This issue should be seriously considered, especially for DNA methylation studies that analyzed mostly candidate CpG sites for which a negative finding would be difficult to find a place in peer-reviewed journals. Future research should put more effort into investigating larger size children populations or implementation of power calculations.[144] Using a non-invasive matrix such as saliva may be an opportunity to collect samples in larger scale children-based population studies.[145]

5 | CONCLUSIONS

Several studies showed evidence that childhood obesity is linked to epigenetic changes. Most of the identified epigenetic signatures have not yet been replicated, indicating that a childhood epigenetics obesity signature is at least complex to unravel, and further investigation is warranted. Based on the identified studies, epigenetics seems to be a more plausible biomarker of childhood obesity rather than its cause; nevertheless, caution is needed because cross-sectional studies were most represented. Interaction between the different epigenetic layers is still based on assumptions rather than solid scientific evidence.

ACKNOWLEDGMENTS

This study is an ancillary endeavor of the Science & Technology in childhood Obesity Policy (STOP) project (H2020 SC2; ref. 774548).

This work is supported by the Bijzonder Onderzoeksfonds Hasselt University through a PhD fellowship (to RA) and the Science and Technology in childhood Obesity Policy grant (grant number 774548—European Commission H2020 [Horizon 2020 Framework Programme] to PV).

CONFLICT OF INTEREST

No conflict of interest statement.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Alfano R, Robinson O, Handakas E, Nawrot TS, Vineis P, Plusquin M. Perspectives and challenges of epigenetic determinants of childhood obesity: A systematic review. *Obesity Reviews*. 2022;23(S1):e13389. doi:10.1111/obr.13389