

3D genomic features across >50 diverse cell types reveal insights into the genomic architecture of childhood obesity

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Abstract

The prevalence of childhood obesity is increasing worldwide, along with the associated common comorbidities of type 2 diabetes and cardiovascular disease in later life. Motivated

by evidence for a strong genetic component, our prior genome-wide association study (GWAS) efforts for childhood obesity revealed 19 independent signals for the trait; however, the mechanism of action of these loci remains to be elucidated. To molecularly characterize these childhood obesity loci we sought to determine the underlying causal variants and the corresponding effector genes within diverse cellular contexts. Integrating childhood obesity GWAS summary statistics with our existing 3D genomic datasets for 57 human cell types, consisting of high-resolution promoter-focused Capture-C/Hi-C, ATAC-seq, and RNA-seq, we applied stratified LD score regression and calculated the proportion of genome-wide SNP heritability attributable to cell type-specific features, revealing pancreatic alpha cell enrichment as the most statistically significant. Subsequent chromatin contact-based fine-mapping was carried out for genome-wide significant childhood obesity loci and their linkage disequilibrium proxies to implicate effector genes, yielded the most abundant number of candidate variants and target genes at the *BDNF*, *ADCY3*, *TMEM18* and *FTO* loci in skeletal muscle myotubes and the pancreatic beta-cell line, EndoC-BH1. One novel implicated effector gene, *ALKAL2* – an inflammation-responsive gene in nerve nociceptors – was observed at the key *TMEM18* locus across multiple immune cell types. Interestingly, this observation was also supported through colocalization analysis using expression quantitative trait loci (eQTL) derived from the Genotype-Tissue Expression (GTEx) dataset, supporting an inflammatory and neurologic component to the pathogenesis of childhood obesity. Our comprehensive appraisal of 3D genomic datasets generated in a myriad of different cell types provides genomic insights into pediatric obesity pathogenesis.

Key points

Question

What are the causal variants and corresponding effector genes conferring pediatric obesity susceptibility in different cellular contexts?

Findings

Our method of assessing 3D genomic data across a range of cell types revealed heritability enrichment of childhood obesity variants, particularly within pancreatic alpha cells. The mapping of putative causal variants to cis-regulatory elements revealed candidate effector genes for cell types spanning metabolic, neural, and immune systems.

Meaning

We gain a systemic view of childhood obesity genomics by leveraging 3D techniques that implicate regulatory regions harboring causal variants, providing insights into the disease pathogenesis across different cellular systems.

eLife assessment

This **important** study presents genome-wide high-resolution chromatin-based 3D genomic interaction maps for over 50 diverse human cell types and integrates these data with pediatric obesity GWAS. The work provides **convincing** evidence that multiple pancreatic islet cell types are key effector cell types. The authors also perform variant-to-gene mapping to nominate genes underlying several GWAS hits. Overall, the results will be of interest to both the fields of 3D genome architecture and pediatric obesity.

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Introduction

The prevalence of obesity has risen significantly worldwide¹, especially among children and adolescents². Obesity is associated with chronic diseases, such as diabetes, cardiovascular diseases, and certain cancers^{3–6}, along with mechanical issues including osteoarthritis and sleep apnea⁷.

Modern lifestyle factors, including physical inactivity, excessive caloric intake, and socioeconomic inequity, along with disrupted sleep and microbiome, represent environmental risk factors for obesity pathogenesis. However, genetics also play a significant role, with the estimated heritability ranging from 40% to 70%^{8–10}. Studies show that body weight and obesity remain stable from infancy to adulthood^{11–14}, but variation between individuals does exist¹⁵. Genome-wide association studies (GWAS) have improved our understanding of the genetic contribution to childhood obesity^{16–21}. However, the functional consequences and molecular mechanisms of identified genetic variants in such GWAS efforts are yet to be fully elucidated. Efforts are now being made to predict target effector genes and explore potential drug targets using various computational and experimental approaches^{22–26}, which subsequently warrant functional follow-up efforts.

With our extensive datasets generated on a range of different cell types, by combining 3D chromatin maps (Hi-C, Capture-C) with matched transcriptome (RNA-seq) and chromatin accessibility data (ATAC-seq), we investigated heritability patterns of pediatric obesity-associated variants and their gene-regulatory functions in a cell type-specific manner. This approach yielded 94 candidate causal variants mapped to their putative effector gene(s) and corresponding cell type(s) setting. In addition, using methods comparable to our prior efforts in other disease contexts^{27–34}, we also uncovered new variant-to-gene combinations within specific novel cellular settings, most notably in immune cell types, which further confirmed the involvement of the immune system in the pathogenesis of obesity in the early stages of life.

Methods

Data and resource

Datasets used in prior studies are listed in **eTable 1**. ATAC-seq, RNA-seq, Hi-C, and Capture-C library generation for each cell type is provided in their original published study and their *pre-processing pipelines and tools* can be found in **eMethods**.

Definition of cis-Regulatory Elements (cREs)

We intersected ATAC-seq open chromatin regions (OCRs) of each cell type with chromatin conformation capture data determined by Hi-C/Capture-C of the same cell type, and with promoters (−1,500/+500bp of TSS) defined by GENCODE v30.

Childhood obesity GWAS summary statistics

Data on childhood obesity from the EGG consortium was downloaded from www.egg-consortium.org. We used 8,566,179 European ancestry variants (consisting of 8,613 cases and 12,696 controls in stage 1, 921 cases and 1,930 controls in stage 2), representing ~55% of the total 15,504,218 variants observed across all ancestries in the original study³⁵. The sumstats file was reformatted by *munge_sumstats.py* to standardize with the weighted variants from HapMap v3 within the LDSC baseline, which reduced the variants to 1,217,311 (7.8% of total).

Cell type specific partitioned heritability

We used LDSC (<http://www.github.com/bulik/ldsc>) v1.0.1 with `--h2` flag to estimate SNP-based heritability of childhood obesity within 4 defined sets of input genomic regions: (1) OCRs, (2) OCRs at gene promoters, (3) cREs, and (4) cREs with an expanded window of ±500 bp. The baseline model LD scores, plink filesets, allele frequencies and variants weights files for the European 1000 genomes project phase 3 in hg38 were downloaded from the provided link (<https://alkesgroup.broadinstitute.org/LDSCORE/GRCh38/>). The cREs of each cell type were used to create the annotation, which in turn were used to compute annotation-specific LD scores for each cell types cREs set.

Genetic loci included in variant-to-genes mapping

19 sentinel signals that achieved genome-wide significance in the trans-ancestral meta-analysis study³⁵ were leveraged for our analyses. Proxies for each sentinel SNP were queried using TopLD³⁶ and LDlinkR tool³⁷ with the GRCh38 Genome assembly, 1000 Genomes phase 3 v5 variant set, European population, and LD threshold of $r^2 > 0.8$, which resulted in 771 proxies, including the 21 SNPs from the 99% credible set of the original study (eTable 2).

GWAS-eQTL colocalization

The summary statistics for the European ancestry subset from the EGG consortium GWAS for childhood obesity was used. Common variants ($MAF \geq 0.01$) from the 1000 Genomes Project v3 samples were used as a reference panel. We used non-overlapped genomic windows of ±250,000 bases extended in both directions from the median genomic position of each of 19 sentinel loci as input. We used ColocQuaL³⁸ to test genome-wide colocalization of all possible variants included in each inputted window against GTEx v8 eQTLs associations for all 49 tissues available from <https://www.gtexportal.org/home/datasets>. A conditional posterior probability of colocalization of 0.8 or greater was imposed.

Results

Enrichment assessment of childhood obesity variants across cell types

To explore the enrichment of childhood obesity GWAS variants across cell types, we carried out Partitioned Linkage Disequilibrium Score Regression (LDSR)³⁹ on all ATAC-seq-defined OCRs for each cell type (Fig. 1A). We observed that 41 of 57 cell types – including 22 metabolic, 21 immune, 7 neural cell types and 7 independent cell lines (eTable 1) – showed at least a degree of

directional enrichment with the total set of OCRs (**Fig. 1B.a**). However, only four cell types – two pancreatic alpha and two pancreatic beta cell-based datasets – had statistically significant enrichments ($P < 0.05$). These enrichments were less pronounced when focusing on promoter OCRs only (**Fig. 1B.b**). To further limit the LD enrichment assessment to just those OCRs that can putatively regulate gene expression via chromatin contacts with gene promoters, we used the putative cREs^{27,29}. This reduced the number of cell types showing at least nominal enrichment (31 of 57), enlarged the dispersion of enrichment ranges across different cell types, increased the 95% confidence intervals (CI) of enrichments, and hence increased the P -value of the resulting regression score. cREs from pancreatic alpha cells derived from single-cell ATAC-seq were the only dataset that remained statically significant (**Fig. 1B.c**).

The original reported LDSR method analyzed enrichment in the 500bp flanking regions of their regulatory categories³⁹. However, when we expanded our analysis to the ± 500 bp window for our cREs, albeit incorporating more weighted variants into the enrichment (represented by larger dots in **Fig. 1B.d**), this resulted in a decrease in the number of cell types yielding at least nominal enrichment (26 cell types), the enrichment range across cell types, the 95% CI, and level of significance. The pancreatic alpha cell observation also dropped below the bar for significance with this expanded window definition.

Consistency and diversity of childhood obesity proxy variants mapped to cREs

Despite the enrichments above only being limited to just a small number of cell types, it is likely that individual loci have differing levels of contributions in various cellular contexts and could not be detected at the genome wide assessment scale. As such we elected to further explore the candidate effector genes that are directly affected by cREs harboring childhood obesity-associated variants by systematically mapping the genomic positions of the LD proxies onto each cell type's cREs, resulting in several scenarios, as illustrated in **Fig. 2A,B**. The resulting 94 proxies clustered at 13 original loci (**eTable 3**). **eFig. 1A** outlines the number of signals at each locus included or excluded based on the criteria we defined for our regions of interest. The *TMEM18* locus yielded the most variants through cREs mapping, with 46 proxies for the two lead independent variants, rs7579427 and rs62104180. The second most abundant locus was *ADCY3*, with 21 proxies for lead variant rs4077678 (**Fig. 2C**). The higher number of variants at one locus did not correlate with implicating more genes or cell types through mapping. The mapping frequency of various variants within a specific locus exhibited substantial differences.

Inspecting individual variants regardless of their locus, we found that 28 of 94 proxies appeared in cREs across multiple cell types, with another 66 observed in just one cell type (**Fig. 2D**). 45 variants of these 66 just contacted one gene promoter, such as at the *GPR1* and *TFAP2B* loci (**eFig. 2E**).

Overall, the number of cell types in which a variant was observed in open chromatin correlated with the number of genes contacted via chromatin loops (**eFig. 3A**). However, we also observed that some variants found in cREs in multiple cell types were more selective with respect to their candidate effector genes (**eFig. 3B-red arrow**), or conversely, more selective across given cell types but implicated multiple genes (**eFig. 3B-blue arrow**). **eFig. 4** outlines our observations at the *TMEM18* locus – an example locus involved in both scenarios.

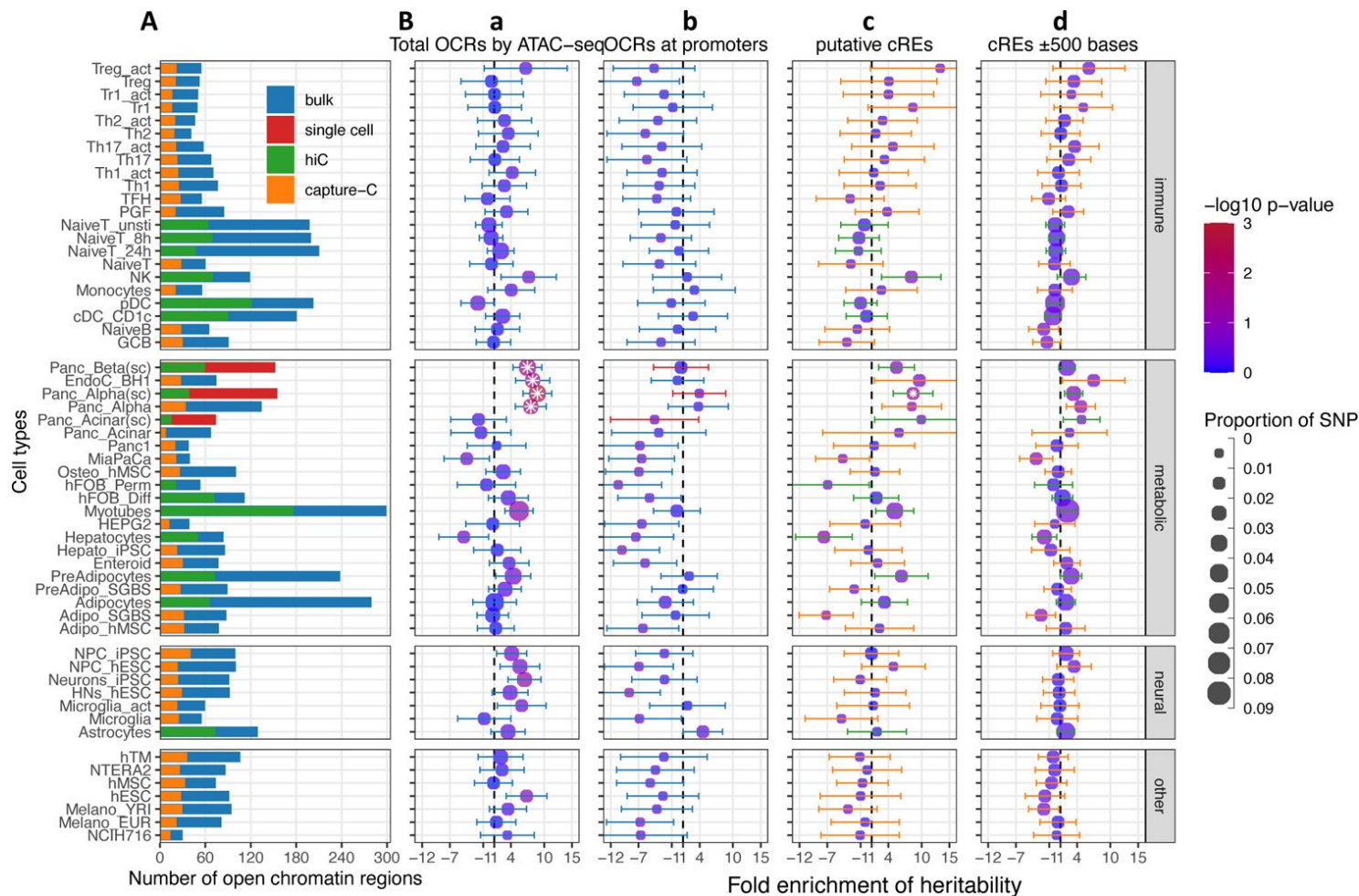


Figure 1

Partitioned Linkage Disequilibrium Score Regression analysis for open chromatin regions of all cell types. A. Bar-plot shows total number of open chromatin regions (OCRs) identified by ATAC-seq for each cell type on bulk cells - blue, or on single cell - red; and portion of OCRs that fall within promoter-interacting region (PIR-OCRs) identified by hi-C - green, or by capture-C - orange. B. 4 panels of dot-plots show heritability enrichment by LDSC analysis for each cell type, with standard error whiskers. Dots' colors corresponding to p-values, dots with white asterisk are significant p-value < 0.05 , dots' sizes corresponding to proportion of SNP contribute for heritability. Dash line at 1, i.e., no enrichment. a) Analysis done on whole OCRs set of each cell type (whiskers colors match with bulk/single cell from bar-plot A); b) On only OCRs that overlapped with promoters (whiskers' colors match with bulk/single cell from bar-plot A); c) On the putative cREs of each cell type (whiskers' colors match with hi-C/capture-C from bar-plot A); d) On the same PIR-OCRs as middle panel with their genomic positions expanded ± 500 bases on both sides (whiskers' colors match with hi-C/capture-C from bar-plot A).

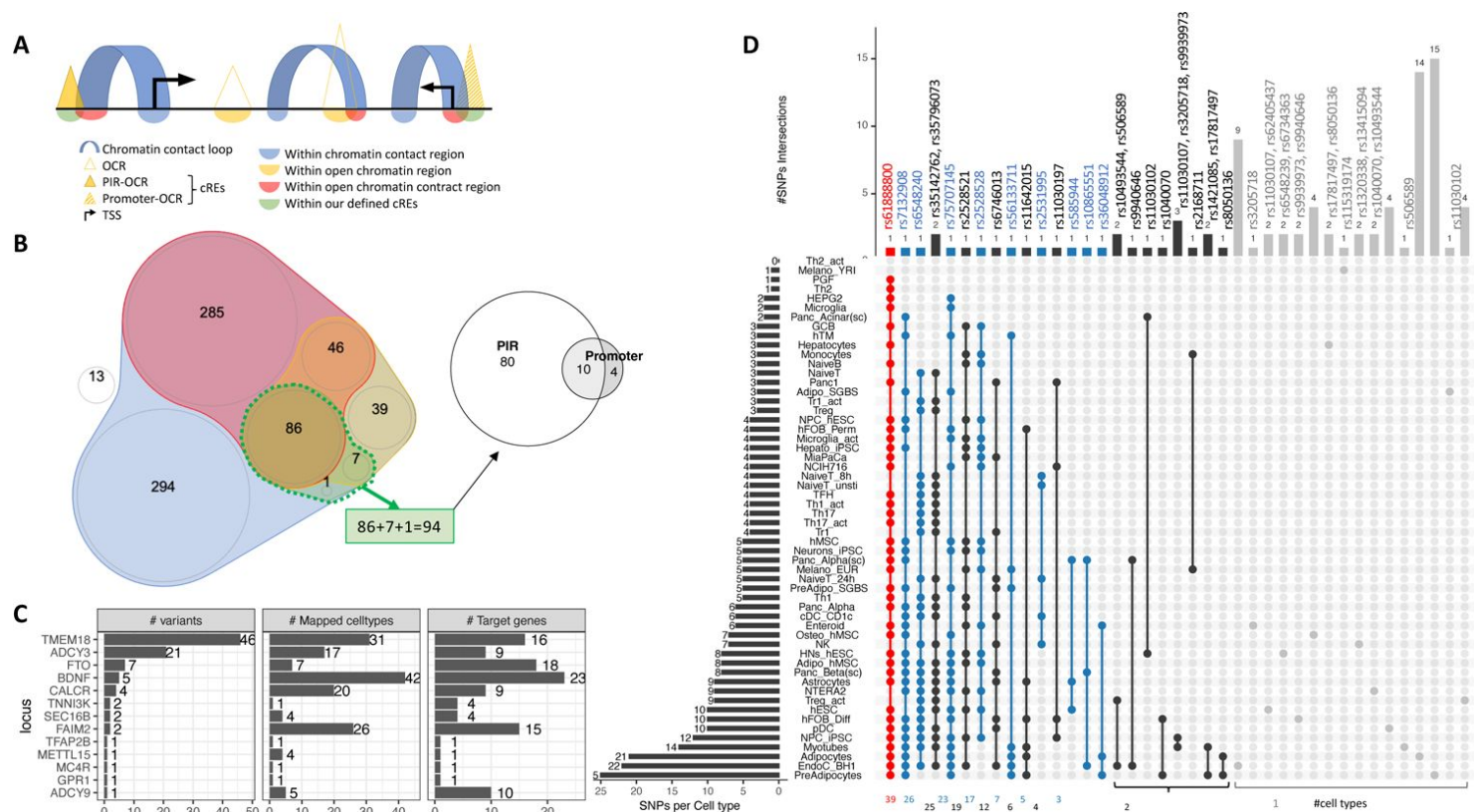


Figure 2

Mapping 771 proxies to the open chromatin regions of each cell type A. Schematic shows 4 different ways OCRs can be subset due to overlapping with chromatin contact sites and gene promoters, colors of sets of OCRs applied for the areas in B panel. B. Venn diagram shows how 771 proxies mapped onto the OCRs: a. Blue area: 758 proxies were located within contact regions of at least one cell type regardless of chromatin state; b. Red area: 417 proxies were located within contact regions marked as open by overlapping with OCR; c. Yellow area: If we only considered open chromatin regions, 178 proxies were included; d. Green area: To focus on just those variants residing within open chromatin and contacting promoter regions in any cell type, we overlapped the genomic positions of these proxies with each cell type's cRE set, yielding 90 variants (3 from the 99% credible set) directly contacting open gene promoters (**eTable 3**), with 10 located within a promoter of one gene but contacting another different gene promoter. There were an additional 4 variants located within gene promoters but in chromatin contact with promoter(s) of nearby transcript(s) of the same gene. C. Bar-plot shows number of proxies, cell types and target genes mapped at each locus. D. The upSet plot shows the degree of overlap across cell types of the variants; ranked from the most common variant (red) – rs61888800 from *BDNF* locus, a well-known 5' untranslated region variant of this gene that is associated with anti-depression and therapeutic response^{81,82} – appeared in 39 cell types, to the group of variants (grey) which appeared in only one cell type.

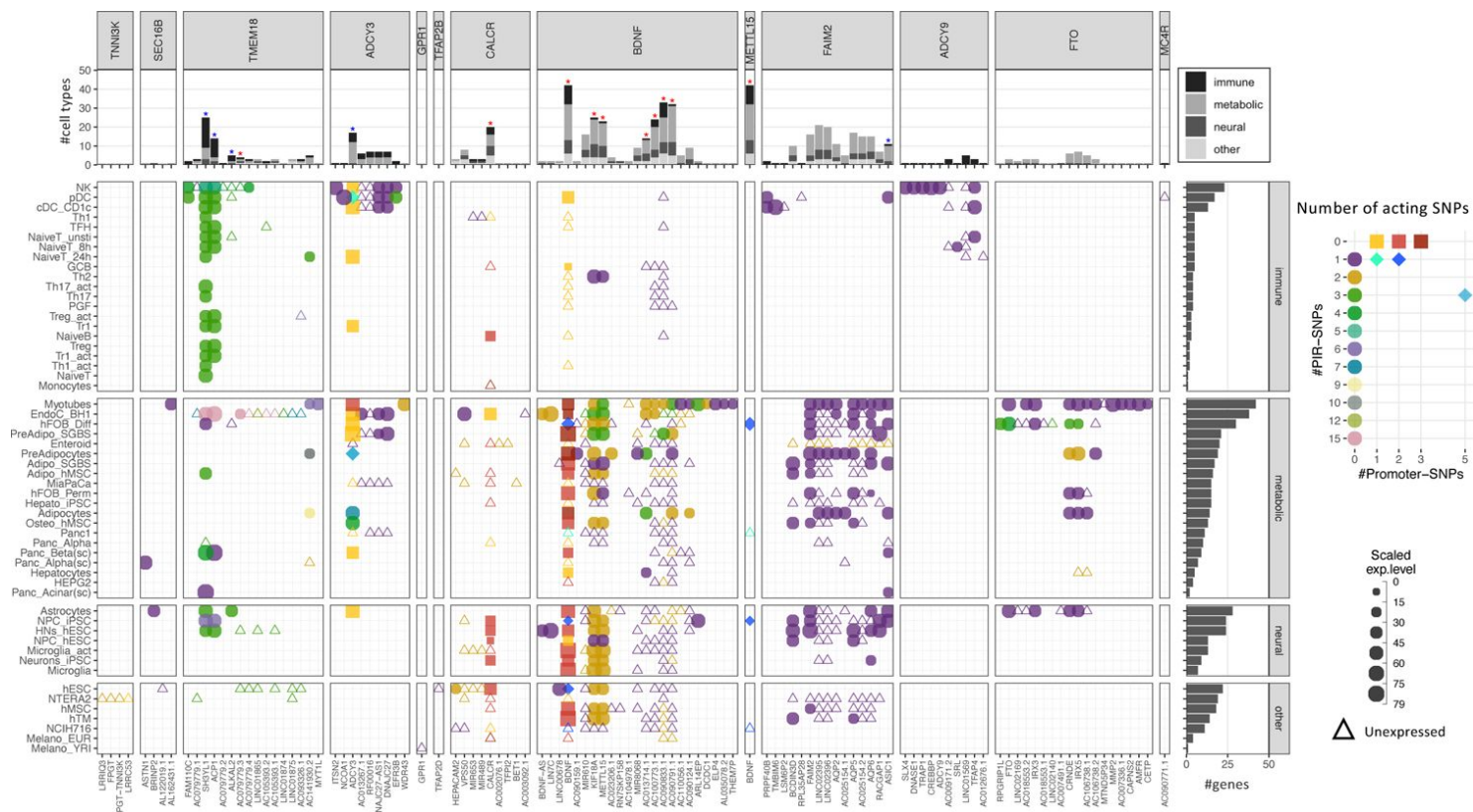


Figure 3

Profiles of 111 implicated genes by 94 proxies through cREs of each cell type Main panel: Bubble plot show corresponding expression level (size) and number of variants (color) target each implicated gene of each cell type. Squares represent genes with variants at their promoters. Circles represent genes with variants contacted through chromatin loops. Some genes were implicated by both types, these “double implications” are represented as diamond shapes, and were identified across several cell types: two cell types (plasmacytoid dendritic cells and pre-differentiated adipocytes) for *ADCY3* gene, and five for *BDNF* (human embryonic stem cells - hESC, differentiated human fetal osteoblast cells - hFOB_Diff, neural progenitor cells derived from induced pluripotent stem cells - NPC_iPSC, PANC-1, and NCIH716 cell lines) Genes with expression undetected in our arrays are shown as triangles. Top panel: bar-plot shows numbers of cell types each gene was implicated within, color-coded by which systems the cell types belong to. Right panel: bar-plot shows numbers of genes implicated by the variants with each cell type.

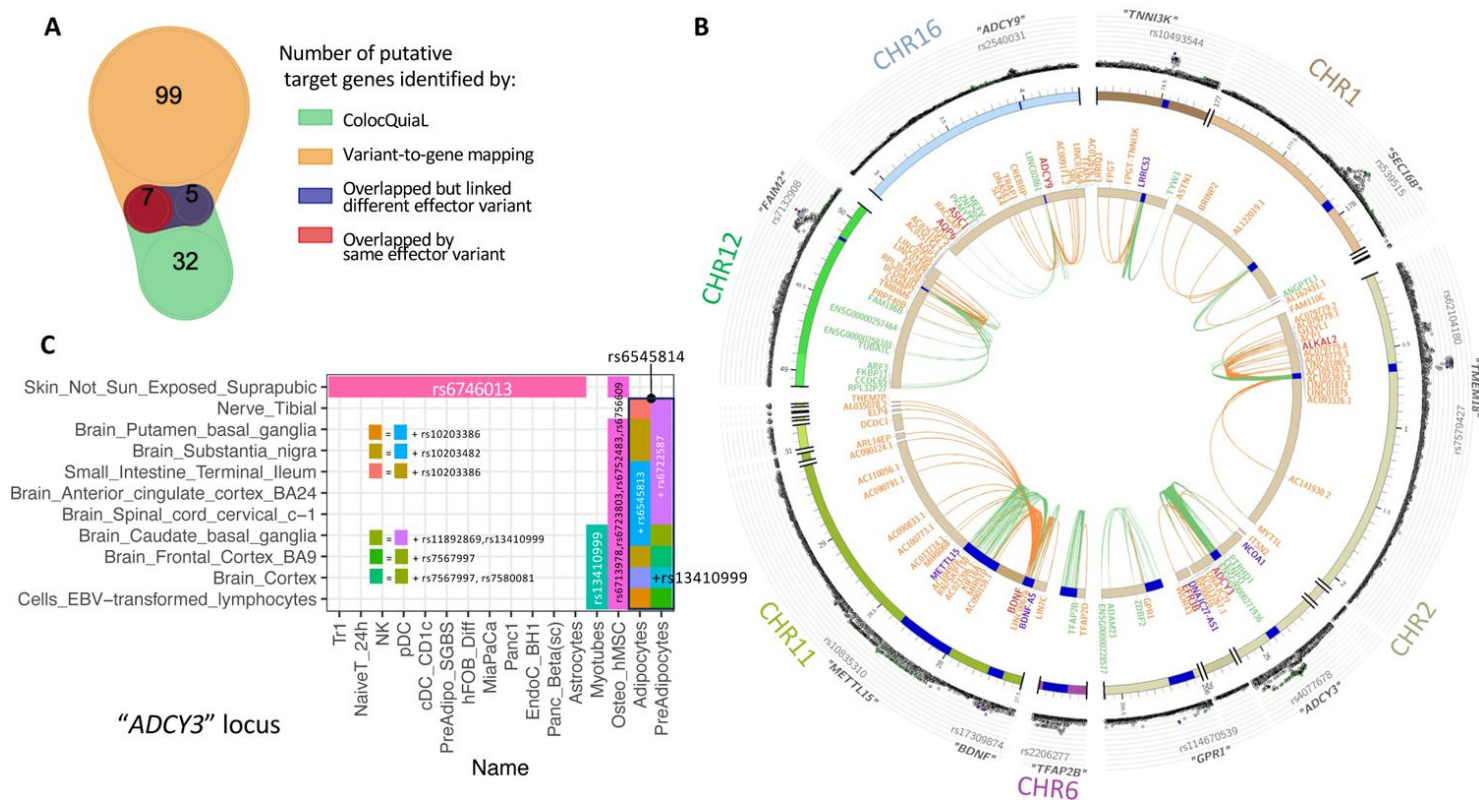


Figure 4

Colocalization of target effector genes with eQTLs A. Venn diagram shows the overlaps between sets of genes yielded by ColocQuiaL and the variant-to-gene mapping process. B. Circos plot of the 10 loci demonstrates the differences in the ranges of associations between the two approaches, with long-ranged chromatin contacts between obesity variants and target genes displayed as orange links and short-range eQTLs colocalizations as green links. Two SNPs – rs35796073, and rs35142762 within the *TMEM18* locus, in linkage disequilibrium with rs7579427 – were estimated with high probability (cond.PP.H4=0.78) of colocalizing with the expression of *ALKAL2* gene in subcutaneous adipose tissue. These pairs of SNP-gene were also identified by our variant-to-gene mapping approach in natural killer cells, plasmacytoid dendritic cells, unstimulated PBMC naïve CD4 T cells and astrocytes. The rs7132908 variant at the *FAIM2* locus colocalized with the expression of *AQP6* in thyroid tissue and with *ASIC1* in prostate tissue, not only with high cond.PP.H4 but also with high individual SNP causal probability (SNP.PP.H4 > 0.95). rs7132908 was the second most consistent observation in our variant-to-gene mapping, namely across 25 different cell types (Figure 2C) and all three systems plus the other independent cell lines. The pair of rs7132908-contacting-*AQP6* was observed in 15 different cell types – 8 metabolic and 4 neural cell types, and 3 independent cell lines. The pair of rs7132908-contacting-*ASIC1* was observed in 11 different cell types - 8 metabolic and 2 neural cell types, and plasmacytoid dendritic cells. The other eQTL signals that overlapped with our variant-to-gene mapping results were: *BDNF* at the *METTL15* locus with its promoter physically contacted by rs11030197 in 4 cell types and its expression significantly colocalized (cond.PP.H4=0.82) in tibial artery; *ADCY9* at its locus with its promoter physically contacted by rs2531995 in natural killer cells and its expression significantly colocalized in skin tissue (“Skin_Not_Sun_Exposed_Suprapubic”, cond.PP.H4=0.97). And *ADCY3* in the C panel. C. ColocQuiaL estimated that these SNPs highly colocalize with the expression of *ADCY3* in 11 different tissues, where the overlapping with the 16 cell types is represented, color-coded by the proxies rs numbers.

Implicated genes cluster at loci strongly associated with childhood obesity consistently across multiple cell types

Mapping the variants across all the cell types resulted in a total of 111 implicated childhood obesity candidate effector genes (Table 1). Among these, 45 genes were specific to just one cell type (eFig. 5A), including 13 in myotubes and 7 in natural killer cells. Conversely and notably, *BDNF* appeared across 42 different cell types. Across the metabolic, neural, and immune systems and seven other cell lines, there were 9 genes consistently implicated in all four categories (top panel Fig. 3 – red stars, eFig. 5B: “all”), while 5 genes were consistently implicated in metabolic, neural, and immune systems (top panel Fig. 3 – blue stars, eFig. 5B: “all_main”). Two genes, *ADCY3* and *BDNF*, had variants both at their promoters and contacted variants in cREs via chromatin loops (eFig. 6).

At the *TMEM18* locus on chr 2p25.3, a highly significant human obesity locus that has long been associated with both adult and childhood obesity, we observed differing degrees of evidence for 16 genes, but noted that rs6548240, rs35796073, and rs35142762 consistently contacted the *SH3YL1*, *ACP1*, and *ALKAL2* promoters across multiple cell types (Fig. 2D – third and fourth column).

At the chr 2p23 locus, *ADCY3* yielded the most contacts (i.e. many proxies contacting the same gene via chromatin loops), suggesting this locus acts as a regulatory hub. However, we observed a similar composition in cell types for four other genes: *DNAJC27*, *DNAJC27-AS1* (both previously implicated in obesity and/or diabetes traits⁴⁰), *AC013267.1*, and *SNORD14* (RF00016). *ITSN2*, *NCOA1*, and *EFR3B* were three genes within this locus that were only implicated in immune cell types. *NCOA1* encodes a prominent meta-inflammation factor⁴¹ known to reduce adipogenesis and shift the energy balance between white and brown fat, and its absence known to induce obesity⁴².

CALCR was the most frequently implicated gene at its locus, supported by 20 cell types across all systems. While within the *BDNF* locus, *METTL15* and *KIF18A* – two non-cell-type-specific genes – plus some lncRNA genes, were contacted by childhood obesity-associated proxies within the same multiple cell types as *BDNF*, again suggesting the presence of a regulatory hub.

At the *FAIM2* locus on chr 12q13.12, we observed known genes associated with obesity, eating patterns, and diabetes-related traits, including *ASIC1*, *AQP2*, *AQP5*, *AQP6*, *RACGAP1*, and *AC025154.2* (*AQP5-AS1*) along with *FAIM2* (Table 1). These genes were harbored within PIR-OCRs of astrocytes, neural progenitors, hypothalamic neurons, and multiple metabolic cell types. Plasmacytoid and CD1c+ conventional dendritic cells were the only two immune cell types that harbored such proxies within their cREs, implicating *ASIC1*, *PRPF40B*, *RPL35AP28*, *TMBIM6*, and *LSM6P2* at the *FAIM2* locus.

The independent *ADCY9* and *FTO* loci are both located on chromosome 16. Genes at the *ADCY9* locus were only implicated in a subset of immune cell types. Interestingly, genes at the *FTO* locus were only implicated in Hi-C datasets (as opposed to Capture C), including 6 metabolic cell types and astrocytes. Most genes at the *FTO* locus were implicated in skeletal myotubes, differentiated osteoblasts, and astrocytes, namely *FTO* and *IRX3*; while *IRX5*, *CRNDE*, and *AC106738.1* were also implicated in adipocytes and hepatocytes.

The most implicated cell types by two sets of analyses

EndoC-BH1 and myotubes are the two cell types in which we implicated the most effector genes, with 38 and 42, respectively – Fig. 3 side panel. This phenomenon is likely proportional in the case of myotubes, given the large number of cREs identified by overlapped Hi-C contact data and

Table 1

PubMed-query known functions for 111 genes implicated by obesity variants.

Locus	Implicated genes	Obesity or related traits	Different traits
TNNI3K	LRRIQ3	(NA)	Associated with opioid usage [PMID:34728798] and MDD [PMID: 31748543]
	FPGT	Predict BMI in Korean pop. [PMID: 28674662]	(NA)
	FPGT-TNNI3K		Associated with MDD [PMID: 31748543]
	LRRC53	Associated with high BMI increased risk heart attack [PMID: 32471361]	(NA)
	ASTN1	Identified as obesity QTL in rat [PMID: 35729251]	Associated with neurodevelopmental traits [PMID: 24381304] and variety of cancers [PMID: 32945491]
	BRINP2	(NA)	Associated with neurodevelopmental traits [PMID: 34267256]
SEC16B	AL122019.1	(NA)	
	AL162431.1		
TMEM18	FAM110C	(NA)	Overexpression induces microtubule aberrancies [PMID: 17499476], involved in cell spreading and migration [PMID: 19698782]
	SH3YL1	Associated with BMI in type 2 diabetes nephropathy [PMID: 33223406]	Influence on T cell activation [PMID: 31427643], involved in different cancer types [PMID: 26305679,24508479]
	ACP1	Associated with early-onset obesity [PMID: 24129437], correlated with cardiovascular risks [PMID: 19570551], drive adipocyte differentiation via control of pdgfra signaling [PMID: 33615467]	Associated with bipolar disorder [PMID: 31830721]
	ALKAL2	Associated with childhood BMI [PMID: 33627773]	Enhance expression in response to inflammatory pain in nociceptors [PMID: 35608912, 35610945]
	MYT1L	Associated with early-onset obesity [PMID: 24129437]	(NA)
	AC079779.1	(NA)	
	AC079779.2		
	AC079779.3		
	AC079779.4		
	LINC01865		
	AC105393.2		
	AC105393.1		

	LINC01874		
	LINC01875		
	AC093326.1		
	AC141930.2		
ADCY3	ITSN2	(NA)	Regulate T-cells function [PMID: 32618424] and help the interaction with B-cells [PMID: 29337666]
	NCOA1	Meta-inflammation gene [PMID: 25647480], reduce adipogenesis, shift the energy balance between white and brown fat [PMID: 31133421]	(NA)
	ADCY3	Regulate/impair MC4R within energy-regulating melanocortin signaling pathway [PMID: 29311635, 32955435]	
	DNAJC27-AS1	Linked to obesity, diabetes traits [PMID: 30131766]	
	DNAJC27	Linked to obesity, diabetes traits [PMID: 30131766]	
	EFR3B	Associated with T1D [PMID: 21980299], down-regulated in rare obesity-related disorder [PMID: 25705109]	
	WDR43	(NA)	Associated with breast cancer [PMID: 27117709]
	AC013267.1	(NA)	
	RF00016		
GPR1	GPR1	Increase expression in obese phenotype [PMID: 34174278]	(NA)
TFAP2B	TFAP2D		Involve in embryogenesis [PMID: 12711551]
CALCR	HEPACAM2	(NA)	Associated with colorectal cancer [PMID: 29659199, 29973580]
	VPS50		Involve in neurodevelopmental disorders and defects [PMID: 30828385, 34037727]
	MIR653		Involve in different types of cancer [PMID: 35777307]
	MIR489	Promote adipogenesis in mice [PMID: 34004251]	(NA)
	CALCR	Associated with BMI and control of food-intake [PMID: 34462445, 34210852, 31955990, 29522093]	
	TFPI2	(NA)	Involved in colorectal cancer [PMID: 35004840, 34092617, 25902909]

Table 1 (continued)

	BET1	Involved in triacylglycerol metabolism [PMID: 24423365]	Associated with muscular dystrophy [PMID: 34310943, 34779586]
	AC003092.1	(NA)	Association with glioblastoma [PMID: 33815468, 30442884]
	AC002076.1	(NA)	
BDNF	LIN7C	Associated in T2D [PMID: 20215397] , obesity [PMID: 23044507]	Associated with psychopathology [PMID: 23044507]
	BDNF-AS	Regulate <i>BDNF</i> and <i>LIN7C</i> expression [PMID: 22960213, 22446693]	(NA)
	BDNF	Regulate eating behavior and energy balance [PMID: 34556834]	
	MIR610	(NA)	Involve in different types of cancer [PMID: 34408418, 29228616, 26885452]
	KIF18A		Involve in different types of cancer [PMID: 35591854, 35286090]
	METTL15	Associated with childhood obesity [PMID: 31504550]	(NA)
	AC090124.1	(NA)	Reported to differentially prognostic of pancreatic cancer [PMID: 34307375]
	ARL14EP		Involve in WAGR syndrome [PMID: 36011342, 31511512]
	DCDC1		Involvement with eyes anomalies [PMID: 34773354, 34703991]
	THEM7P		Associated with mechanisms underlying inguinal hernia [PMID: 34392144]
	AL035078.2		(NA)
	ELP4		
	LINC00678		
	AC023206.1		
	RN7SKP158		
	AC104978.1		
	MIR8068		
	AC013714.1		
	AC100773.1		
	AC090833.1		
	AC090791.1		
	AC110056.1		
	AL035078.2		

Table 1 (continued)

FAIM2	PRPF40B	(NA)	Splicing regulator involved in T-cell development [PMID: 31088860, 34323272]	
	TMBIM6	Deficiency leads to obesity by increasing Ca2+-dependent insulin secretion [PMID: 32394396]	Immune cell function and survival [PMID: 26470731]	
	BCDIN3D	Associated with obesity, T2D [PMID: 20215397]	(NA)	
	FAIM2	Associated with childhood obesity [PMID: 31504550]		
	AQP2	Associated with obesity, diabetes [PMID: 33367818]		
	AQP5	Associated with non-obese diabetes [PMID: 25635992,22320885]	Responsible for transporting water, involve in Sjogren's syndrome [PMID: 25635992, 31557796]	
	AQP6	Down-regulated in retina in diabetes [PMID: 21851171]	Associated with renal diseases [PMID: 30654539]	
	RACGAP1	Involve in diabetes nephropathy [PMID: 35222021]	(NA)	
	ASIC1	Inhibition increase food intake and decrease energy expenditure [PMID: 35894166]		
	LSM6P2	(NA)		
	RPL35AP28			
	LINC02396			
	LINC02395			
	AC025154.1			
	AC025154.2			
ADCY9	SLX4	(NA)	Associated with blood pressure [PMID: 30671673]	
	DNASE1	Associated with obesity hypertension [PMID: 33351325]	(NA)	
	TRAP1	Involve in global metabolic network, deletion reduce obesity incidence [PMID: 25088416]		
	CREBBP	Associated with high adiposity and low cardiometabolic risk [PMID: 33619380]		
	ADCY9	Asociated with BMI, obesity [PMID: 33619380, 23563607]		
	SRL	(NA)	Involve in cardiac dysfunction [PMID: 22119571]	
	LINC01569		Associated with cancer and endometriosis [PMID: 35341703, 34422671]	
	TFAP4		Associated with BMI, birth weight,	(NA)

Table 1 (continued)

		maternal glycemic [PMID: 35708509]	
	AC012676.1	(NA)	Involve in hepatocellular carcinoma [PMID: 35210216]
	AC009171.2	(NA)	
FTO	FTO	Most extensively studied obesity locus [PMID: 34556834]	(NA)
	IRX3	Obesogenic effects in adipocytes [PMID: 26760096] , brain [PMID: 24646999] , pancreas[93]	
	IRX5		
	AC018553.1	(NA)	Associated with melanoma [PMID: 35611195]
	CRNDE	Regulator of angiogenesis in obesity-induced diabetes [PMID: 31863035]	(NA)
	MMP2	Involve in obesity-relate angiogenesis [PMID: 35919566]	
	CAPNS2	(NA)	
	AMFR	Involve in hepatic lipid metabolism [PMID: 33591966]	(NA)
	CETP	Involve in monogenic hyperalphalipoproteinemia [PMID: 34878751]	
	RPGRIP1L	Hypomorphism of this ciliary gene linked to morbid obesity [PMID: 27064284, 30597647, 29657248]	
	LINC02169	(NA)	Associated with occupational exposure to gases/fumes and mineral dust [PMID: 31152171]
	AC007491.1	(NA)	
	AC018553.2		
	LINC02140		
	AC106738.1		
AC106738.2			
MTND5P34			
AC007336.1			
MC4R	AC090771.1	(NA)	

Table 1 (continued)

ATAC-seq open regions (**Fig. 1A** [↗](#)), but not for EndoC-BH1. Albeit harboring an average number of cREs compared to other cell types, EndoC-BH1 cells were consistently among the top-ranked heritability estimates for the childhood obesity variants resulting from the EGG consortium GWAS (**Fig. 1** [↗](#)) and harbored a significant number of implicated genes by the mapping of proxies. Interestingly, the pancreatic alpha cell type – shown above to be the most significant for heritability estimate by LDSC – revealed only 6 implicated genes contacted by the defined proxies, namely *BDNF* and five lncRNA genes.

Pathway analysis

Of the 111 implicated genes in total, PubMed query revealed functional studies for 66 genes. The remaining were principally lncRNA and miRNA genes with currently undefined functions (**Table 1** [↗](#)). To investigate how our implicated genes could confer obesity risk, we performed several pathway analyses keeping them either separated for each cell type or pooling into the respective metabolic, neural, or immune system sets. **eFig. 7** shows simple Gene Ontology (GO) biological process terms enrichment results.

Leveraging the availability of our expression data generated via RNA-seq (available for 46 of 57 cell types), we performed pathway analysis. Given that our gene sets from the variant-to-gene process was stringently mapped, the sparse enrichment from normal direct analyses is not ideal for exploring obesity genetic etiology. Thus, we incorporated two methods from the *pathfindR* package⁴³ [↗](#) and our customized *SPIA* (details in **eMethods**). The result of 60 enriched KEGG terms is shown in **eFig. 8** (**eTable 4**), with 13 genes in 14 cell types for *pathfindR* and 39 enriched KEGG terms shown in **eFig. 9** (**eTable 5**), with 10 genes in 42 cell types for customized *SPIA*. There were 20 overlapping pathways between the two approaches (yellow rows in **eTable 4&5**) including many signaling pathways such as the GnRH (hsa04912), cAMP (hsa04024), HIF-1 (hsa04066), Glucagon (hsa04922), Relaxin (hsa04926), Apelin (hsa04371), and Phospholipase D (hsa04072) signaling pathways. They were all driven by one or more of these 5 genes: *ADCY3*, *ADCY9*, *CREBBP*, *MMP2*, and *NCOA1*. Interestingly, we observed the involvement of natural killer cells in nearly all the enriched KEGG terms from *pathfindR* due to the high expression of the two adenylyl cyclase encoded genes, *ADCY3* and *ADCY9*, along with *CREBBP*. The *SPIA* approach disregarded the aquaporin genes (given they appear so frequently in so many pathways that involve cellular channels) but highlighted the central role of *BDNF* which single-handedly drove four signaling pathways: the Ras, Neurotrophin, PI3K-Akt, and MAPK signaling pathways. This also revealed the role of *TRAP1* in neurodegeneration.

These two approaches did not discount the role of *FAIM2* and *CALCR*. However, their absence was mainly due to the content of the current KEGG database. On the other hand, these approaches accentuated the role of the *MMP2* gene at the *FTO* locus in skeletal myotubes, given its consistency within the GnRH signaling pathway (**eFig. 10**), which is in line with previous studies linking its expression with obesity⁴⁴ [↗](#)–⁴⁶ [↗](#).

Supportive evidence by colocalization of target effector genes with eQTLs

The GTEx consortium has characterized thousands of eQTLs, albeit in heterogeneous bulk tissues⁴⁷ [↗](#). To assess how many observed gene-SNP pairs agreed with our physical variant-to-gene mapping approach in our multiple separate cellular settings, we performed colocalization analysis using ColocQuiaL³⁸ [↗](#).

282 genes were reported to be associated with the variants within 13 loci from our variant-to-genes analysis. We found 114 colocalizations for ten of our loci that had high conditioned posterior probabilities (cond.PPH4.abf ≥ 0.8), involving 44 genes and 41 tissues among the eQTLs. We extracted the posterior probabilities for each SNP within each colocalization and selected the 95% credible set as the likely causal variants (complete list in **eTable 6**). Despite sensitivity differences

and varying cellular settings, when compared with our variant-to-gene mapping results, colocalization analysis yielded consistent identification for 21 pairs of SNP-gene interactions when considering the analyses across all our cell types, composed of 20 SNPs and 7 genes. Details of these SNP-gene pairs are shown in **Fig. 4A** and **B**.

Of these 20 SNPs, 15 were at the *ADCY3* locus, in LD with sentinel variant rs4077678, and all implicated *ADCY3* as the effector gene in 29 cell types – 15 metabolic, 6 immune, 4 neural cell types and 4 independent cell lines (**Fig. 4C**). Indeed, missense mutations have been previously reported for this gene in the context of obesity^{48,49} while another member of this gene family, *ADCY5*, has also been extensively implicated in metabolic traits⁵⁰.

Predicting transcription factors (TFs) binding disruption at implicated genes contributing to obesity risk

TFs regulate gene expression by binding to DNA motifs at enhancers and silencers, where any disruption by a SNP can potentially cause dysregulation of a target gene. Thus, we used *motifbreakR* (R package) to predict such possible events at the loci identified by our variant-to-gene mapping. Each variant was predicted to disrupt the binding of several different TFs, thus requiring further literature cross-examination to select the most probable effects. For example, rs7132908 (consistently contacting *FAIM2* in 25 cell types) was predicted to disrupt the binding of 12 different transcription factors. Among them, SREBF1 (**eFig. 11A**) was the only TF that concurred with evidence that it regulates *AQP2* and *FAIM2* at the same enhancer⁵¹. The full prediction list can be found in **eTable 7**.

To narrow down the list of putative TF binding sites at each variant position, we leveraged the ATAC-seq footprint analysis using the RGT suite⁵². The final set of Motif-Predicted Binding Sites (MPBS) within each cell type ATAC-seq footprints was used to overlap with the genomic locations of the OCRs, and then overlapped with our obesity variants, resulting in annotated 29 variants. Mosaic plot in **eFig. 11B** shows the number and proportions of variants predicted by *motifbreakR* and/or overlapped with MPBS. Insignificant *P*-value from Fisher's exact test indicated the independence of the two analyses. Only seven variants were found within the cREs for the same TF motifs predicted to be disrupted by *motifbreakR* (**eFig. 11C**). **eFig. 12** outlines the seven variants that *motifbreakR* and ATAC-seq footprint analysis agreed on the TF bindings they might disrupt.

Discussion

Given the challenge of uncovering the underlying molecular mechanisms driving such a multifactorial disease as obesity, our approach leveraging GWAS summary statistics, RNA-seq, ATAC-seq, and promoter Capture C / Hi-C offers new insights. This is particularly true as it is becoming increasingly evident that multiple effector genes can operate in a temporal fashion at a given locus depending on cell state, including at the *FTO* locus⁵³. Our approach offers an opportunity to implicate relevant cis-regulatory regions across different cell types contributing to the genetic etiology of the disease. By assigning GWAS signals to candidate causal variants and corresponding putative effector genes via open chromatin and chromatin contact information, we enhanced the fine-mapping process with an experimental genomic perspective to yield new insights into the biological pathways influencing childhood obesity.

LD score regression is a valuable method that estimates the relationship between linkage disequilibrium score and the summary statistics of GWAS SNPs to quantify the separate contributions of polygenic effects and various confounding factors that produce SNP-based heritability of disease. The general positive heritability enrichment across our open chromatin features spanning multiple cell types (**Fig. 1B**) reinforces the notion that obesity etiology involves many systems in our body.

While obesity has long been known to be a risk factor for pancreatitis and pancreatic cancer, the significant enrichment of pancreatic alpha and beta cell related 3D genomic features for childhood obesity GWAS signals demonstrates the bidirectional relationship between obesity and the pancreas; indeed, it is well established that insulin has obesogenic properties.

Moreover, the comorbidity of obesity and diabetes (either causal or a result of the overlap between SNPs associated with these two diseases) is tangible. When focusing on genetic annotation of the cREs only, the association with obesity became more diverse across cell types, especially in metabolic cells. Interestingly, the lack of enrichment (only 8 of 57 cell types yielded no degree of enrichment) of obesity SNPs heritability in open gene promoters (**Fig. 1B** [↗](#).b) reveals that cRE regions harboring obesity SNPs are more involved in gene regulation than disruption, and therefore potentially contributing more weight to the manifestation of the disease.

Of course, we should factor in the effective sample sizes of the GWAS efforts that are wide-ranging (2,000-24,000 – given that the N for each variant is different within a single dataset, thus contributing to the weights and *P*-value of each SNP when the algorithm calculates the genome-wide heritability), which could result in noise and negative enrichment observed in the analysis – a methodology limitation of partial linkage regression that has been extensively discussed in the field⁵⁴ [↗](#). Thus, it is crucial to interpret the enrichment (or lack thereof) of disease variants in a certain cellular setting with an *ad hoc* biological context.

From mapping the common proxies of 19 independent sentinel SNPs that were genome-wide significantly associated with childhood obesity to putative effector genes through chromatin contacting cREs, one striking finding was the several potential “hubs” of putatively core effector genes, whose occurrence spread across three human physiological systems. With the data available from so many cell types, our approach connected new candidate causal variants to known obesity-related genes and new implications of cell modality for previously known associations.

A potential application of this association could be to fine-tune the effect of a drug toward controlling appetite. An example of bringing new aspects to the old is for the signal within the *FTO* locus that contacted *IRX3* and *IRX5*: previous studies have suggested these obesogenic effects operate in adipocytes⁵⁵ [↗](#), brain⁵⁶ [↗](#), or pancreas⁵⁷ [↗](#); here we confirmed this association in adipocytes and uncover the presence of distal chromatin contacts in myotubes for the first time.

Besides the above-mentioned genes with known associations with obesity, we discovered newly implicated genes. For example, the *LRRIQ3* gene at the *TNNI3K* locus had its open promoter contacted by two SNPs, rs1040070 and rs10493544, in NTERA2 cells only. The published studies⁵⁸ [↗](#),⁵⁹ [↗](#) that associated *LRRIQ3* with major depressive disorder and opioid usage acknowledged the overlapping promoter of this gene, albeit in the opposite direction, with a run-through transcript of *FPGT-TNNI3K* – previously shown to be associated with BMI in European⁶⁰ [↗](#) and Korean populations⁶¹ [↗](#).

It is apparent that not all the implicated genes we report would contribute equally to the susceptibility of obesity pathogenesis. Each locus comprises genes whose functions are obviously related to obesity or similar traits like BMI, fat weight, etc., while other genes are not so directly obvious in their relation to these traits.

It is encouraging that for implicated genes within these multi-cell-type loci across different physiological systems we could find previous associations to the corresponding cell types or systems. Examples are the two aforementioned genes at the *TMEM18* locus (*SH3YL1* and *ACPI*)⁶² [↗](#)–⁶⁶ [↗](#) with the broad spectrum of their functions, *HEPACAM2* implicated in the NCIH716 cell line at the *CALCR* locus⁶⁷ [↗](#),⁶⁸ [↗](#), and *LRRIQ3* in the NTERA2 cell line at the *TNNI3K* locus⁶⁹ [↗](#).

Chronic inflammation is an essential characteristic of obesity pathogenesis. Adipose tissue-resident immune cells have been observed, leading to an increased focus in recent years on their potential contribution to metabolic dysfunction. On the other hand, neurological or psychological conditions, such as stress, induce the secretion of both glucocorticoids (increase motivation for food) and insulin (promotes food intake and obesity). Pleasure feeding then reduces activity in the stress-response network, reinforcing the feeding habit. It has been shown that voluntary behaviors, stimulated by external or internal stressors or pleasurable feelings, memories, and habits, can override the basic homeostatic controls of energy balance.⁷⁰ The potential link between the immune system and metabolic disease, and moreover, through the neural system, was tangible in our findings.

Two of the three SNPs which ranked the third most consistent in our variant-to-gene mapping (**Fig. 2C**) – rs35796073 and rs35142762 – contacted the *ALKAL2* promoter (supported by GTEx evidence to colocalize with *ALKAL2* expression). The anaplastic lymphoma kinase (encoded by *ALK* gene) is a receptor tyrosine kinase, belongs to the insulin receptor family, and has been reported to promote nerve cell growth and differentiation.^{71,72} Despite *ALKAL2* (*ALK* and *LTK* ligand 2) being studied principally in the context of immunity, a recent study using the EGCUT biobank GWAS identified *ALK* as a candidate thinness gene and genetic deletion showed that its expression in hypothalamic neurons acts as a negative regulator in controlling energy expenditure via sympathetic control of adipose tissue lipolysis.⁷³ *ALKAL2* – encoding a high-affinity agonist of *ALK/LTK* receptors – which has been reported to enhance expression in response to inflammatory pain in nociceptors.^{74,75} – has been recently implicated as a novel candidate gene for childhood BMI by transcriptome-wide association study,⁷⁶ and achieved genome-wide significance in a GWAS study contrasting persistent healthy thinness with severe early-onset obesity using the STILTS and SCOOP cohorts.⁷⁷ The finding that overexpression of *ALKAL2* could potentiate neuroblastoma progression in the absence of *ALK* mutation⁷⁸ echoes the relationship between *ADCY3* and *MC4R*,⁷⁹ where a peripheral gene, *ADCY3*, can regulate/impair the function of a core gene, i.e. *MC4R*, within the energy-regulating melanocortin signaling pathway.⁸⁰

Our approach implicates putative target genes based on a mechanism of regulation for these variants to alter gene expression – through regulator TF(s) that bind to these contact sites. A potential limitation of the predictions from *motifbreakR* and matching TF motifs to ATAC-seq footprint by the RGT toolkit is that they were both based on the position probability matrixes of Jaspur and Hocomoco, which come from public motif databases. The ATAC-seq footprint analysis also carries sequence bias that can lead to false positive discovery. Thus, our attempt to call such regulators by predicting TF binding disruption can only serve as nominations – but warrant further functional follow up.

Another limitation of this work is the diversity in data quality among different samples, since different datasets were sampled and collected at different time points, from different patients, using different protocols, with libraries sequenced at different depths and qualities, and initially preprocessed with different pipelines and parameters. Thus, it is crucial to keep in mind that the discrepancy in data points might have resulted from variations in data quality. Importantly, any association discovered must be validated functionally before effector genes of the genetic variants can be leveraged to develop new therapies. Their putative function(s) must be characterized, together with the mechanism whereby the given variant's alleles differentially affect the expression of the targeted genes. The next step is to explore how the target genes affect the trait of interest more directly.

Our results have provided a set of leads for future exploratory experiments in specific cellular settings in order to further expand our knowledge of childhood obesity genomics and hence equip us with more effective means to overcome the burden of this systematic disease.

Conclusion

Our approach of combining RNA-seq, ATAC-seq, and promoter Capture C/Hi-C datasets with GWAS summary statistics offers a systemic view of the multi-cellular nature of childhood obesity, shedding light on potential regulatory regions and effector genes. By leveraging physical properties, such as open chromatin status and chromatin contacts, we enhanced the fine-mapping process and gained new insights into the biological pathways influencing the disease. Although further functional validation is required, our findings provide valuable leads together with their cellular contexts for future research and the development of more effective strategies to address the burden of childhood obesity.

Data Availability

All data produced in the present study are available upon reasonable request to the authors

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Summary:

This paper studies the genetic factors contributing to childhood obesity. Through a comprehensive analysis integrating genome-wide association study (GWAS) data with 3D genomic datasets across 57 human cell types, consisting of Capture-C/Hi-C, ATAC-seq, and RNA-seq, the study identifies significant genetic contributions to obesity using stratified LD score regression, emphasizing the enrichment of genetic signals in pancreatic alpha cells and identification of significant effector genes at obesity-associated loci such as BDNF, ADCY3, TMEM18, and FTO. Additionally, the study implicated ALKAL2, a gene responsive to inflammation in nerve nociceptors, as a novel effector gene at the TMEM18 locus. This suggests a role for inflammatory and neurological pathways in obesity's pathogenesis which was supported through colocalization analysis using eQTL derived from the GTEx dataset. This comprehensive genomic analysis sheds light on the complex genetic architecture of childhood obesity, highlighting the importance of cellular context for future research and the development of more effective strategies.

Strengths:

Overall, the paper has several strengths, including leveraging large-scale, multi-modal datasets, using computational reasonable tools, and having an in-depth discussion of the significant results.

Weaknesses:

(1) The results are somewhat inconclusive or not validated.

The overall results are carefully designed, but most of the results are descriptive. While the authors are able to find additional evidence either from the literature or explain the results with their existing knowledge, none of the results have been biologically validated. Especially, the last three result sections (signaling pathways, eQTLs, and TF binding) further extended their findings, but the authors did not put the major results into any of the figures in the main text.

(2) Some technical details are missing.

While the authors described all of their analysis steps, a lot of the time, they did not mention the motivation. Sometimes, the details were also omitted. For example:

- The manuscript would benefit from a detailed explanation of the methods used to define cREs, particularly the process of intersecting OCRs with chromatin conformation data. The current description does not fully clarify how the cREs are defined.
- How did the authors define a contact region?
- The manuscript would benefit from an explanation regarding the rationale behind the selection of the 57 human cell types analyzed. It is essential to clarify whether these cell types have unique functions or relevance to childhood development and obesity.
- I wonder whether the used epigenome datasets are all from children. Although the authors use literature to support that body weight and obesity remain stable from infancy to adulthood, it remains uncertain whether epigenomic data from other life stages might overlook significant genetic variants that uniquely contribute to childhood obesity.
- Given that the GTEx tissue samples are derived from adult donors, there appears to be a mismatch with the study's focus on childhood obesity. If possible, identifying alternative validation strategies or datasets more closely related to the pediatric population could strengthen the study's findings.

(3) The writing needs to improve.

<https://doi.org/10.7554/eLife.95411.1.sa1>

Author response:

"Overall, the paper has several strengths, including leveraging large-scale, multi-modal datasets, using computational reasonable tools, and having an in-depth discussion of the significant results."

We thank the reviewer for the very supportive comments.

Based on the comments and questions, we have grouped the concerns and corresponding responses into three categories.

(1) The scope and data selection

"The results are somewhat inconclusive or not validated."

The overall results are carefully designed, but most of the results are descriptive. While the authors are able to find additional evidence either from the literature or explain the results with their existing knowledge, none of the results have been biologically validated. Especially, the last three result sections (signaling pathways, eQTLs, and TF binding) further extended their findings, but the authors did not put the major results into any of the figures in the main text."

The goal of this manuscript is to provide a list of putative childhood obesity target genes to yield new insights and help drive further experimentation. Moreover, the outputs from signaling pathways, eQTLs, and TF binding, although noteworthy and supportive of our method, were not particularly novel. In our manuscript we placed our focus on the novel findings from the analyses. We did, however, report the part of the eQTLs analysis concerning *ADCY3*, which brought new insight to the pathology of obesity, in Figure 4C.

"The manuscript would benefit from an explanation regarding the rationale behind the selection of the 57 human cell types analyzed. It is essential to clarify whether these cell types have unique functions or relevance to childhood development and obesity."

We elected to comprehensively investigate the GWAS-informed cellular underpinnings of childhood development and obesity. By including a diverse range of cell types from different tissues and organs, we sought to capture the multifaceted nature of cellular contributions to obesity-related mechanisms, and open new avenues for targeted therapeutic interventions.

There are clearly cell types that are already established as being key to the pathogenesis of obesity when dysregulated: adipocytes for energy storage, immune cell types regulating inflammation and metabolic homeostasis, hepatocytes regulating lipid metabolism, pancreatic cell types intricately involved in glucose and lipid metabolism, skeletal muscle for glucose uptake and metabolism, and brain cell types in the regulation of appetite, energy expenditure, and metabolic homeostasis.

While it is practical to focus on cell types already proven to be associated with or relevant to obesity, this approach has its limitations. It confines our understanding to established knowledge and rules out the potential for discovering novel insights from new cellular mechanisms or pathways that could play significant roles in the pathogenesis of obesity. Therefore, it was essential to reflect known biology against the unexplored cell types to expand our overall understanding and potentially identify innovative targets for treatment or prevention.

"I wonder whether the used epigenome datasets are all from children. Although the authors use literature to support that body weight and obesity remain stable from infancy to adulthood, it remains uncertain whether epigenomic data from other life stages might overlook significant genetic variants that uniquely contribute to childhood obesity."

The datasets utilized in our study were derived from a combination of sources, both pediatric and adult. We recognize that epigenetic profiles can vary across different life stages but our principal effort was to characterize susceptibility BEFORE disease onset.

"Given that the GTEx tissue samples are derived from adult donors, there appears to be a mismatch with the study's focus on childhood obesity. If possible, identifying alternative validation strategies or datasets more closely related to the pediatric population could strengthen the study's findings."

We thank the reviewer for raising this important point. We acknowledge that the GTEx tissue samples are derived from adult donors, which might not perfectly align with the study's focus on childhood obesity. The ideal strategy would be a longitudinal design that follows individuals from childhood into adulthood to bridge the gap between pediatric and adult data, offering systematic insights into how early-life epigenetic markers influence obesity later in life. In future work, we aim to carry out such efforts, which will represent substantial time and financial commitment.

Along the same lines, the Developmental Genotype-Tissue Expression (dGTEx) Project is a new effort to study development-specific genetic effects on gene expression at 4 developmental windows spanning from infant to post-puberty (0-18 years). Donor recruitment began in August 2023 and remains ongoing. Tissue characterization and data production are underway. We hope that with the establishment of this resource, our future research in the field of pediatric health will be further enhanced.

"Figure 1B: in subplots c and d, the results are either from Hi-C or capture-C. Although the authors use different colors to denote them, I cannot help wondering how much difference between Hi-C and capture-C brings in. Did the authors explore the difference between the Hi-C and capture-C?"

Thank you for your comment. It is not within the scope of our paper to explore the differences between the Hi-C and Capture-C methods. In the context of our study, both methods serve the same purpose of detecting chromatin loops that bring putative enhancers to sometimes genomically distant gene promoters. Consequently, our focus was on utilizing these methods to identify relevant chromatin interactions rather than comparing their technical differences.

(2) Details on defining different categories of the regions of interest

"Some technical details are missing.

While the authors described all of their analysis steps, a lot of the time, they did not mention the motivation. Sometimes, the details were also omitted."

We will add a section to the revision to address the rationale behind different OCRs categories.

"Line 129: should "-1,500/+500bp" be "-500/+500bp"?"

A gene promoter was defined as a region 1,500 bases upstream to 500 bases downstream of the TSS. Most transcription factor binding sites are distributed upstream (5') from TSS, and the assembly of transcription machinery occurs up to 1000 bases 5' from TSS. Given our interest in SNPs that can potentially disrupt transcription factor binding, this defined promoter length allowed us to capture such SNPs in our analyses.

"How did the authors define a contact region?"

Chromatin contact regions identified by Hi-C or Capture-C assays are always reported as pairs of chromatin regions. The Supplementary eMethods provide details on the method of processing and interaction calling from the Hi-C and Capture-C data.

"The manuscript would benefit from a detailed explanation of the methods used to define cREs, particularly the process of intersecting OCRs with chromatin conformation data. The current description does not fully clarify how the cREs are defined."

"In the result section titled "Consistency and diversity of childhood obesity proxy variants mapped to cREs", the authors introduced the different types of cREs in the context of open chromatin regions and chromatin contact regions, and TSS. Figure 2A is helpful in some way, but more explanation is definitely needed. For example, it seems that the authors introduced three chromatin contacts on purpose, but I did not quite get the overall motivation."

We apologize for the confusion. Our definition of cREs is consistent throughout the study. Figure 2A will be the first Figure 1A in the revision in order to aid the reader.

The 3 representative chromatin loops illustrate different ways the chromatin contact regions (pairs of blue regions under blue arcs) can overlap with OCRs (yellow regions under yellow triangles – ATAC peaks) and gene promoters.

[1] The first chromatin loop has one contact region that overlaps with OCRs at one end and with the gene promoter at the other. This satisfies the formation of cREs; thus, the area under the yellow ATAC-peak triangle is green.

[2] The second loop only overlapped with OCR at one end, and there was no gene promoter nearby, so it is unqualified as cREs formation.

[3] The third chromatin loop has OCR and promoter overlapping at one end. We defined this as a special cRE formation; thus, the area under the yellow ATAC-peak triangle is green.

To avoid further confusion for the reader, we will eliminate this variation in the new illustration for the revised manuscript.

"Figure 2A: The authors used triangles filled differently to denote different types of cREs but I wonder what the height of the triangles implies. Please specify."

The triangles are illustrations for ATAC-seq peaks, and the yellow chromatin regions under them are OCRs. The different heights of ATAC-seq peaks are usually quantified as intensity values for OCRs. However, in our study, when an ATAC-seq peak passed the significance threshold from the data pipeline, we only considered their locations, regardless of their intensities. To avoid further confusion for the reader, we will eliminate this variation in the new illustration for the revised manuscript.

"Figure 1B-c. the title should be "OCRs at putative cREs". Similarly in Figure 1B-d."

cREs are a subset of OCRs.

- In the section "Cell type specific partitioned heritability", the authors used "4 defined sets of input genomic regions". Are you corresponding to the four types of regions in Figure 2A?

Figure 2A will be the first Figure 1A in the revision and will be modified to showcase how we define OCRs and cREs.

"It seems that the authors described the 771 proxies in "Genetic loci included in variant-to-genes mapping" (ln 154), and then somehow narrowed down from 771 to 94 (according to ln 199) because they are cREs. It would be great if the authors could describe the selection procedure together, rather than isolated, which made it quite difficult to understand."

In the Methods section entitled "Genetic loci included in variant-to-genes mapping," we described the process of LD expansion to include 771 proxies from 19 sentinel obesity-significantly associated signals. Not all of these proxies are located within our defined cREs. Figure 2B, now Figure 2A in the revision, illustrates different proportions of these proxies located within different types of regions, reducing the proxy list to 94 located within our defined cREs.

"Figure 2. What's the difference between the 771 and 758 proxies?"

13 out of 771 proxies did not fall within any defined regions. The remaining 758 were located within contact regions of at least one cell type regardless of chromatin state.

(3) Typos

"In the paragraph "Childhood obesity GWAS summary statistics", the authors may want to describe the case/control numbers in two stages differently. "in stage 1" and "921 cases" together made me think "1,921" is one number."

This will be amended in the revision.

"Hi-C technology should be spelled as Hi-C. There are many places, it is miss-spelled as "hi-C". In Figure 1, the author used "hiC" in the legend. Similarly, Capture-C sometime was spelled as "capture-C" in the manuscript."

"At the end of the fifth row in the second paragraph of the Introduction section: "exisit" should be "exist".

"In Figure 2A: "Within open chromatin contract region" should be "Within open chromatin contact region".

These typos and terminology inconsistencies will be amended in the revision.

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