Environment-wide and epigenome-wide association study of adiposity in 'Children of 1997' birth cohort: abridged secondary publication

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KEY MESSAGES

1. We identified several potential targets for obesity prevention including maternal exposure to second-hand smoke, consumption of artificially sweetened beverages, earlier puberty, and binge eating. We also identified several methylation loci associated with adiposity.
2. Identification of potential drivers of adiposity in Hong Kong Chinese individuals helps development of health policy interventions and future research.

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Introduction

Early-life adiposity persists into adulthood and increases the risks of multiple chronic diseases. Most nutritional studies focus on specific dietary factors; however, dietary patterns often co-occur and are difficult to distinguish from many other exposures. Environment-wide association studies (EWAS) enable various exposures across the human environmental exposome to be tested in a high-throughput manner, similar to genome-wide association studies that explore genetic effects.[1]

Effective treatments for obesity are limited, partly owing to limited understanding of the underlying molecular pathways. DNA methylation, which refers to the addition of a methyl group at the 5′ position of a cytosine residue in DNA, can modulate gene expression and thus influence an individual's susceptibility to obesity or obesity-related chronic diseases.[2] Unlike genetic variants, DNA methylation statuses are modifiable and may change in response to environmental factors or disease. Therefore, study results of Western populations may not be generalisable to Chinese populations because of differences in environmental exposure and economic development history.

Hong Kong can provide unique insights concerning health determinants, because it has mixed Western and Chinese cultures. Most Chinese people in Hong Kong are first-, second-, or third-generation migrants from neighbouring Guangdong Province in southern China. Their dietary habits are similar to those in southern China but also influenced by Western culture. Although active smoking among Chinese mothers was rare, maternal exposure to second-hand smoke during pregnancy was common before the implementation of a smoking ban in public places and workplaces in 2007 in Hong Kong. Moreover, most long-term studies involve populations of European descent in developed countries. Thus, a study of young Chinese people in Hong Kong may help to determine whether these associations reflect specific socioeconomic contexts or biological factors. For example, the associations of breastfeeding with childhood and adolescent adiposity in the 'Children of 1997' birth cohort are higher than associations typically present in Western settings.[3] We thus conducted an environment-wide and epigenome-wide association study to identify potential drivers of adiposity.

Methods

This study was based on the 'Children of 1997' birth cohort in Hong Kong.[4] Participants were recruited after birth in April and May 1997 at all 49 Maternal and Child Health Centres (MCHCs) in Hong Kong, which provide free check-ups and immunisations; the study included 88% of births in that period. A self-administered questionnaire in Chinese was used to collect baseline information concerning family, education, and birth characteristics, as well as infant feeding and second-hand smoke data. The initial study was primarily designed to provide a short-term assessment of the effects of second-hand smoke, and it included follow-up via the MCHCs until 18 months. In 2005, with support from the Health and Health Services Research Fund and Health and Medical Research Fund, we further collected data regarding infant characteristics, serious morbidities, childhood adiposity, pubertal development, migration history, and socioeconomic

status. Regular updates about subsequent growth were obtained from the Student Health Service. In 2007, with support from The University of Hong Kong University Research Committee Strategic Research Theme of Public Health, we re-established contact with the cohort and conducted several follow-up studies, including three questionnaire/telephone surveys and an in-person Biobank clinical follow-up at age ~17.5 years. More than 3500 participants attended the Biobank clinical follow-up, and their blood samples were stored. In 2020 (at age ~23 years), we conducted a follow-up survey to obtain updated information about anthropometric measurements.

Body mass index (BMI) was assessed by bioelectrical impedance analysis using a Tanita segmental body composition monitor (Tanita BC-545, Tanita, Tokyo, Japan). Waist and hip circumferences were measured twice with a tape measure. At age ~23 years, 700 participants were randomly selected from individuals with available blood samples and BMI below the 25th centile or above the 75th centile to complete a questionnaire including anthropometric measurements. A total of 308 participants replied and provided their waist, hip, height, and body weight information.

DNA methylation was examined in 288 of the 308 participants. Two samples with a sex mismatch were excluded; thus, 286 samples (168 women and 118 men) were included in the EWAS. After excluding non-CpG probes and probes located on sex chromosomes, 843 393 probes remained for analyses. Environmental exposures were defined broadly. After excluding exposures with ≥50% missing values, 370 exposures were included and classified into 13 categories: demographic, socioeconomic, family history, infant feeding, maternal information, maternal diet, diet, health status, physical activity, moods and feelings, school and academic performance, sleep, and pubertal timing. Univariable linear regression was used to assess associations of each of the 370 exposures with indicators of adiposity. Exposures that reached the Bonferroni-corrected significance threshold ($P<0.05/370 = 1.36*10^{-4}$) were included for multivariable linear regression that controlled for potential confounders. Exposures that had change-in-estimate ratios >50% were excluded. We replicated associations in the follow-up survey (n=308) and excluded associations with discordant directions.

In the epigenome-wide association study, because of the heteroscedasticity in methylation beta-values, robust linear regression models were used to determine associations of each CpG with BMI and waist-to-hip ratio (WHR) at age ~23 years, with adjustment for potential confounders. A Benjamini-Hochberg–corrected false discovery rate of <0.05 was considered statistically significant.

Results

A total of 3618 participants who attended the Biobank clinical follow-up at age ~17.5 years were included for analysis (Fig 1). After Bonferroni correction, 30 associations with BMI and 20 associations with WHR remained. After controlling for confounders, 25 associations with BMI and 11 associations with WHR remained. At age ~23 years, 25 associations with BMI and 8 associations with WHR had a consistent direction.

At age ~17.5 years, higher BMI was associated with sex, maternal exposure to second-hand smoke, diet, physical activity, health status, earlier puberty, and binge eating, whereas lower BMI was associated with consumptions of sweets and chocolate and a history of nightmares. At age ~23 years, most exposures showed consistent directions of association. However, the amount and frequency of chocolate consumption were not associated with BMI. Associations with WHR were similar to associations with BMI. Sex, consumption of artificially sweetened beverages (ASBs), and health status were associated with WHR at age ~17.5 years and age ~23 years.

In the epigenome-wide association study of 286 participants, 21 CpGs were identified for BMI in the genes *RBM16*, *SCN2B*, *AGPAT4*, *TFCP2*, *SLC24A4*, *TECPR2*, *KSR1*, *RPTOR*, *GTF3C3*, *ZNF827*, *TXNDC15*, *C2*, and *RPS6KA2*, whereas 18 CpGs were identified for WHR in the genes *LANCL2*, *C6orf195*, *MIR4535*, *CTRL*, *LYRM9*, *DCDC2*, *DIRC3*, *RPS6KA2*, *LPP*, *NFIC*, *MIR7641-2*, *ZNF141*, *RNF213*, *OPA3*, and *RRS1* (Fig 2). cg14630200 in *RPS6KA2* was a shared CpG for both BMI and WHR.

Discussion

We confirmed the risk factors for adiposity (maternal exposure to second-hand smoke and consumption of ASBs) and replicated null associations for sugar-sweetened beverages, breastfeeding, and milk consumption frequency. We also identified several CpGs associated with BMI and WHR.

Children who consume more ASBs have a higher BMI. However, association between sugar-sweetened beverages and adiposity was not found. The difference might have arisen because few participants regularly consumed sugar-sweetened beverages (only 6.8% reported daily consumption),[5] whereas 43% of participants reported daily consumption of ASBs. In addition, binge eating increased childhood BMI. Earlier puberty was associated with a higher subsequent BMI in girls. Clarification regarding this bi-directional association is worthwhile.

In the epigenome-wide association study, *RPS6KA2* was a shared gene for BMI and WHR, consistent with the results of previous studies.[2]

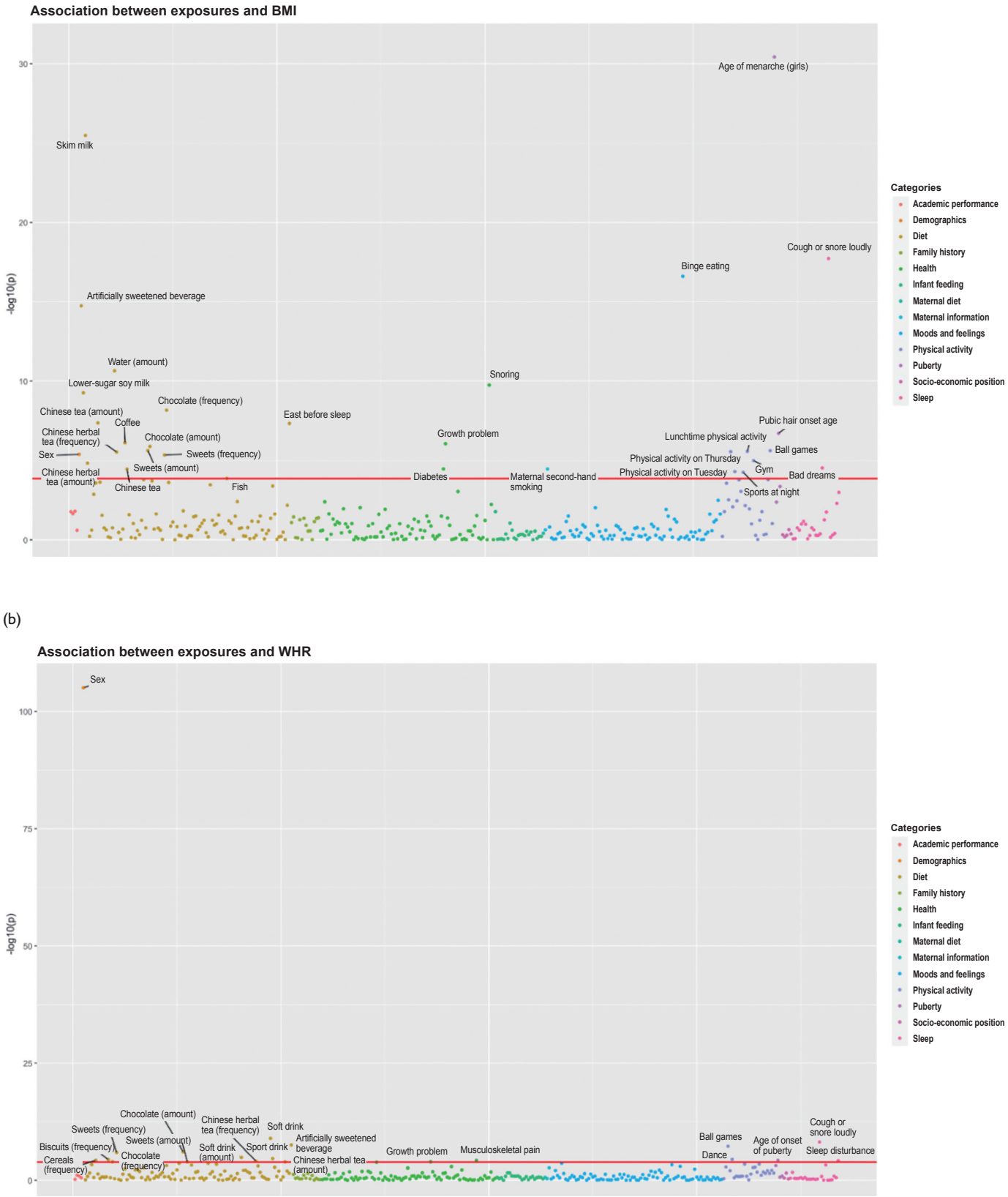

FIG 1. Associations of all exposures with (a) body mass index (BMI) and (b) waist-to-hip ratio (WHR) at age ~17.5 years among 3618 participants from the 'Children of 1997' birth cohort in the Biobank clinical follow-up. Cut-off lines indicate Bonferroni-corrected P value thresholds

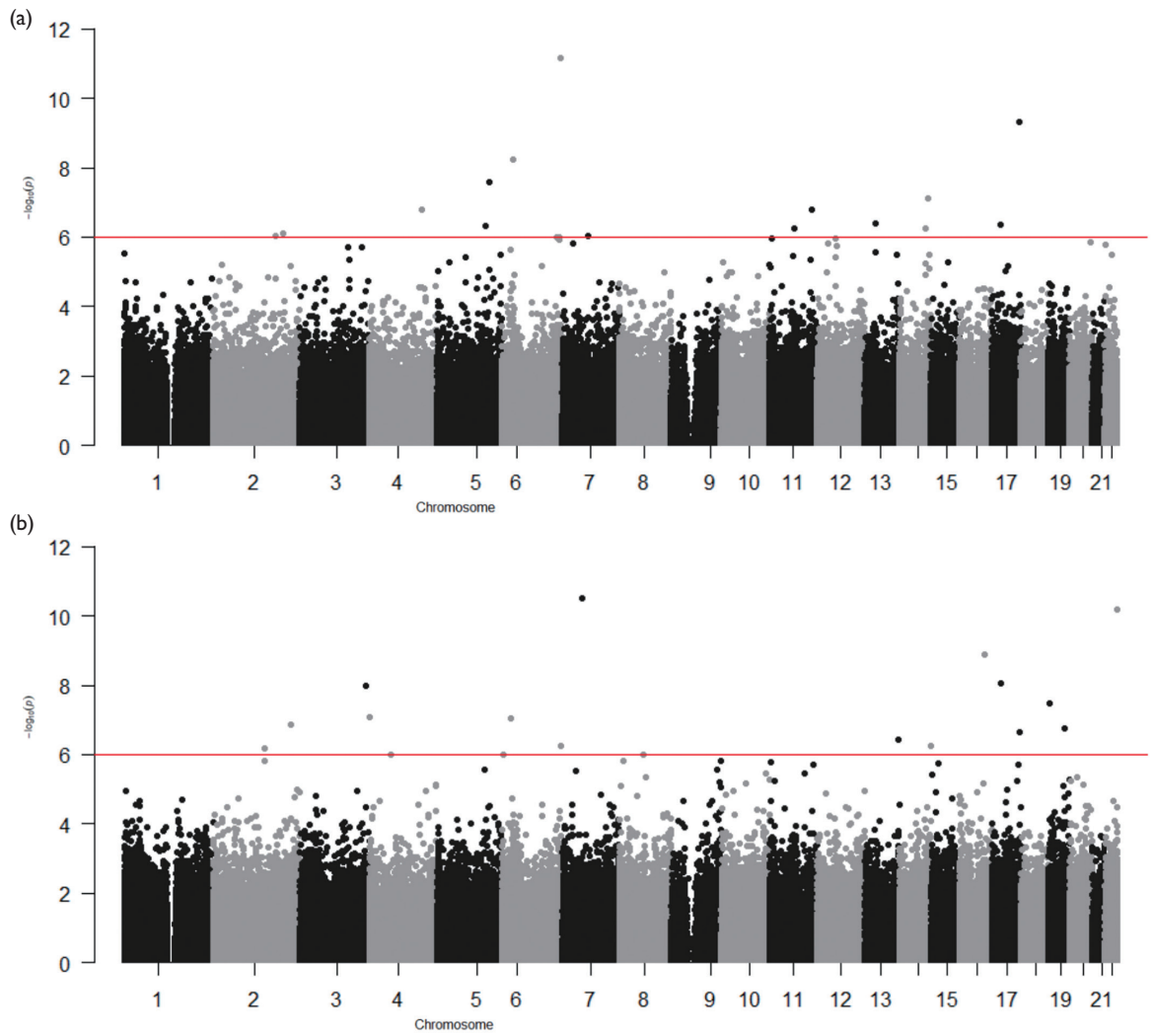

FIG 2. Epigenome-wide associations with (a) body mass index and (b) waist-to-hip ratio at age ~23 years among 286 participants from the 'Children of 1997' birth cohort in the follow-up survey

Genes *ZNF827*, *MIR7641-2*, *RPTOR*, *KSR1*, *GTF3C3*, and *NFIC* were associated with obesity or obesity-related disorders. *KSR1* is reportedly associated with the regulation of glucose homeostasis, and *GTF3C3* is reportedly associated with obesity-related dysglycaemia. *NFIC*, which encodes nuclear factor I-C, regulates adipocyte differentiation. *OPA3* is a novel regulator of mitochondrial function and controls thermogenesis and abdominal fat mass.

There are several limitations to the study. The sample sizes in the EWAS and epigenome-wide association study were relatively small. Results of the present study should be replicated in larger populations. The use of questionnaires to ascertain exposures is susceptible to recall bias and social desirability bias. Although level of confounding was minimal, residual confounding is likely to exist. Therefore, the associations are regarded as potential risk factors, rather than causal relationships.

Conclusions

Environmental exposures (eg, consumption of ASBs and binge eating) and epigenetics in early-life adiposity were associated with adiposity. If these associations are confirmed to be causal relationships, they may provide novel targets for interventions.

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Disclosure

The results of this research have been previously published in:

1. Zhao J, Fan B, Huang J, et al. Environment- and epigenome-wide association study of obesity in 'Children of 1997' birth cohort. Elife 2023;12:e82377.

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