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RESEARCH ARTICLE

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Epigenome-wide mediation analysis identified Cytosine-phosphate-Guanine sites linking environmental factors with diabetes indicators

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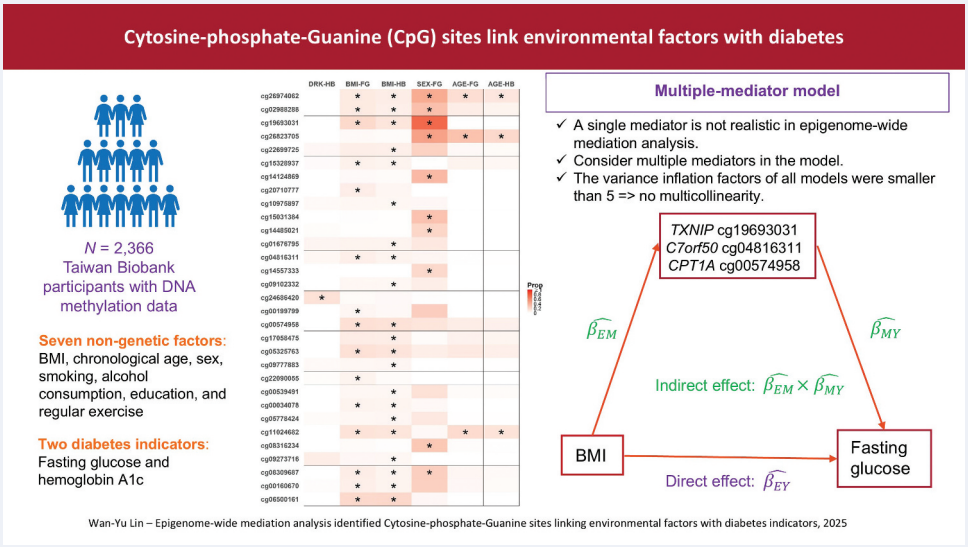
ABSTRACT

Aims: Environmental factors can alter DNA methylation (DNAm) levels, influence gene expression, and then change fasting glucose (FG) or hemoglobin A1c (HbA1c).

Methods: Through analyzing DNAm data of 2366 Taiwan Biobank individuals aged between 30 and 70 years, I evaluated the role of DNAm in mediating the associations of seven non-genetic factors (BMI, chronological age, sex, smoking, drinking alcoholic beverages, education, and regular exercise) with FG and HbA1c.

Results: Among 846,232 Cytosine-phosphate-Guanine (CpG) sites, the single-mediator model explored that 21, 15, 10, 3, 3, and 1 CpGs significantly mediated ($p < 6.6E\text{-}9$) the BMI-HbA1c, BMI-FG, sex-FG, age-FG, age-HbA1c, and drinking-HbA1c associations, respectively. The multiple-mediator model considered all significant mediators and selected the model with the smallest Akaike Information Criterion, and identified 8 CpGs that linked exposures (BMI, sex, age, and drinking) to diabetes indicators. Seven out of the 8 CpGs have been reported to be associated with diabetes, FG, HbA1c, or insulin resistance in previous epigenome-wide association studies.

Conclusion: Four of the 8 CpGs (cg19693031, cg04816311, cg00574958, and cg11024682) were associated with the expression of genes implicated in diabetes and metabolism, including the *TXNIP*, *GPR146*, *CPT1A*, and *SREBF1* genes. These findings highlight the underlying epigenetic mechanism linking non-genetic factors with diabetes.



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1. Introduction

Obesity, aging, male sex, smoking, lower educational attainment, and less physical activity are all associated with an increased risk of developing diabetes. Obesity is a primary risk factor for diabetes by leading to both insulin resistance and β-cell dysfunction [1]. Aging is also a critical risk factor for diabetes due to increased insulin resistance and decreased pancreatic function [2]. Moreover, males are more likely to develop diabetes than females. Around the world, 17.7 million more males are estimated to have diabetes than

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Article highlights

- Some non-genetic or environmental factors, such as BMI, age, and smoking, are associated with diabetes. DNA methylation may mediate the association of environmental factors with fasting glucose or hemoglobin A1c.
- I aim to explore the role of DNA methylation in mediating the associations of seven non-genetic factors (BMI, chronological age, sex, smoking, alcohol consumption, education, and regular exercise) with fasting glucose and hemoglobin A1c.
- The multiple-mediator model identified 8 CpGs that link environmental factors with diabetes indicators. These CpGs were mapped to genes implicated in metabolism, including *TXNIP*, *CPT1A*, and *SREBF1*.
- These findings highlight the underlying epigenetic mechanism linking non-genetic factors with diabetes.

females [3]. Smokers are 30%-40% more likely to develop diabetes than nonsmokers [4]. Lower educational attainment is associated with a higher risk of diabetes [5]. While educational level has no direct biological effect on diabetes, it may change lifestyles and influence the risk of diabetes. Ultimately, regular physical activity helps regulate blood sugar levels and reduces the risk of developing diabetes [6]. Mounting evidence supports a link between these six non-genetic factors and diabetes.

Compared with the six abovementioned factors, the relationship between alcohol consumption and diabetes is relatively controversial. Some studies reported that drinking alcohol increased the risk of developing diabetes [7], while others found that alcohol consumption reduced the risk of diabetes [8]. Moreover, several studies did not find any clear relationship between alcohol and diabetes [9].

DNA methylation (DNAm) is regarded as a bridge between environmental factors and diabetes indicators. Unlike the DNA sequence, DNAm is dynamic throughout the lifetime and can be altered by environmental factors. DNAm may affect gene expression without changing the hereditary DNA sequence. A hypothesis is that environmental stimuli alter DNAm, and then DNAm changes influence gene expression and diabetes traits. Previous studies have reported associations between diabetes and DNAm of the *IGFBP2*, *MSI2*, *FTO*, *TXNIP*, *SREBF1*, *PHOSPHO1*, *SOCS3*, and *ABCG1* genes [10–13].

To evaluate the underlying epigenetic mechanism linking these seven non-genetic factors to diabetes, I here performed an epigenome-wide mediation analysis using high-dimensional mediation hypotheses testing (HDMT) [14]. Moreover, I mapped the Cytosine-phosphate-Guanine (CpG) sites significantly mediating the association of the seven environmental factors with two diabetes indicators (fasting glucose [FG] and hemoglobin A1c [HbA1c]) to several well-known protein-coding genes.

2. Materials and methods

2.1. The Taiwan Biobank data

Since 2012, the Taiwan Biobank (TWB) has recruited ~200,000 community-based volunteers from Taiwan's residents [15]. Participants were aged 30 to 70 years without a history of cancer diagnosis. After signing written informed consent, participants provided blood and urine samples and completed lifestyle questionnaire surveys. Moreover, TWB's health professionals conducted physical health examinations for each individual.

The blood and urine samples were analyzed by laboratories certified to ISO standards and accredited by the College of American Pathologists (CAP). The TWB questionnaire included personal information, dietary habits, and environmental exposures. Epidemiologists designed the questionnaire, and several workgroups evaluated the logical flows and clarity of the questions. Moreover, the TWB performed a preliminary study to validate the questionnaire. The reliability of the TWB questionnaire was evaluated by comparing participants' responses at baseline and follow-up surveys [16].

Between 2016 and 2021, TWB randomly selected 2474 participants for DNAm quantification. The sampling was performed based on the county populations and the male-to-female ratio in Taiwan. The Illumina Infinium MethylationEPIC BeadChip (Illumina, Inc., San Diego, CA), comprising 865,859 CpG sites, was used to quantify DNA methylation levels in the individuals. The Infinium MethylationEPIC BeadChip demonstrated a strong correlation between replicates ($r^2 > 0.98$), indicating high assay reproducibility (https://support.illumina.com).

The TWB did not survey the use of diabetes medication. To avoid confounding due to medication use, I excluded 102 individuals who had been diagnosed with diabetes by a physician (who might be taking diabetes medication). A total of 2372 individuals were retained for further analysis.

2.2. Quality control for DNA methylation data

The raw intensity (.idat) files for the 2474 TWB individuals were generated using the Illumina Infinium MethylationEPIC BeadChip (Illumina, Inc., San Diego, CA). CpGs were mapped to genes using an annotation object generated by the 'IlluminaHumanMethylationEPICanno.ilm10b4.hg19' R package (version 0.6.0) [17]. Sample-wise normalization was performed to account for background and dye biases, as well as other unwanted technical variations introduced during batched data processing. Normalization was conducted using the "noob" method (normal-exponential convolution using out-of-band probes) [18], implemented in the "minfi" package [19]. The "minfi" package is widely used for analyzing Illumina DNA methylation array data [19], and the "noob" method can reduce within- and between-platform artifacts while improving the sensitivity [18].

2.2.1. Sample-wise quality control

Illumina proposed the terminology "detection *p*-value" to quantify the quality of DNAm data. A small "detection *p*-value" implies that the measured methylation intensity will likely be a reliable signal rather than background noise. By contrast, a large "detection *p*-value" (say, >0.01) usually implies a poor quality for the signal [20]. I calculated the average "detection *p*-value" to summarize signal quality across ~860,000 CpGs for each individual. The largest average "detection p-value" (across 865,859 CpG sites) for 2372 samples was 0.00137, which was smaller than the acceptable cutoff of

average "detection *p*-value" 0.01 [20]. Therefore, all 2372 samples were considered of good quality at this stage.

Sex prediction was based on comparing the median total intensity of the *X*-chromosome-mapped probes ($Med(X)$) and the median total intensity of the *Y*-chromosome-mapped probes ($Med(Y)$). Subjects with $log_2Med(Y) - log_2Med(X) < -2$ were predicted as females. Six individuals with a mismatch between self-reported sex and DNAm-predicted sex were excluded from further analysis. Ultimately, 2366 individuals were included in the analysis.

Methylation levels were estimated by the intensity of methylated beads (*M*) and unmethylated beads (*U*). Methylation beta (β) values were calculated with the recommendation from Illumina, i.e., $\beta = M/(M + U + \alpha)$, where α was specified as 100 to avoid division by near-zero given small *M* and *U*.

2.2.2. CpG-wise quality control

I also assessed signal quality across all 2366 samples at each CpG site. Methylation *β*-values measured with "detection *p*-values" higher than 0.05 were unacceptable and regarded as missing values (where 0.05 is the acceptable cutoff of individual "detection *p*-value" [21]). A total of 862,277 CpGs (99.58% among 865,859 CpGs) with missing rates <1% were considered to present satisfactory quality. For each missing β value (with "detection *p*-value" higher than 0.05), I imputed it as the average of all known β values at that CpG site. This approach, known as the "mean" method, is considered superior to its more sophisticated counterparts [22].

Due to "*X* inactivation," one of the two *X* chromosomes is silenced in females to equalize the number of active *X*-chromosome genes between males and females [23]. Therefore, 19,627 CpGs on the sex chromosomes were excluded from the subsequent analysis. Finally, 846,232 (= 865,859 − 19,627) CpGs remained in the analysis.

Given the importance of accounting for cellular heterogeneity, I estimated 12 immune-cell fractions using the Houseman constrained projection deconvolution method [24,25], which was implemented in the EpiDISH package [26]. The 12 immune cells included basophils, memory B-cells, naïve B-cells, memory CD4 + T-cells, naïve CD4 + T-cells, memory CD8 + T-cells, naïve CD8 + T-cells, eosinophils, monocytes, natural killer, regulatory T-cells, and neutrophils.

2.3. Seven environmental factors

The TWB surveyed seven non-genetic factors more comprehensively. The factors that demonstrated significant associations ($p < 0.05$) with FG or HbA1c were further analyzed with mediation analysis to investigate whether DNAm mediated these associations. The seven non-genetic factors were listed as follows.

(1) Sex: males vs. females.
(2) Chronological age: in years.
(3) Body mass index (BMI): in kg/m^2.
(4) Smoking status: yes vs. no. Smoking was defined as smoking cigarettes for at least six months.
(5) Drinking status (DRK): yes vs. no. Drinking was defined as having a weekly intake of more than 150 mL of alcoholic beverages for at least 6 months.
(6) Regular exercise (SPO): yes vs. no. Regular exercise was defined as performing 30 minutes of "exercise" at least three times a week. "exercise" refers to leisure-time activities such as mountain climbing, yoga, dancing, jogging, swimming, cycling, weight training, etc.
(7) Educational attainment: in degrees. Educational attainment is an integer ranging from 1 to 7: 1 for "illiterate"; 2 for "no formal education but literate"; 3 for "primary school graduate"; 4 for "junior high school graduate"; 5 for "senior high school graduate"; 6 for "college graduate"; and 7 for "Master's or higher degree."

2.4. Statistical analysis

I first performed a regression analysis to evaluate the association between the seven non-genetic factors and the two diabetes indicators in the TWB. Because the DNAm data were collected from peripheral blood, analyses accounting for cellular heterogeneity are necessary [27].

2.4.1. Single-mediator model

Taking the relationship between BMI (exposure) and FG as an example, I considered an *M* model and a *Y* model as follows,

$$\mathrm{CpG} = \beta_{0M} + \beta_{EM}BMI + \boldsymbol{\beta}_{\boldsymbol{CM}}^{'}\boldsymbol{Covariates} + \varepsilon_M(M\ \mathrm{model}) \quad (1)$$

$$\mathrm{FG} = \beta_{0Y} + \beta_{EY}BMI + \beta_{MY}CpG + \boldsymbol{\beta}_{\boldsymbol{CY}}^{'}\boldsymbol{Covariates} + \varepsilon_Y(Y\ \mathrm{model}) \quad (2)$$

where ***Covariates*** was a vector containing the seven non-genetic factors and 11 immune-cell fractions (although 12 immune-cell fractions were estimated, neutrophils was not included in models due to multicollinearity). I adjusted for the seven non-genetic factors because they were reported to be associated with diabetes in previous studies, including sex [28], age [29], BMI [30], smoking [31], DRK [32], SPO [33], and education [34]. The random error terms of the *M* model and *Y* model are denoted as ε_M and ε_Y, respectively.

To detect a non-zero mediation effect, the testing hypothesis is $H_0 : \beta_{EM} \times \beta_{MY} = 0$ vs. $H_1 : \beta_{EM} \times \beta_{MY} \neq 0$. The null hypothesis including the product of two parameters is called "the composite null hypothesis," and the H_0 can be categorized as three classes: (1) $H_{10} : \beta_{EM} \neq 0, \beta_{MY} = 0$; (2) $H_{01} : \beta_{EM} = 0, \beta_{MY} \neq 0$; and (3) $H_{00} : \beta_{EM} = 0, \beta_{MY} = 0$. Let $\pi_{10}, \pi_{01}, and \pi_{00}$ be the proportions of the three classes of null hypotheses. An appropriate large-scale single mediator hypothesis testing relies on an accurate estimation of $\pi_{10}, \pi_{01}, and \pi_{00}$. I chose the HDMT [14] as the method for the epigenome-wide mediation analysis. According to a recent simulation study comparing six methods [35], the performance of HDMT is relatively more robust to the three proportions ($\pi_{10}, \pi_{01}, and \pi_{00}$) than its competitors. Analysis was

conducted by using the software R (version 4.2.2) and the R package "medScan" (https://cran.r-project.org/web/packages/medScan/index.html).

2.4.2. Multiple-mediator model

Suppose K "single mediators" were identified from models (1) and (2). I further fitted a "multiple-mediator model" as follows,

$$\mathrm{FG} = \beta_{0Y} + \beta_{EY}BMI + \beta_{M_1Y}CpG_1 + \ldots + \beta_{M_KY}CpG_K + \beta'_{\boldsymbol{CY}}\boldsymbol{Covariates} + \varepsilon_Y(Y\ \mathrm{model}) \quad (3)$$

Including all K single mediators in a model may lead to overfitting or multicollinearity. To address this issue, I employed stepwise regression to identify the model with the smallest Akaike Information Criterion (AIC). Specifically, the R command "step" was used to select critical CpG predictors for the Y model [model (3)]. If the variance inflation factor (VIF) of model (3) was larger than 5 (an alarm of multicollinearity), the least significant CpG site was removed from model (3) until VIF <5. Finally, suppose S CpGs remained in model (3), i.e.,

$$\mathrm{FG} = \beta_{0Y} + \beta_{EY}BMI + \beta_{M_1Y}CpG_1 + \ldots + \beta_{M_SY}CpG_S + \beta'_{\boldsymbol{CY}}\boldsymbol{Covariates} + \varepsilon_Y(Y\ \mathrm{model}) \quad (4)$$

The following are their respective M models (M_1, … , M_S models have been fitted in the aforementioned model (1)),

$$CpG_1 = \beta_{0M_1} + \beta_{EM_1}BMI + \beta'_{\boldsymbol{CM}_1}\boldsymbol{Covariates} + \varepsilon_{M_1}(M_1\ \mathrm{model}) \quad (5)$$

…

$$CpG_S = \beta_{0M_S} + \beta_{EM_S}BMI + \beta'_{\boldsymbol{CM}_S}\boldsymbol{Covariates} + \varepsilon_{M_S}(M_S\ \mathrm{model}) \quad (6)$$

The direct effect of BMI on FG is $\widehat{\beta_{EY}}$, and the mediation effect (or indirect effect) of BMI on FG is $\sum_{j=1}^{S}\widehat{\beta_{EM_j}} \times \widehat{\beta_{M_jY}}$, where ^ represents the estimate of regression coefficient from M model or Y model. The total impact of BMI on FG is $\widehat{\beta_{EY}} + \sum_{j=1}^{S}\widehat{\beta_{EM_j}} \times \widehat{\beta_{M_jY}}$, i.e., the sum of direct and indirect effects. The mediation proportion attributable to CpG_j is $\widehat{\beta_{EM_j}} \times \widehat{\beta_{M_jY}} \Big/ \left\{\widehat{\beta_{EY}} + \sum_{j=1}^{S}\widehat{\beta_{EM_j}} \times \widehat{\beta_{M_jY}}\right\}$, where $j = 1, \ldots, S$.

3. Results

3.1. Demographic summary of the Taiwan Biobank data

Table 1 presents the demographic summary of the 2366 TWB individuals, among whom 1172 (49.5%) were males, while 1194 (50.5%) were females. Chronological ages ranged from 30 to 70 years, with a mean of 49.36 years and a standard deviation (SD) of 11.03 years. Regarding body composition, the average BMI was 24.34 kg/m^2, with a SD of 3.66 kg/m^2. With respect to lifestyle factors, 11.33% of individuals had smoked cigarettes for at least 6 months, 6.97% reported weekly intake of more than 150 mL of alcoholic beverages for at least 6 months, and 43.62% had performed 30 minutes of physical exercise at least 3 times a week. Furthermore, educational attainment, on average, fell between a senior high school degree and a college degree. Finally, the mean FG was 94.05 mg/dL (SD = 15.15) and the mean HbA1c was 5.65% (SD = 0.59%).

Table 1. Demographic summary of the 2,366 Taiwan Biobank individuals.

	Taiwan Biobank individuals[a]
N	2,366 (1,172 males and 1194 females)
Chronological age (in years)	49.36 ± 11.03 (range: 30–70)
Body mass index (in kg/m^2)	24.34 ± 3.66
Smoking status (yes vs. no)[b]	268 (11.33%)
Drinking status (yes vs. no) [c]	165 (6.97%)
Regular exercise (yes vs. no)[d]	1,032 (43.62%)
Educational attainment (in degrees)[e]	5.59 ± 0.91
Fasting glucose (in mg/dL)	94.05 ± 15.15
Hemoglobin A1c (HbA1c, in %)	5.65 ± 0.59

[a]Data are presented as "sample size (percentage)" or "mean ± standard deviation".
[b]Smoking status: yes vs. no. Smoking was defined as smoking cigarettes for at least six months.
[c]Drinking status: yes vs. no. Drinking was defined as having a weekly intake of more than 150 mL of alcoholic beverages for at least 6 months.
[d]Regular exercise: yes vs. no. Regular exercise was defined as performing 30 minutes of "exercise" at least three times a week. "exercise" refers to leisure-time activities such as mountain climbing, yoga, dancing, jogging, swimming, cycling, weight training, etc.
[e]Educational attainment: in degrees. Educational attainment is an integer ranging from 1 to 7: 1 for "illiterate"; 2 for "no formal education but literate"; 3 for "primary school graduate"; 4 for "junior high school graduate"; 5 for "senior high school graduate"; 6 for "college graduate"; and 7 for "Master's or higher degree".

3.2. Association of seven environmental factors with two diabetes indicators

Table 2 shows 14 exposure-outcome associations. When all seven non-genetic factors were included in a model, males had significantly larger FG than females ($p < 0.001$). Older age ($p < 0.001$), higher BMI ($p < 0.001$), and cigarette smoking ($p < 0.05$) were all associated with an increased FG and HbA1c. Alcohol consumption and regular exercise were associated with a decreased HbA1c ($p < 0.05$). By contrast, educational attainment was not associated with FG or HbA1c ($p > 0.05$). Nine out of the 14 exposure-outcome associations were significant at $p < 0.05$. I further investigated whether some CpGs mediated these 9 exposure-outcome associations.

3.3. Single-mediator model analysis results

The single-mediator model required $846,232 \times 9 = 7,616,088$ tests, with 846,232 CpGs analyzed, and 9 significant exposure-outcome associations (9 * from Table 2). Using the Bonferroni correction, CpG sites with p-values less than $0.05/7,616,088 =$ 6.6E-9 were considered significant single mediators.

The single-mediator model (equations 1 and 2) identified 31 CpG sites exhibiting significant mediation effects ($p < 6.6E-9$) for 6 exposure-outcome associations (DRK-HbA1c, BMI-FG, BMI-HbA1c, SEX-FG, AGE-FG, and AGE-HbA1c). No significant mediators were found for the other 3 exposure-outcome associations (i.e., SMK-FG, SMK-HbA1c, and SPO-HbA1c). Figure 1 presents the heatmap plot of the 31 CpGs' mediation

Table 2. Fourteen exposure-outcome associations.

Outcome / exposure	Fasting glucose (in mg/dL)[a]		Hemoglobin A1c (HbA1c, in %)	
	Regression estimate (standard error)	*p*-value	Regression estimate (standard error)	*p*-value
Sex (male vs. female)	2.8166 (0.7486)	1.7E-4***	0.0284 (0.0287)	0.323
Chronological age (in years)	0.1917 (0.0358)	9.7E-8***	0.0105 (0.0014)	2.6E-14***
Body mass index (in kg/m^2)	0.6752 (0.0871)	1.3E-14***	0.0318 (0.0033)	4.6E-21***
Smoking status (yes vs. no)	3.7410 (1.0370)	3.2E-4***	0.0887 (0.0398)	0.026*
Drinking status (yes vs. no)	0.1654 (1.2373)	0.894	−0.1207 (0.0475)	0.011*
Regular exercise (yes vs. no)	−0.9342 (0.6576)	0.156	−0.0504 (0.0253)	0.046*
Educational attainment (in degrees)	−0.0242 (0.3554)	0.946	−0.0251 (0.0136)	0.066

[a]Statistical significance is marked with *, **, and ***, representing a *p*-value less than 0.05, 0.01, and 0.001, respectively. Given the importance of accounting for cellular heterogeneity, in the regression model, I adjusted 11 immune-cell fractions estimated by the EpiDISH package [26]: basophils, memory B-cells, naïve B-cells, memory CD4 + T-cells, naïve CD4 + T-cells, memory CD8 + T-cells, naïve CD8 + T-cells, eosinophils, monocytes, natural killer, and regulatory T-cells. Neutrophils was not adjusted due to multicollinearity. The largest variance inflation factor (VIF) was 161.05 when the model included neutrophils, whereas the largest VIF reduced to 2.26 when neutrophils was excluded.

proportions. Supplementary Table S1 summarizes the total, direct, and mediation effects as well as the *p*-values.

An expression quantitative trait methylation (eQTM) analysis could not be performed here because the TWB did not collect gene expression data. I resorted to the eQTM Atlas (https://shiny.crc.pitt.edu/eqtm_browser/), which summarizes CpG sites that are significantly associated with gene expression. Fifteen out of the 31 CpGs have been reported to be associated with the expression of genes implicated in metabolism. Twenty-two CpGs have been found to be associated with diabetes, FG, HbA1c, or insulin resistance, from previous epigenome-wide association studies (EWAS) [36–46]. Cg22699725 demonstrated a causal relationship with HbA1c [38], and cg00574958 presented a causal effect on diabetes [40]. The single-mediator model assumes only one CpG site linking the exposure to the outcome, which is unrealistic in epigenetic studies. Therefore, I conducted a further analysis using a multiple-mediator model.

3.4. Multiple-mediator model analysis results

Although 31 CpG sites showed significant mediation effects ($p < 6.6E\text{-}9$) on FG or HbA1c according to the single-mediator model, many of these CpGs were excluded from model (3) due to multicollinearity. Finally, I kept 8 CpGs to minimize the AIC while maintaining the VIF <5. Table 3 lists the total, direct, and mediation effects of the eight CpGs, among which 4 CpGs are associated with the expression of some genes according to eQTM Atlas (https://shiny.crc.pitt.edu/eqtm_browser/), and 7 CpGs have been reported to be associated with diabetes, FG, HbA1c, or insulin resistance, from previous EWAS [36,37,41,42,45]. Among them, cg00574958 has been found to demonstrate a causal effect on diabetes [40].

Cg19693031, cg04816311, cg00574958, and cg11024682 were associated with the expression of the *TXNIP*, *GPR146*, *CPT1A*, and *SREBF1* genes, respectively. A study involving over 1500 adults from southwest Germany demonstrated that cg19693031 at *TXNIP* played a crucial role in the pathophysiology of diabetes [48]. *GPR146* is a receptor of proinsulin C-peptide, which can be an effective treatment for diabetes-related complications [49]. The *CPT1A* gene is associated with diabetes, as it plays a crucial role in fatty acid metabolism [50]. A study including 15,734 Danish individuals found that *SREBF1* sequence variation is associated with an increased risk of diabetes [51].

4. Discussion

Many EWAS have identified dozens of CpG sites associated with diabetes-related traits [36–46,52]. These sites are often located on or near genes involved in glucose metabolism. For example, a meta-analysis including five European cohorts identified 76 CpGs that were differentially methylated in patients with diabetes compared with controls. Sixty-four out of 76 (84.2%) CpGs were replicated in an independent cohort of Indian Asians [52]. However, there are relatively few studies on epigenome-wide mediation analysis. A recent study showed 50, 46, 12, and 7 CpGs mediated the effects of psychosocial stress on BMI, waist circumference, C-reactive protein, and high-density lipoprotein cholesterol, respectively [53]. To my knowledge, the current work represents a novel epigenome-wide scan to identify CpGs that link environmental factors with diabetes indicators.

The analysis result of FG was in agreement with the result of HbA1c. For example, cg19693031 at *TXNIP* presented the largest proportion in mediating the association between BMI and FG/HbA1c (Table 3). According to a previous Chinese study with 286 pairs of diabetes cases and matched controls, BMI was positively correlated with the DNAm levels of several CpG sites at the *ABCG1* gene ($\widehat{\beta_{EM_j}} > 0$). Furthermore, the DNAm levels of these CpGs were positively associated with incident diabetes risk ($\widehat{\beta_{M_jY}} > 0$, and therefore the mediation effect $\widehat{\beta_{EM_j}} \times \widehat{\beta_{M_jY}} > 0$) [54]. Moreover, a larger BMI is associated with "hypomethylation" (decreased DNA methylation) at *TXNIP* cg19693031 ($\widehat{\beta_{EM_j}} < 0$), and hypomethylation at cg19693031 is related to an increased diabetes risk ($\widehat{\beta_{M_jY}} < 0$, and therefore the mediation effect $\widehat{\beta_{EM_j}} \times \widehat{\beta_{M_jY}} > 0$) [55]. These findings suggest that the mediating effects of CpGs at *TXNIP* are positive when linking BMI to diabetes, which is consistent with our results (*ABCG1* cg06500161 in Supplementary Table S1; *TXNIP* cg19693031 in Table 3).

Three CpG sites (cg19693031 at *TXNIP*, cg00574958 at *CPT1A*, and cg04816311 at *C7orf50*) have been widely reported to be associated with diabetes by studies in sub-Saharan

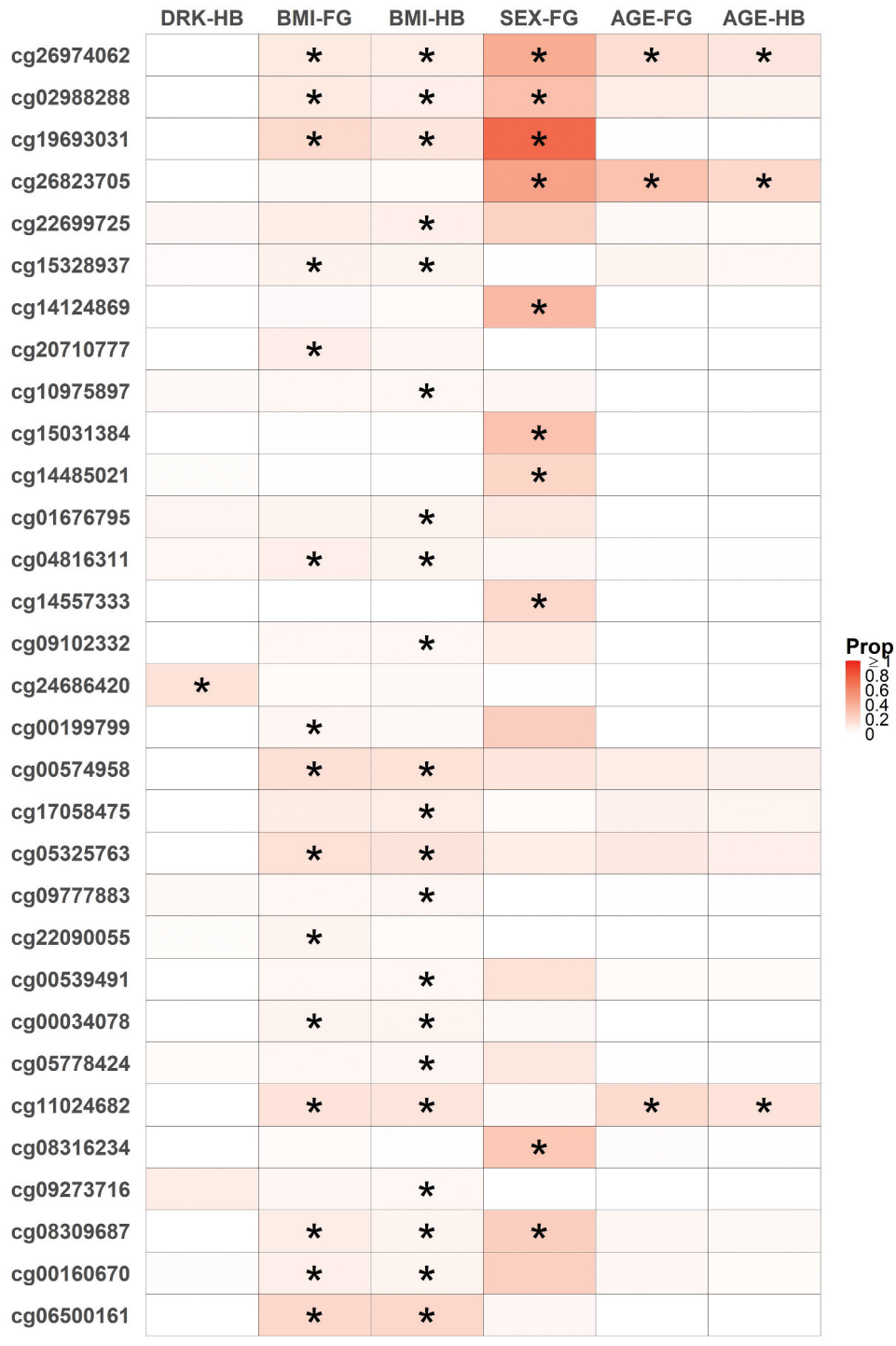

Figure 1. Results of the single-mediator model: heatmap plot of the 31 CpGs' mediation proportions.

The left vertical axis lists 31 CpGs, and the top horizontal axis presents the exposure-outcome combination. The color in each cell marks the strength of the mediation proportion (= mediation effect/total effect), ranging from 0 to 1. "*" denotes that the mediation effect is significant at $p < 0.05/(846232 \times 9) = 6.6E\text{-}9$, where 846,232 CpGs were analyzed, and 9 significant exposure-outcome associations were investigated.

African, Asian, European, British, and Arab populations [40,56–58]. Cg19693031 received much attention for its regulation of *TXNIP* expression and its dose-dependent relationship with HbA1c [59]. Another well-known CpG, cg00574958, is essential for regulating *CPT1A* expression [60]. Carbohydrate and fat intake can have a causal impact on the risk of metabolic diseases through *CPT1A* cg00574958 [42]. I found here that *CPT1A* cg00574958 mediated the effect of BMI on FG and HbA1c at mediation proportions of 11.89% and 12.26%, respectively. Because BMI can reflect carbohydrate and fat intake, my result is generally in line with previous findings [42].

This study has some limitations. Exposures, DNAm levels, and FG/HbA1c were measured concurrently by the TWB. Therefore, I cannot claim causal effects exist between the

Table 3. Results of 8 CpGs estimated through the multiple-mediator model: total effects, direct effects, and mediation effects.

CpG	Evidence from eQTM Atlas	Evidence from EWAS	Evidence from Mendelian randomization	Chr.	Gene	DRK-HbA1c[a]	BMI-FG	BMI-HbA1c	SEX-FG	AGE-FG	AGE-HbA1c
T = Total effect						T = −0.1207	T = 0.6752	T = 0.0318	T = 2.8166	T = 0.1917	T = 0.0105
D = Direct effect = Total effect − mediation effect						D = −0.1026	D = 0.4041	D = 0.0213	D = 0.1135	D = 0.0858	D = 0.0065
M = Mediation effect MP = Mediation proportion = Mediation effect/Total effect											
cg26974062		Associated with insulin resistance across ancestry [36]		1	*TXNIP*					*M* = 0.0234 MP = 0.1221	*M* = 0.0011 MP = 0.1048
cg19693031	Associated with the expression of the *TXNIP* gene	Associated with insulin resistance across ancestry [36]	No evidence of a causal effect of cg19693031 on HbA1c [47]	1	*TXNIP*		*M* = 0.1264 MP = 0.1872	*M* = 0.0042 MP = 0.1321	*M* = 1.8958 MP = 0.6731		
cg26823705		Associated with FG and HbA1c [37]		1	*NBPF20*					*M* = 0.0465 MP = 0.2426	*M* = 0.0014 MP = 0.1333
cg14124869				3	*ABHD5*				*M* = 0.8073 MP = 0.2866		
cg04816311	Associated with the expression of the *GPR146* gene	Associated with HbA1c [41]	No evidence of the causal effect on diabetes [40]	7	*C7orf50*		*M* = 0.0644 MP = 0.0954	*M* = 0.0024 MP = 0.0755			
cg24686420		Associated with diabetes [EWAS Catalog]		8	*SPIDR*	*M* = −0.0181 MP = 0.1500					
cg00574958	Associated with the expression of the *CPT1A* gene	Associated with diabetes [42]	cg00574958 demonstrated a causal effect on diabetes [40]	11	*CPT1A*		*M* = 0.0803 MP = 0.1189	*M* = 0.0039 MP = 0.1226			
cg11024682	Associated with the expression of the *SREBF1* gene	A classifier for diabetes stratification [45]	No evidence of the causal effect on diabetes [40]	17	*SREBF1*					*M* = 0.0360 MP = 0.1878	*M* = 0.0015 MP = 0.1429
Cumulative mediation proportions						0.1500	0.4015	0.3302	0.9597	0.5525	0.3810

[a]DRK: Status of drinking alcoholic beverages (yes vs. no).

exposures, CpGs, and diabetes traits. Reverse causation is currently not addressed. Furthermore, replication of the identified eight CpGs in an independent cohort is still required to strengthen the findings.

This work has several strengths. To my knowledge, this was the first study to use HDMT to formally assess the mediating effects of genome-wide CpGs on the relationship between exposures and diabetes-related outcomes. Furthermore, I utilized multiple-mediator models to jointly consider all significant mediators. The multiple-mediator model analysis can preserve the most predictive CpGs and eliminate those with inferior explanatory ability. Lastly, compared with most DNAm research on individuals of European ancestry, this study provided insights from a less-studied ethnicity.

5. Conclusion

In summary, this work identified 8 CpGs that link environmental factors to diabetes indicators. Among them, 7 CpGs have been reported to be associated with diabetes, FG, HbA1c, or insulin resistance in previous GWAS [36,37,41,42,45]. All CpGs' mediation effects were in the same direction as the total effect (shown in Table 3 and Supplementary Table S1). This work's findings highlight the role of epigenetics in linking environmental factors to diabetes outcomes. In addition to using the HDMT [14] approach to identify individual mediators, I performed stepwise regression to pinpoint 8 predictive and uncorrelated CpG sites. Findings from this study help elucidate the mechanisms by which CpGs partially mediate the associations between exposures (DRK, BMI, sex, and age) and FG/HbA1c.

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Dr. Wan-Yu Lin is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Author contributions

Wan-Yu Lin: Conceptualization, Data curation, Investigation, Funding acquisition, Project administration, Resources, Formal analysis, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Disclosure statement

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Ethical declaration

The Ethics and Governance Council of Taiwan Biobank and the Institutional Review Board on Biomedical Science Research/IRB-BM at Academia Sinica approved the TWB project. Written informed consent was obtained from each participant, in accordance with institutional requirements and the principles outlined in the Declaration of Helsinki. TWB approved my access to the data on 18 February 2020, under application number TWBR10810-07. I also received the approval to perform this study from the Research Ethics Committee of the National Taiwan University Hospital (NTUH-REC no. 201805050RINB).

Data availability statement

The participant-relevant data supporting the findings of this study are made available by the Taiwan Biobank. To access the data, applicants should submit a request through the official website: https://www.twbiobank.org.tw/.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Klein S, Gastaldelli A, Yki-Jarvinen H, et al. Why does obesity cause diabetes? Cell Metab. 2022;34(1):11–20. doi: 10.1016/j.cmet.2021.12.012
2. Aguayo-Mazzucato C. Functional changes in beta cells during ageing and senescence. Diabetologia. 2020;63(10):2022–2029. doi: 10.1007/s00125-020-05185-6
3. Kautzky-Willer A, Leutner M, Harreiter J. Sex differences in type 2 diabetes. Diabetologia. 2023;66(6):986–1002. doi: 10.1007/s00125-023-05891-x
4. Lehrer S, Rheinstein PH. Diabetes, cigarette smoking and transcription factor 7-like 2 (Tcf7L2) in the UK Biobank cohort. Bull Acad Natl Med. 2021;205(9):1146–1150. doi: 10.1016/j.banm.2021.09.001
5. Sacerdote C, Ricceri F, Rolandsson O, et al. Lower educational level is a predictor of incident type 2 diabetes in European countries: the EPIC-InterAct study. Int J Epidemiol. 2012;41(4):1162–1173. doi: 10.1093/ije/dys091
6. Syeda USA, Battillo D, Visaria A, et al. The importance of exercise for glycemic control in type 2 diabetes. Am J Med Open. 2023;9:100031. doi: 10.1016/j.ajmo.2023.100031
7. Han TS, Zhang S, Duan W, et al. Eighteen-year alcohol consumption trajectories and their association with risk of type 2 diabetes and its related factors: the China health and nutrition survey. Diabetologia. 2019;62(6):970–980. doi: 10.1007/s00125-019-4851-z
8. Teratani T, Morimoto H, Sakata K, et al. Dose-response relationship between tobacco or alcohol consumption and the development of diabetes mellitus in Japanese male workers. Drug Alcohol Depen. 2012;125(3):276–282.
9. Feskens EJM, Kromhout D. Cardiovascular risk-factors and the 25-year incidence of diabetes-mellitus in middle-aged men - the zutphen study. Am J Epidemiol. 1989;130(6):1101–1108.

10. Nadiger N, Veed JK, Nataraj PC, et al. DNA methylation and type 2 diabetes: a systematic review. Clin Epigenet. 2024;16(1):67. doi: 10.1186/s13148-024-01670-6
11. Wittenbecher C, Ouni M, Kuxhaus O, et al. Insulin-like growth factor binding protein 2 (IGFBP-2) and the risk of developing type 2 diabetes. Diabetes. 2019;68(1):188–197. doi: 10.2337/db18-0620
12. Jeon JP, Koh IU, Choi NH, et al. Differential DNA methylation of MSI2 and its correlation with diabetic traits. PLOS ONE. 2017;12(5):e0177406. doi: 10.1371/journal.pone.0177406
13. Walaszczyk E, Luijten M, Spijkerman AMW, et al. DNA methylation markers associated with type 2 diabetes, fasting glucose and HbA(1c) levels: a systematic review and replication in a case-control sample of the lifelines study. Diabetologia. 2018;61(2):354–368. doi: 10.1007/s00125-017-4497-7
14. Dai JY, Stanford JL, LeBlanc M. A multiple-testing procedure for high-dimensional mediation hypotheses. J Am Stat Assoc. 2022;117(537):198–213. doi: 10.1080/01621459.2020.1765785
•• **An appropriate large-scale single mediator hypothesis testing.**
15. Wei CY, Yang JH, Yeh EC, et al. Genetic profiles of 103,106 individuals in the Taiwan Biobank provide insights into the health and history of Han Chinese. NPJ Genom Med. 2021;6(1):10. doi: 10.1038/s41525-021-00178-9
•• **Introduced the Taiwan Biobank data.**
16. Feng YA, Chen CY, Chen TT, et al. Taiwan Biobank: a rich biomedical research database of the Taiwanese population. Cell Genom. 2022;2(11):100197.
• **Introduced the Taiwan Biobank data.**
17. Fortin JP, Hansen KD. IlluminaHumanMethylationEPICanno.Ilm10b4.hg19: annotation for Illumina's epic methylation arrays. R package version 060. 2017.
18. Triche TJ, Weisenberger DJ, Van Den Berg D, et al. Low-level processing of Illumina Infinium DNA methylation BeadArrays. Nucleic Acids Res. 2013;41(7):e90. doi: 10.1093/nar/gkt090
19. Fortin JP, Triche TJ, Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. Bioinformatics. 2017;33(4):558–560. doi: 10.1093/bioinformatics/btw691
20. Maksimovic J, Phipson B, Oshlack A. A cross-package bioconductor workflow for analysing methylation array data. F1000Res. 2016;5:1281. doi: 10.12688/f1000research.8839.2
21. van Andel MM, Groenink M, van den Berg MP, et al. Genome-wide methylation patterns in Marfan syndrome. Clin Epigenet. 2021;13(1):217. doi: 10.1186/s13148-021-01204-4
22. Di Lena P, Sala C, Prodi A, et al. Methylation data imputation performances under different representations and missingness patterns. BMC Bioinf. 2020;21(1):268. doi: 10.1186/s12859-020-03592-5
23. Fang H, Disteche CM, Berletch JB. X inactivation and escape: epigenetic and structural features. Front Cell Dev Biol. 2019;7:219. doi: 10.3389/fcell.2019.00219
24. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinf. 2012;13(1):86. doi: 10.1186/1471-2105-13-86
25. Salas LA, Zhang Z, Koestler DC, et al. Enhanced cell deconvolution of peripheral blood using DNA methylation for high-resolution immune profiling. Nat Commun. 2022;13(1):761. doi: 10.1038/s41467-021-27864-7
26. Luo Q, Dwaraka VB, Chen QW, et al. A meta-analysis of immune-cell fractions at high resolution reveals novel associations with common phenotypes and health outcomes. Genome Med. 2023;15(1):59. doi: 10.1186/s13073-023-01211-5
27. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. Genome Biol. 2014;15(2):R31. doi: 10.1186/gb-2014-15-2-r31
28. Ciarambino T, Crispino P, Leto G, et al. Influence of gender in diabetes mellitus and its complication. Int J Mol Sci. 2022;23(16):8850. doi: 10.3390/ijms23168850
29. Yan ZH, Cai MJ, Han X, et al. The interaction between age and risk factors for diabetes and prediabetes: a community-based cross-sectional study. Diabet Metab Synd Ob. 2023;16:85–93. doi: 10.2147/DMSO.S390857
30. Chen Y, Zhang XP, Yuan J, et al. Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. BMJ Open. 2018;8(9):e021768. doi: 10.1136/bmjopen-2018-021768
31. Maddatu J, Anderson-Baucum E, Evans-Molina C. Smoking and the risk of type 2 diabetes. Transl Res. 2017;184:101–107. doi: 10.1016/j.trsl.2017.02.004
32. Li X, Hur J, Smith-Warner SA, et al. Alcohol intake, drinking pattern, and risk of type 2 diabetes in three prospective cohorts of U.S. women and men. Diabetes Care. 2025;48(7):1189–1197. doi: 10.2337/dc24-1902
33. Colberg SR, Sigal RJ, Yardley JE, et al. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. Diabetes Care. 2016;39(11):2065–2079. doi: 10.2337/dc16-1728
34. Whitaker SM, Bowie JV, McCleary R, et al. The association between educational attainment and diabetes among men in the United States. Am J Mens Health. 2014;8(4):349–356. doi: 10.1177/1557988313520034
35. Du J, Zhou X, Clark-Boucher D, et al. Methods for large-scale single mediator hypothesis testing: possible choices and comparisons. Genet Epidemiol. 2023;47(2):167–184. doi: 10.1002/gepi.22510
•• **Demonstrated that the performance of HDMT is more robust than its competitors.**
36. Fragoso-Bargas N, Elliott HR, Lee-Odegard S, et al. Cross-ancestry DNA methylation marks of insulin resistance in pregnancy: an integrative epigenome-wide association study. Diabetes. 2023;72(3):415–426. doi: 10.2337/db22-0504
37. Seo H, Park JH, Hwang JT, et al. Epigenetic profiling of type 2 diabetes mellitus: an epigenome-wide association study of DNA methylation in the Korean Genome and epidemiology study. Genes (Basel). 2023;14(12):2207. doi: 10.3390/genes14122207
38. Yousri NA, Albagha OME, Hunt SC. Integrated epigenome, whole genome sequence and metabolome analyses identify novel multi-omics pathways in type 2 diabetes: a Middle Eastern study. BMC Med. 2023;21(1):347. doi: 10.1186/s12916-023-03027-x
39. Chen Z, Miao F, Braffett BH, et al. DNA methylation mediates development of HbA1c-associated complications in type 1 diabetes. Nat Metab. 2020;2(8):744–762. doi: 10.1038/s42255-020-0231-8
40. Cardona A, Day FR, Perry JRB, et al. Epigenome-wide association study of incident type 2 diabetes in a British population: EPIC-Norfolk study. Diabetes. 2019;68(12):2315–2326. doi: 10.2337/db18-0290
41. Wang Z, Peng H, Gao W, et al. Blood DNA methylation markers associated with type 2 diabetes, fasting glucose, and HbA1c levels: an epigenome-wide association study in 316 adult twin pairs. Genomics. 2021;113(6):4206–4213. doi: 10.1016/j.ygeno.2021.11.005
42. Lai CQ, Parnell LD, Smith CE, et al. Carbohydrate and fat intake associated with risk of metabolic diseases through epigenetics of CPT1A. Am J Clin Nutr. 2020;112(5):1200–1211. doi: 10.1093/ajcn/nqaa233
• **Showed that carbohydrate and fat intake can have a causal impact on the risk of metabolic diseases through *CPT1A* cg00574958.**
43. Sarnowski C, Huan TX, Ma YY, et al. Multi-tissue epigenetic analysis identifies distinct associations underlying insulin resistance and Alzheimer's disease at CPT1A locus. Clin Epigenet. 2023;15(1):173. doi: 10.1186/s13148-023-01589-4
44. Smyth LJ, Dahlstrom EH, Syreeni A, et al. Epigenome-wide meta-analysis identifies DNA methylation biomarkers associated with diabetic kidney disease. Nat Commun. 2022;13(1):7891. doi: 10.1038/s41467-022-34963-6
45. Krause C, Sievert H, Geissler C, et al. Critical evaluation of the DNA-methylation markers ABCG1 and SREBF1 for type 2 diabetes stratification. Epigenomics. 2019;11(8):885–897. doi: 10.2217/epi-2018-0159
46. Dayeh T, Tuomi T, AImgren P, et al. DNA methylation of loci within ABCG1 and PHOSPHO1 in blood DNA is associated with future type 2 diabetes risk. EpigeneticsUs. 2016;11(7):482–488. doi: 10.1080/15592294.2016.1178418
47. Miller RG, Mychaleckyj JC, Onengut-Gumuscu S, et al. DNA methylation is associated with glycemic control over 28 years in type 1

diabetes: findings from the Pittsburgh epidemiology of diabetes complications (EDC) study. BMJ Open Diab Res Ca. 2023;11(1): e003068.

48. Florath I, Butterbach K, Heiss J, et al. Type 2 diabetes and leucocyte DNA methylation: an epigenome-wide association study in over 1,500 older adults. Diabetologia. 2016;59(1):130–138. doi: 10.1007/s00125-015-3773-7
    - **Demonstrated that *TXNIP* cg19693031 played a crucial role in the pathophysiology of diabetes.**
49. Kolar GR, Grote SM, Yosten GLC. Targeting orphan G protein-coupled receptors for the treatment of diabetes and its complications: C-peptide and GPR146. J Intern Med. 2017;281(1):25–40. doi: 10.1111/joim.12528
50. Ren Q, Guo M, Yang F, et al. Association of CPT1A gene polymorphism with the risk of gestational diabetes mellitus: a case-control study. J Assist Reprod Genet. 2021;38(7):1861–1869. doi: 10.1007/s10815-021-02143-y
51. Grarup N, Stender-Petersen KL, Andersson EA, et al. Association of variants in the sterol regulatory element-binding factor 1 (SREBF1) gene with type 2 diabetes, glycemia, and insulin resistance: a study of 15,734 Danish subjects. Diabetes. 2008;57(4):1136–1142. doi: 10.2337/db07-1534
52. Fraszczyk E, Spijkerman AMW, Zhang Y, et al. Epigenome-wide association study of incident type 2 diabetes: a meta-analysis of five prospective European cohorts. Diabetologia. 2022;65(5):763–776. doi: 10.1007/s00125-022-05652-2
53. Opsasnick LA, Zhao W, Ratliff SM, et al. Epigenome-wide mediation analysis of the relationship between psychosocial stress and cardiometabolic risk factors in the Health and retirement study (HRS). Clin Epigenet. 2024;16(1):180. doi: 10.1186/s13148-024-01799-4
    - **An epigenome-wide mediation analysis based on 2,668 adults from the Health and Retirement Study.**
54. Qie R, Chen Q, Wang T, et al. Association of ABCG1 gene methylation and its dynamic change status with incident type 2 diabetes mellitus: the rural Chinese cohort study. J Hum Genet. 2021;66(4):347–357. doi: 10.1038/s10038-020-00848-z
55. Ma H, Wang X, Liang ZX, et al. BMI change during childhood, DNA methylation change at TXNIP, and glucose change during midlife. Obesity. 2023;31(8):2150–2158. doi: 10.1002/oby.23806
56. Meeks KAC, Henneman P, Venema A, et al. Epigenome-wide association study in whole blood on type 2 diabetes among sub-Saharan African individuals: findings from the RODAM study. Int J Epidemiol. 2019;48(1):58–70. doi: 10.1093/ije/dyy171
57. Chambers JC, Loh M, Lehne B, et al. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian asians and europeans with incident type 2 diabetes: a nested case-control study. Lancet Diabetes Endocrinol. 2015;3(7):526–534. doi: 10.1016/S2213-8587(15)00127-8
58. Al Muftah WA, Al-Shafai M, Zaghlool SB, et al. Epigenetic associations of type 2 diabetes and BMI in an Arab population. Clin Epigenet. 2016;8(1):13. doi: 10.1186/s13148-016-0177-6
59. Dawes K, Philibert W, Darbro B, et al. Additive and interactive genetically contextual effects of HbA1c on cg19693031 methylation in type 2 diabetes. Genes (Basel). 2022;13(4):683. doi: 10.3390/genes13040683
60. Irvin MR, Zhi DG, Joehanes R, et al. Epigenome-wide association study of fasting blood lipids in the genetics of lipid-lowering drugs and diet network study. Circulation. 2014;130(7):565±. doi: 10.1161/CIRCULATIONAHA.114.009158