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Epigenome-wide association study of short-term temperature fluctuations based on within-sibship analyses in Australian females

Yao Wu [a,1], Rongbin Xu [a,1], Shanshan Li [a], Ee Ming Wong [b,c], Melissa C. Southey [b,c,d], John L. Hopper [e], Michael J. Abramson [a], Shuai Li [b,e,f,g,2], Yuming Guo [a,2,*]

[a] School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC 3004, Australia
[b] Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, VIC 3800, Australia
[c] Department of Clinical Pathology, Melbourne Medical School, The University of Melbourne, Melbourne, VIC 3010, Australia
[d] Cancer Epidemiology Division, Cancer Council Victoria, VIC 3004, Australia
[e] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, VIC 3010, Australia
[f] Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge CB1 8RN, UK
[g] Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, VIC 3052, Australia

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ABSTRACT

Background: Temperature fluctuations can affect human health independent of the effect of mean temperature. However, no study has evaluated whether short-term temperature fluctuations could affect DNA methylation.
Methods: Peripheral blood DNA methylation for 479 female siblings of 130 families were analysed. Gridded daily temperatures data were obtained, linked to each participant's home address, and used to calculate nine different metrics of short-term temperature fluctuations: temperature variabilities (TVs) within the day of blood draw and preceding one to seven days (TV 0–1 to TV 0–7), diurnal temperature range (DTR), and temperature change between neighbouring days (TCN). Within-sibship design was used to perform epigenome-wide association analyses, adjusting for daily mean temperatures, and other important covariates (e.g., smoking, alcohol use, cell-type proportions). Differentially methylated regions (DMRs) were further identified. Multiple-testing comparisons with a significant threshold of 0.01 for cytosine-guanine dinucleotides (CpGs) and 0.05 for DMRs were applied.
Results: Among 479 participants (mean age ± SD, 56.4 ± 7.9 years), we identified significant changes in methylation levels in 14 CpGs and 70 DMRs associated with temperature fluctuations. Almost all identified CpGs were associated with exposure to temperature fluctuations within three days. Differentially methylated signals were mapped to 68 genes that were linked to human diseases such as cancer (e.g., colorectal carcinoma, breast carcinoma, and metastatic neoplasms) and mental disorder (e.g., schizophrenia, mental depression, and bipolar disorder). The top three most significantly enriched gene ontology terms were Response to bacterium (TV 0–3), followed by Hydrolase activity, acting on ester bonds (TCN), and Oxidoreductase activity (TV 0–3).
Conclusions: Short-term temperature fluctuations were associated with differentially methylated signals across the human genome, which provides evidence on the potential biological mechanisms underlying the health impact of temperature fluctuations. Future studies are needed to further clarify the roles of DNA methylation in diseases associated with temperature fluctuations.

1. Introduction

Climate change is already exacerbating unstable weather conditions across the globe (IPCC, 2012). In the past two decades, temperature fluctuations have been identified as a risk factor for both mortality and morbidity independent of the effect of mean temperature (Cheng et al.,

* Corresponding author at: Department of Epidemiology and Preventive Medicine, School of Public Health and Preventive Medicine, Monash University, Level 2, 553 St Kilda Road, Melbourne, VIC 3004, Australia.
E-mail address: yuming.guo@monash.edu (Y. Guo).
[1] Authors contributed equally to this work.
[2] Co-senior authors.

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2014). The health impact of temperature fluctuations can be multifaceted. For example, the diurnal temperature range (DTR), representing the changes in temperature during a day, has been widely reported to increase mortality/morbidity risk, particularly for cardiovascular and respiratory diseases (Cheng et al., 2014). Another indicator——temperature change between the neighbouring days (TCN), has been associated with increased mortality risk (Zhan et al., 2017). To capture the combined and lagged effects of both DTR and TCN, temperature variability (TV) has been increasingly used to assess the health impact of temperature fluctuations within a short exposure window (e.g., from two to seven days) (Guo et al., 2016). Numerous studies have reported a persistent association between TV (up to seven days) and mortality/morbidity risk and identified children, the elderly, people with respiratory disease as vulnerable populations (Wu et al., 2021b; Zhao et al., 2018).

The pathophysiological mechanism underlying the health impact of temperature fluctuations remains unclear. Current hypotheses involve limited thermoregulation and acclimatization to unstable weather, following inflammatory response and oxidative stress (Chen et al., 2018; Halonen et al., 2010; Liu et al., 2015; Schneider et al., 2008; Zhao et al., 2019). DNA methylation is an epigenetic modification that is associated with the normal regulation of gene expression and cellular processes (Suzuki and Bird 2008). The alterations of DNA methylation are highly associated with many environmental exposures, such as greenness, air pollution, and ambient temperature (Martin and Fry 2018; Rider and Carlsten 2019; Xu et al., 2021a; b). Previous studies have described the critical role of DNA methylation in the regulation of inflammation and oxidative stress (Hedman et al., 2016; Wierda et al., 2010), such role has been proposed as the potential mechanisms behind the health impact of temperature (Bind et al., 2014; Bui et al., 2020; Lechner et al., 2005).

An increasing number of studies have linked the environmental temperature to human epigenetic modifications, identifying 15 genes that are differentially methylated in response to temperature exposure (Xu et al., 2020). However, previous studies focused primarily on the mean temperature during different exposure windows (e.g., daily, monthly, or yearly mean temperature) (Xu et al., 2020). Given that the health impacts of temperature fluctuation are independent of mean temperature, the former might show independent epigenetic effect of mean temperature. To the best of our knowledge, no study to date has assessed the association between short-term temperature fluctuations and DNA methylation, which could have great importance to reveal the underlying mechanisms behind the health impact of temperature fluctuations. In this study, we aimed to assess the association between short-term exposure to temperature fluctuations and genome-wide DNA methylation using data from a twin and family study in Australia.

2. Methods

2.1. Study population

The study sample consisted of participants from the Australian Mammographic Density Twins and Sisters Study (AMDTSS). Details of the AMDTSS have been described previously (Li et al., 2019b; Li et al., 2018; Li et al., 2015; Odefrey et al., 2010; Xu et al., 2021a; b; Xu et al., 2021c). Briefly, female twins and their sisters were recruited through the Australian Twin Registry between 2004 and 2009. The inclusion criteria were as follows: (1) aged between 40 and 70; (2) not pregnant or breastfeeding; (3) without breast cancer. The present analyses included 479 women from 130 families with DNA methylation measurements, comprising 66 monozygotic (MZ) twin pairs, 66 dizygotic (DZ) twin pairs, and 215 sisters of these twins.

The study was approved by the Human Research Ethics Committee of The University of Melbourne and the Monash University Human Research Ethics Committee. Written informed consents were signed by every participant.

2.2. Data collection

2.2.1. DNA methylation data

Peripheral blood was drawn from participants for DNA methylation measurements. Genomic DNA was first isolated from dried blood spots (or Guthrie Cards) and then used for methylation measurement by the Infinium HumanMethylation450K BeadChip array. Under a standard pipeline from the Bioconductor *minifi* package, we processed the raw methylation data following procedures below: background correction and control normalization using Illumina's reference factor-based normalization methods (*preprocessIllumina*), subset-quantile within array normalization correction for the technical differences between the type I and II array designs (Maksimovic et al., 2012), and minimising technical batch effects (or non-biological experimental variation) using an empirical Bayes method (ComBat) (Johnson et al., 2007). Quality control was performed by several procedures: removing cytosine-guanine dinucleotides (CpGs) with a detection p-value > 0.01 (indicating a poor-quality signal) in one or more samples; filtering out the probes from the sex chromosomes, probes binding to documented SNPs, probes mapping to multiple places in the genome (cross-reactive), negative control probes, and probes with bead count<3 in at least 5 % of samples. Eventually, a total of 411,394 CpGs were included for downstream analyses. For each CpG, methylated and unmethylated intensity values were used to measure DNA methylation levels and generate Beta values for analysis. Beta value quantifies the proportion of methylation and has a value range between 0 (completely unmethylated) and 1 (fully methylated). Outliers were defined as DNA methylation levels at any one CpG site that are three times beyond the inter-quartile range from the 25th or 75th percentiles compared to the rest of the population and were removed during analyses (Seeboth et al., 2020).

2.2.2. Meteorological data

We obtained daily temperatures (maximum temperature and minimum temperature [T_{max} and T_{min}]) and relative humidity data at a spatial resolution of $0.05^{\circ}\times0.05^{\circ}$ across Australia from the SILO database hosted by the Queensland Department of Environment and Science (DES) (SILO 2020). All data were linked to each participant's home address. The daily mean temperature was computed as the average of the daily T_{max} and T_{min}. We applied several widely used indices to reflect short-term temperature fluctuation: DTR, TCN, and TV (from TV 0–1 to TV 0–7) (Cheng et al., 2014; Guo et al., 2016; Zhan et al., 2017). DTR was the difference between daily T_{max} and T_{min}. TCN was calculated as the difference of mean temperature between two neighbouring days. TV was defined as the standard deviation (SD) of T_{max} and T_{min} within different exposure windows (Guo et al., 2016). For example, we calculated TV 0–3 as the SD of all T_{max} and T_{min} for the current and preceding three days ($T_{max\text{-}lag0}$, $T_{min\text{-}lag0}$, $T_{max\text{-}lag1}$, $T_{min\text{-}lag1}$, $T_{max\text{-}lag2}$, $T_{min\text{-}lag2}$, $T_{max\text{-}lag3}$, $T_{min\text{-}lag3}$). All values were calculated based on the date of the blood draw.

2.2.3. Assessment of covariates

Demographic and lifestyle characteristics data including birth date, education level, weight, height, smoking history, and alcohol use habits were collected using telephone-administered questionnaires by trained interviewers. As described previously (Xu et al., 2021c), both smoking and alcohol use were categorized into three groups (never, past, and current) by asking the frequency of cigarettes and alcohol consumed. Body mass index (BMI) was defined as weight divided by squared height (kg/m^2). Chronological age was calculated as the number of years from the birth date to the day when the blood sample was collected. As DNA methylation levels vary by the leukocyte composition, we estimated the cell-type makeup ($CD8^+$ T, $CD4^+$ T, natural killer [NK], B cells, monocytes, and granulocytes) of each sample using the Houseman algorithm, with the function *estimateCellCounts* embedded in the minfi package (Houseman et al., 2012). The survey year and season (spring, summer, autumn, winter) were defined based on the date of the blood draw and

the official season definition provided by the Australia Bureau of Meteorology.

2.3. Statistical analyses

2.3.1. CpG-specific analyses

We applied a within-sibship design to control for complex sources of confounders, including shared familial effects which shaped the environmental exposure and population stratification. For each of nine temperature indices, we fitted a generalized estimating equation (GEE) model to assess the associations between each of nine temperature indices and CpG-specific DNA methylation level whilst handling correlated data structures arising from similarities between family members. The equation was as follows:

$$E(Meth_{ij}) = \beta_0 + \beta_{within}{}^{*}(Expo_{ij} - \overline{Expo_i}) + \beta_{between}{}^{*}\overline{Expo_i} + \beta^{*}X_{ij} \quad (1)$$

where $E(Meth_{ij})$ stands for the expected value of DNA methylation level at one CpG site of subject *j* in family *i*. $Expo_{ij}$ denotes the exposure level of subject *j* in family *i*. $\overline{Expo_i}$ is the average exposure level of all subjects in family *i*. β_{within} and $\beta_{between}$ stand for the within- and between-family effect estimates associated with every one-unit increase in exposure, respectively. We also added a set of covariates (X_{ij}) to the model, including mean temperature, relative humidity, proportions of six cell types, chronological age, education level, BMI, smoking and drinking habits, survey year, and season. Mean temperature and relative humidity were added to the model as potential confounders because they have a relationship with both the temperature fluctuation and DNA methylation (Bind et al., 2014). Chronological age, BMI, smoking and drinking habits were regressed out as they were known covariates associating with DNA methylation (Ryan et al., 2020). Blood cell composition was added to the model to account for potential confounding by cell type due to their distinct methylation signatures (Montano et al., 2016). We also performed sensitivity analysis by removing cell composition in the model to check whether cell type adjustment changed effect size significantly. We adjusted for survey year and season to account for long-term trends and seasonal variation. We adopted a natural cubic spline with 4 degrees of freedom for the 21-day moving average of both relative humidity and mean temperature. We focused on within-family effects (β_{within}) which were less biased by both measured and unmeasured shared familial factors than inter-family effect estimates ($\beta_{between}$) (Xu et al., 2021c).

For each exposure index, p-values for all CpG sites were adjusted for epigenome-wide multiple testing using the Benjamini–Hochberg method (Benjamini and Hochberg 1995). CpG sites with a false discovery rate (FDR) < 0.05 were considered to be significant. A Quantile-Quantile (Q-Q) plot with a genomic inflation factor (known as λ) was used to assess the degree of test statistic inflation (Li et al., 2018; Van der Most et al., 2017).

2.3.2. Differentially methylated region (DMR) analyses

We used two methods with different algorithms to investigate differentially methylated regions (DMRs): DMRcate and comb-p (Lent et al., 2018; Pedersen et al., 2012; Peters et al., 2015; Xu et al., 2021d). The DMRcate statistical model identifies the regions of enrichment by computing a smoothed kernel association z-statistics of all CpGs in a seed region against a null comparison, then ranks them by Fisher's multiple comparison statistics. The recommended bandwidth of 1000 and scaling factor for bandwidth of 2 were used for DMRcate. For individual CpGs, we used a FDR < 0.05 as the significance threshold. DMR was defined as consisting of at least two consecutive CpGs and having a FDR < 0.01. The comb-p method, on the contrary, identifies DMRs based on the output of a probe-by-probe analysis (Pedersen et al., 2012; Vangeel et al., 2017). Briefly, Comb-p calculates Stouffer-Liptak-Kechris (SLK)-corrected p-values for each CpG based on autocorrelation between CpGs, then identifies regions of enrichment using a peak-finding algorithm. Finally, one-step Šidák multiple-testing correction is applied to SLK-corrected p-values. We used a FDR threshold of 0.05 for the initial selection of enriched regions, and a Šidák significant threshold of 0.01 for the final identification of DMRs consisting of at least two consecutive CpGs. As DMRcate and Comb-p apply distinct statistical algorithms to identify DMRs, significant DMRs were defined as DMRs identified by both MDRcate and comb-p methods to reduce false-positive results.

2.3.3. Gene-diseases associations

We mapped significant CpGs to gene Entrez identifiers using 450 K array annotation, then evaluated gene-disease associations (GDAs) using DisGeNET database (Version 7.0 https://www.disgenet.org/) (Piñero et al., 2016). DisGeNET is an open-access platform containing over one billion gene-disease associations between >21 thousand genes and over 30 thousand diseases, disorders, traits, or phenotypes. We selected the top five associated diseases for each gene according to GDA scores which consider the number and type of curated sources, and the number of publications reporting the association.

2.3.4. Functional characterization of CpG sites

For each temperature fluctuation index, gene set enrichment analysis for differentially methylated CpG sites and regions was performed based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms. We included all significant CpGs identified from CpG-specific analysis and CpGs located in the significant DMRs. A standard hypergeometric test (HGT) was applied to explore GO terms and KEGG pathways, accounting for probe number and multi-gene bias (Maksimovic et al., 2021). Gene-specific p-values for over-representation were produced. *ClusterProfiler* (version 4.0) was used to eliminate redundant GO terms resulting from the parent–child semantic relationship and obtain a representative term (Wu et al., 2021a). GO terms or KEGG pathways with a FDR for enrichment < 0.05 were considered significant.

All analyses were performed in R software (version 4.1.0) with packages "geepack" (for GEE, version 1.3–2), "ENmix" (for comb-p analyses, version 1.28.8), "DMRcate" (for DMRcate analyses, version 2.6.0), "missMethyl" (for gene set enrichment analyses, version 1.26.1) and "ClusterProfiler" (version 4.0.5).

3. Results

3.1. Characteristics of study participants and measurement of exposure

The characteristics of participants are shown in Table 1. The average age of 479 participants was 56.4 years (SD: 7.9 years). Most of the participants (60.8 %) had never smoked and less than a third (30.7 %) were former smokers. There were 235 current drinkers, accounting for nearly 50 % of all participants. The education level of participants ranged from primary and secondary schools (41.3 %) to university (28.2 %). Most of the participants were located in the southeast and east coastal regions of Australia (Fig. 1).

Table 2 shows the distribution of nine temperature fluctuation metrics and other meteorological conditions. All environmental exposures were matched with the date of blood draw. Among 479 participants, the mean (±SD) was 11.2 (±4.3) ℃ for DTR, ranging from 0.3 ℃ to 23.9 ℃; and −0.2 (±2.4) ℃ for TCN, ranging from −10.7 ℃ to 6.1 ℃; and 6.7 (±2.1) C for TV 0–1, ranging from 2.1 C to 13.3 C. TV decreased slightly with longer lengths of exposure and became minimal on TV 0–7. The daily mean temperature varied from 4.1 ℃ to 31.8 ℃, with an average daily value of 17.6 ℃. The exposure indices were correlated with each other, with the highest Pearson correlation observed for TV 0–6 and TV 0–7 (0.98) (Fig. S1). Correlation coefficients between nine exposure indices and cell types were statistically non-significant or weak (Table S1). Compared to TCN, both DTR and TV showed larger variability (Fig. 1).

Table 1
Characteristics of participants.

Variables	ALL (n = 479)	DZ (n = 132)	MZ (n = 132)	Sister (n = 215)
Age, mean (SD)	56.4 (7.9)	57.0 (7.2)	55.6 (8.4)	56.56 (8.0)
BMI, mean (SD)	26.8 (5.7)	26.8 (5.7)	26.3 (5.4)	27.11 (5.9)
Smoking status (%)				
Current	41 (8.6)	10 (7.6)	12 (9.1)	19 (8.8)
Former	147 (30.7)	37 (28.0)	37 (28.0)	73 (34.0)
Never	291 (60.8)	85 (64.4)	83 (62.9)	123 (57.2)
Drinking status (%)				
Current	235 (49.1)	65 (49.2)	69 (52.3)	101 (47.0)
Former	52 (10.9)	15 (11.4)	8 (6.1)	29 (13.5)
Never	192 (40.1)	52 (39.4)	55 (41.7)	85 (39.5)
Education level, %				
Secondary or below	198 (41.3)	63 (47.7)	50 (38.2)	85 (39.7)
Vocational	144 (30.1)	29 (22.0)	37 (28.2)	69 (32.2)
University	135 (28.2)	40 (30.3)	44 (33.6)	60 (28.0)
Cell type, mean (SD)				
CD8 + T	9.0 (3.7)	8.7 (4.0)	9.1 (3.6)	9.0 (3.6)
CD4 + T	15.2 (5.7)	15.1 (5.6)	15.4 (6.3)	15.1 (5.5)
Natural killer (NK)	6.5 (4.3)	7.0 (4.3)	6.7 (4.7)	6.0 (4.0)
B cells	5.4 (2.3)	5.7 (2.7)	5.2 (2.1)	5.4 (2.2)
Monocytes	6.8 (2.2)	7.0 (2.4)	6.5 (2.0)	6.9 (2.2)
Granulocytes	59.4 (7.9)	58.9 (8.2)	59.3 (8.3)	59.8 (7.6)
Survey year, %				
2005	139 (29.0)	31 (23.5)	55 (41.7)	53 (24.7)
2006	223 (46.6)	77 (58.3)	51 (38.6)	95 (44.2)
2007	98 (20.5)	18 (13.6)	24 (18.2)	56 (26.0)
2008	19 (4.0)	6 (4.5)	2 (1.5)	11 (5.1)
Season, %				
Spring	158 (33.0)	31 (23.5)	46 (34.8)	81 (37.7)
Summer	99 (20.7)	38 (28.8)	21 (15.9)	40 (18.6)
Autumn	119 (24.8)	35 (26.5)	36 (27.3)	48 (22.3)
Winter	103 (21.5)	28 (21.2)	29 (22.0)	46 (21.4)

Definition of abbreviations: SD, standard deviation; DZ, monozygotic; MZ, dizygotic.

3.2. Findings from CpG-specific analyses

We identified 14 CpGs significantly (FDR < 0.05) associated with different temperature fluctuations indices after epigenome-wide multiple testing adjustment. These CpGs were located in 10 unique genes (Fig. 2). The most significant association (with the lowest p-value) was observed for cg08485565 (*KCNK4*), corresponding to a 0.27 % increase in methylation level associated with per IQR increase in TCN. Both TV 0–1 and TV 0–2 were associated with cg06811300 (*ATXN1*) and cg13148126 (NTN1). No significant associations were found for longer TV exposures (from TV 0–3 to TV 0–7) (Figure S2 and Table 3). The calculated lambdas and QQ plots suggested no signficant inflation of p-values (Figure S3). For most CpGs identified, the directions of the coefficients for TV of different exposure windows were the same, but opposite of the direction of TCN (Figure S4). After removing cell types in the model, the effect sizes changed slightly (Table S2).

3.3. Findings from DMR analyses

As summarized in Table 4, we identified 161 DMRs using DMRcate and 80 DMRs using comb-p significantly associated with different temperature fluctuation exposures. Among them, 70 DMRs were identified by both DMRcate and comb-p (both Šidák p-value < 0.01 and FDR < 0.01). Most of 70 DMRs were associated with exposure to TCN (27 DMRs), followed by TV 0–5 exposure (16 DMRs). Detailed information on identified DMRs is shown in Table S3. For example, *CIZ1* DMRs (untranslated regions and promoter) showed an average increase in methylation level of 1.72 % associated with per IQR increase in TCN. Methylation levels of DMRs located in *GNA12* (gene body) decreased by about 1.48 % with per IQR increase in TV 0–4. Among all the identified DMRs, the association with the lowest p-value was observed for DMRs located in intergenic regions, showing a mean methylation difference of 2.17 % associated with per IQR increase in TV 0–3. There were no differentially methylated positions overlapping with DMRs.

3.4. Annotated genes and their related human diseases or phenotypes

All the identified CpGs and DMRs mapped to 68 genes. A total of 32 genes with changed methylation levels were found in relation to TCN, while only 5 genes were found to be associated with DTR. As shown by Fig. 3, DTR and TCN were associated with non-overlapping genes, while both TV with short exposure window (TV 0–2 and TV 0–3) and TV with long exposure window (TV 0–5 to TV 0–7) were more likely to be associated with same genes.

As for gene-disease associations, 51 out of 68 genes were linked to 124 diseases and 47 phenotypes. For example, we identified ZNF518B, a protein coding gene functioning in gene silencing, associated with *arthritis*; PTPRN2, coding for the protein tyrosine phosphatase family, associated with *atopic dermatitis*; and NTN1 associated with *subarachnoid hemorrhage*. Except for LGALS9C, NT5M, and PLD6, all genes were related to multiple diseases. Most identified diseases were associated with cancer (e.g., colorectal carcinoma, breast carcinoma, and metastatic neoplasms) and mental disorder (e.g., schizophrenia, mental depression, and bipolar disorder) (Table S4).

3.5. Gene set enrichment analyses

Based on our GO term and KEGG pathway analyses, 24 enriched GO annotations were identified, while no significant KEGG pathways were observed (all FDR > 0.05). Most identified GO terms were related to DTR (nine GO terms), followed by TV 0–4 (seven GO terms) and TV 0–2/TV 0–5 (both five GO terms). TV 0–2 were more likely to share GO terms with TV 0–3; TV 0–4 shared three GO terms with TV 0–5. The top 3 most significantly enriched GO terms were *Response to bacterium* (TV 0–3), followed by *Hydrolase activity, acting on ester bonds* (TCN), and *Oxidoreductase activity* (TV 0–3) (Fig. 4).

4. Discussion

To the best of our knowledge, this is the first study of genome-wide DNA methylation in relation to temperature fluctuations. Our study identified significant changes in methylation levels in 14 CpGs and 70 DMRs associated with short-term temperature fluctuations. Almost all identified CpGs were associated with exposure to temperature fluctuations within 3 days. Differentially methylated signals were mapped to 51 genes linked to human diseases like cancer and mental disorders.

Although no previous studies have analysed the DNA methylation in relation to temperature fluctuations, similar magnitudes of methylation changes associated with mean temperature were found in a previous study (Xu et al., 2021a). According to the epigenome-wide association study (EWAS) Atlas, many CpGs identified in our study have been linked with diseases associated with temperature fluctuations (Li et al., 2019a). For example, the DNA methylation level of cg07618733 was found to display a large, but not significant, difference between adolescent boys suffering from depressive and sleep symptoms and healthy controls (Ammala et al., 2019). However, no clear evidence was found for females in previous studies. Another study from Poland showed that rapid temperature changes were more likely to be associated with an increased risk of mental disorders (Lickiewicz et al., 2020). The top differentially methylated CpG site associated with metabolic syndrome was cg04457354 (Burghardt et al., 2018). Fasting blood glucose, one of the components of metabolic syndrome, was shown to be associated with outdoor temperature, with adipose tissue being the potential mediator. As temperature increases, brown adipose tissue, a cold-activated tissue with a high rate of glucose uptake (Cannon and Nedergaard 2004; Poher et al., 2015), may be inactivated and thus leading to insulin resistance (Lichtenbelt et al., 2009). Notably, our analyses didn't replicate the results for CpGs identified by a previous

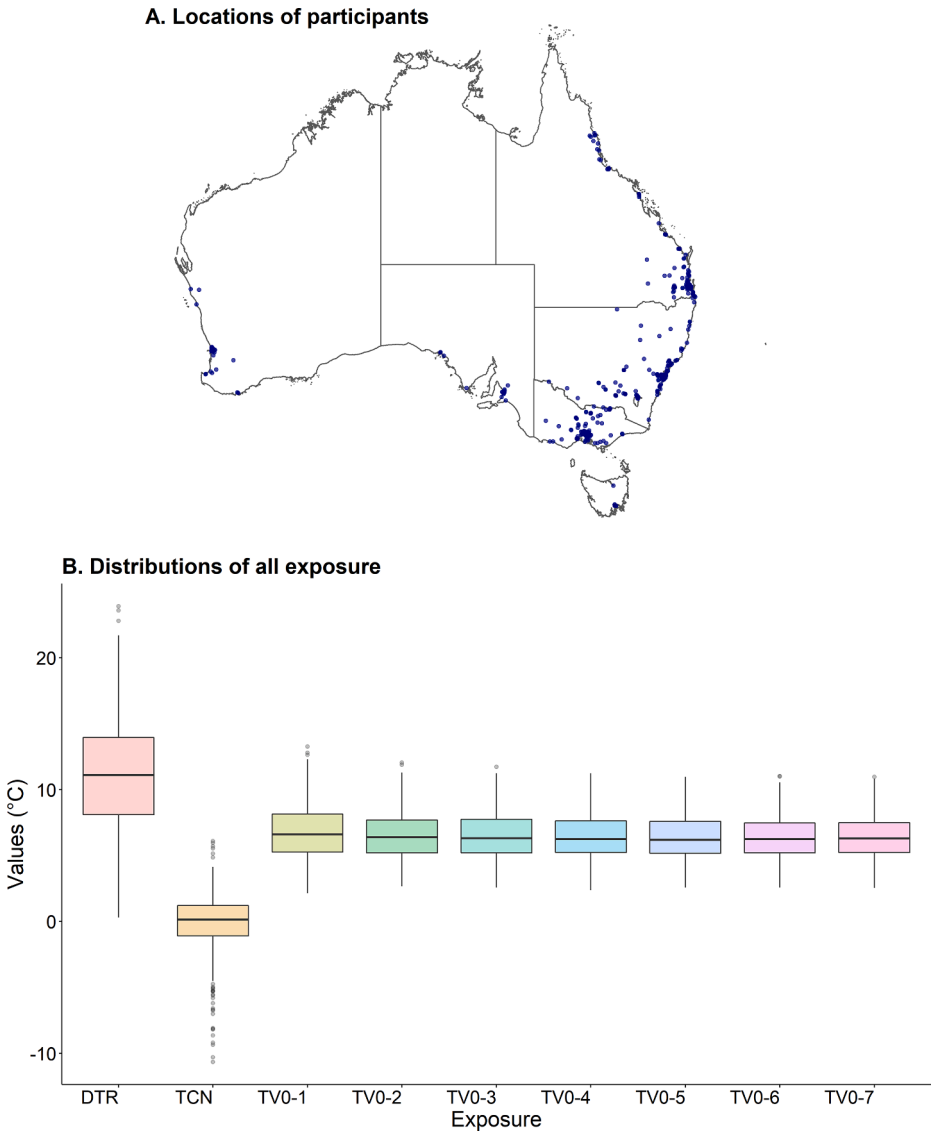

Fig. 1. Address of 479 participants and distribution of TCN, DTR, TV 0–1, and TV 0–7. Definition of abbreviations: TV, temperature variability; DTR, diurnal temperature range; TCN, temperature change between the neighbouring days.

Table 2
Summary statistics of nine temperature fluctuation metrics and other meteorological factors.

Variables	Mean	Standard deviation	Minimum	Percentiles			Maximum
				25th	50th	75th	
Mean temperature, ℃	17.6	5.4	4.1	13.5	17.4	21.5	31.8
Relative humidity, %	69.6	11.4	25.5	62.9	71.7	77.0	97.9
Temperature fluctuations, ℃							
DTR	11.2	4.3	0.3	8.1	11.1	14.0	23.9
TCN	−0.2	2.4	−10.7	−1.1	0.1	1.2	6.1
TV 0–1	6.7	2.1	2.1	5.3	6.6	8.1	13.3
TV 0–2	6.5	1.8	2.7	5.2	6.4	7.7	12.1
TV 0–3	6.5	1.7	2.6	5.2	6.3	7.8	11.7
TV 0–4	6.4	1.6	2.4	5.2	6.3	7.6	11.2
TV 0–5	6.4	1.6	2.6	5.2	6.2	7.6	11.0
TV 0–6	6.4	1.6	2.6	5.2	6.2	7.5	11.0
TV 0–7	6.4	1.5	2.5	5.2	6.3	7.5	11.0

Definition of abbreviations: TV, temperature variability; DTR, diurnal temperature range; TCN, temperature change between the neighbouring days.

study on the association between mean temperature and DNA methylation (Xu et al., 2021a), implying that temperature fluctuation might act by a different mechanism from mean temperature, or at least, target different CpGs. We did not observe an overlap between differentially methylated positions and regions. The possible reason is that DMRcate considers all neighbouring CpGs when smoothing and Comb-p examines regional clustering of neighbouring CpGs with low p-values. The two methods perform best when series of CpG-specific estimates were

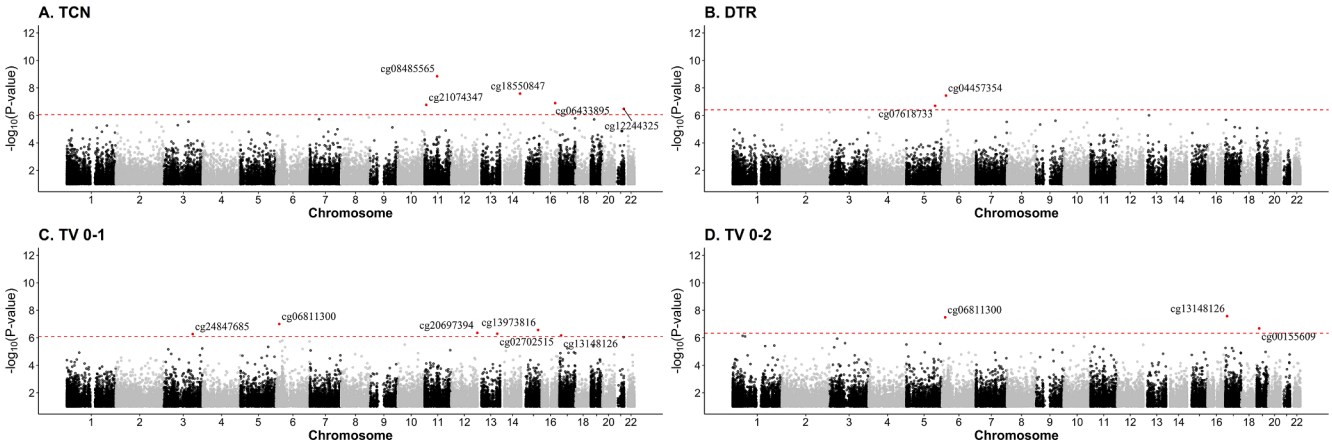

Fig. 2. Manhattan plots for the association between temperature variation and genome-wide DNA methylation. Notes: The red horizontal line represents the threshold where false discovery rate = 0.05. Definition of abbreviations: TV, temperature variability; DTR, diurnal temperature range; TCN, temperature change between the neighbouring days.

Table 3
Differentially methylated CpGs significantly associated with different exposure of temperature fluctuation.

Exposure	CpG	CHR	Position	Gene/nearest gene	Location in gene	Relation to CpG islands	Coefficient (per IQR increase)	SE	*p*-value	FDR
DTR	cg07618733	Chr5	145,562,428	LARS	TSS200	Open sea	2.17×10^{-3}	4.18×10^{-4}	2.01×10^{-7}	0.041
	cg04457354	Chr6	20,447,442	E2F3	Body	Open sea	-1.22×10^{-2}	2.22×10^{-3}	3.64×10^{-8}	0.015
TCN	cg21074347	Chr11	9,115,009	FLJ46111	TSS1500	Shore	1.29×10^{-2}	2.46×10^{-3}	1.71×10^{-7}	0.018
	cg08485565	Chr11	64,057,771	KCNK4	TSS1500	Shore	2.67×10^{-3}	4.42×10^{-4}	1.43×10^{-9}	0.001
	cg18550847	Chr14	100,610,570	EVL	3′UTR	Island	5.34×10^{-3}	9.59×10^{-4}	2.60×10^{-8}	0.005
	cg06433895	Chr16	68,873,850	TANGO6#	IGR	Shelf	-6.08×10^{-3}	1.15×10^{-3}	1.28×10^{-7}	0.018
	cg12244325	Chr21	45,883,864	LRRC3#	IGR	Island	7.08×10^{-3}	1.39×10^{-3}	3.35×10^{-7}	0.028
TV 0–1	cg06811300	Chr6	16,504,474	ATXN1	5′UTR	Open sea	-8.81×10^{-3}	1.65×10^{-3}	1.00×10^{-7}	0.041
	cg24847685	chr3	147,087,652		IGR	opensea	-6.64×10^{-3}	1.34×10^{-3}	5.42×10^{-7}	0.045
	cg20697394	chr12	132,918,304		IGR	shelf	-1.33×10^{-2}	2.65×10^{-3}	4.40×10^{-7}	0.045
	cg02702515	chr13	100,008,029	UBAC2	Body	opensea	5.05×10^{-3}	1.02×10^{-3}	5.09×10^{-7}	0.045
	cg13973816	chr15	85,174,718	SCAND2	Body	island	2.73×10^{-3}	5.37×10^{-4}	2.74×10^{-7}	0.045
	cg13148126	chr17	8,924,758	NTN1	TSS200	island	-1.65×10^{-3}	3.37×10^{-4}	6.87×10^{-7}	0.047
TV 0–2	cg06811300	Chr6	16,504,474	ATXN1	5′UTR	Open sea	-1.01×10^{-2}	1.82×10^{-3}	3.27×10^{-8}	0.007
	cg13148126	Chr17	8,924,759	NTN1	TSS200	Island	-1.89×10^{-3}	3.39×10^{-4}	2.68×10^{-8}	0.007
	cg00155609	Chr19	12,912,527	PRDX2	5′UTR	Island	3.77×10^{-3}	7.27×10^{-4}	2.12×10^{-7}	0.029

Definition of abbreviations: CpG, cytosine-guanine dinucleotide; CHR, chromosome; SE, standard error; IGR, intergenic region; FDR, false discovery rate; TSS, transcription starting site; 3′UTR, the three prime untranslated region.
* Position refers to Genome Research Consortium human genome build 37 (GRCh37)/UCSC human genome 19 (hg19). # Nearest gene.

consistent but small. As both DMRcate and comb-p identify DMRs based on association results of neighbouring probes, a significant DMR does not necessarily overlap a significant CpG site in that region (Lee et al., 2019).

Several studies found that cg21074347 and cg18550847 were differentially methylated between samples (newborns, children, and adults) with asthma and healthy controls (Cardenas et al., 2019; Forno et al., 2019; Hoang et al., 2020; Nicodemus-Johnson et al., 2016; Peng et al., 2019; Reese et al., 2019). As reported in a previous study (Wu et al., 2021b), TV was identified as a risk factor for asthma. Sudden temperature change may induce the desquamation of mucosal epithelial cells followed by the cooling of the upper respiratory tract, resulting in the dysfunction of local respiratory defences. Nevertheless, we cannot conclude that the above CpGs mediate the association between temperature fluctuations and relevant diseases, more studies are needed to explore the roles of DNA methylation in the health impact of temperature fluctuations.

Our study also found that several differentially methylated signals were located within genes with either temperature sensitivity or a known biochemical process involved in temperature fluctuation-related

Table 4
Summary of differentially methylated regions significantly associated with different exposure of temperature fluctuation.

Exposure	No. of significant DMRs		
	DMRcate (FDR < 0.01)	**comb-p** (Šidák p-value < 0.01)	**comb-p overlaps with DMRcate**
DTR	14	9	6
TCN	62	29	27
TV 0–1	15	8	6
TV 0–2	19	12	11
TV 0–3	19	12	10
TV 0–4	21	16	13
TV 0–5	30	16	16
TV 0–6	29	16	15
TV 0–7	25	16	14
Total (unique)	161	80	70

Definition of abbreviations: DMR, differentially methylated region; TV, temperature variability; DTR, diurnal temperature range; TCN, temperature change between the neighbouring days.

diseases. For example, *KCNK4* (Potassium Two Pore Domain Channel Subfamily K Member 4) encodes a protein that functions as a voltage-insensitive potassium channel. A rising temperature (above 37 ℃), within the range of ambient temperature, could increase the frequency of channel opening (Plant 2012). Another gene, *PRDX2* (Peroxiredoxin 2), encodes a member of the peroxiredoxin family of antioxidant enzymes that work against oxidative stress (De Franceschi et al., 2011). When the DNA methylation levels were higher, the expression of *PRDX2* decreased, which could limit the antioxidant capacity (Hong et al., 2018). Previous animal studies found that increased temperature was associated with oxidative stress (Castro et al., 2020; Madeira et al., 2013; Wiens et al., 2017). Our results suggested *PRDX2* could be involved in the oxidative stress response induced by temperature fluctuations, especially TCN. Besides, several genes identified in our studies were linked to breast cancer risk locus, such as *FGFR2* and *ATXN1* (Fletcher et al., 2013; Ke et al., 2015). Although there was no evidence of the association between temperature fluctuation and breast cancer, previous studies showed that females and those aged 45–65 years generated higher cancer mortality risks associated with TV compared with males and those aged above 65 years (Yi et al., 2021).

As suggested by our results, differentially methylated signals associated with temperature fluctuations were found at multiple genomic regions mapped to genes related to mental diseases. Numerous studies have reported the association between ambient temperature and mental diseases. A study in China showed declined mental health attributable to increase in long-term temperature fluctuation (Xue et al., 2019). Another study reviewed the association between extreme weather and mental health, and showed that weather changes could induce psychopathological phenomena such as seasonal affective disorders to weather sensitivity and weather-related pain (Cianconi et al., 2020). More commonly, a strong link between weather fluctuations and specific mental symptom patterns was found, although the latter was below the pathological threshold in most of the cases (Thompson et al., 2018). Although no previous studies, to our knowledge, have directly assessed the association between temperature fluctuations within a short period and mental diseases, we can also speculate, based on our findings, that DNA methylation might be involved in the health impact of unstable weather.

The potential biological implication of the health impacts of temperature fluctuations could also be supported by our knowledge-based gene set enrichment analyses. Our results showed that TV 0–2 shared 3 GO terms with TV 0–3, and TV 0–4 shared 3 GO terms with TV 0–5. Although both high correlation and functional similarities among TV indices could lead to the overlap in GO terms, our findings suggest functional similarities of TV with nearby exposure windows contributed more to the overlapped terms because of the relatively less overlapped results among other TV indices (Wang et al., 2007). On the contrary, there might exist fewer functional similarities between TV and DTR, as no overlaps were found between them. Several identified GO terms in our study have been described to involve inflammatory responses and oxidative stress. For example, oxidoreductase (term: *oxidoreductase activity*) is responsible for the generation of oxidants that are implicated in the development of many diseases (Kotera et al., 2009). One of the subclasses of oxidoreductase, xanthine oxidoreductase, was found to regulate the intracellular generation of reactive oxygen species (Ives et al., 2015). The latter acts as a mediator of *NLRP3* inflammasome activation (Gross et al., 2011; Ives et al., 2015). Immune regulatory cytokine (term: *immune effector*) could exacerbate allergic inflammatory responses in airway (Lacy and Moqbel 2001). Secretory granules (term: *secretory granule*) provided a place to store histamine during the inflammatory process (White 1999). There was also a close linkage among oxidant products, inflammation, and immune responses. For example, the redox milieu contributes to the adaptive immune response induced by T cells that are integral to the inflammatory responses (Ohl et al., 2016). As the inflammatory response and oxidative stress were reported as a potential pathophysiological mechanism underlying the health impact of temperature fluctuations (D'Amato et al., 2018; Halonen et al., 2010; Liu et al., 2015; Schneider et al., 2008; Zhao et al., 2019), significantly enriched GO terms in relation to inflammation and oxidative stress identified in this study supported previous findings of health outcomes associated with temperature fluctuations and suggest additional biological relevance of our differential methylation signals.

Our study has several strengths. First, this is the first study of DNA methylation in relation to temperature fluctuation. This study suggests potential biochemical mechanisms underlying the association between temperature fluctuations and mortality/morbidity, which could improve the understanding of the health impact of temperature fluctuations. Second, we are benefiting from using twin families that can control for shared familial genetic factors. As a result, we can obtain a less biased within-family association of temperature fluctuations with DNA methylation. Third, we evaluated the impact of inter-day and intra-day temperature fluctuations using DTR, TCN, and TV of multiple exposure windows ranging from two to eight days. By doing this, we captured a comprehensive impact of short-term temperature fluctuations on DNA methylation.

However, some limitations should be acknowledged. All participants in this study were female. Our study had a relatively small sample size, which prevent us from exploring the modification effect of season. Besides, considering that the power to discover new temperature fluctuation-associated differentially methylated signals depends on the sample size, more studies with large sample sizes are needed to further identify locus or DMRs with small effect sizes. As global null hypothesis, that all exposure-specific null hypotheses are true simultaneously, is of limited interest in this study, we didn't correct for multiple comparisons across different exposure indices. As a result, the interpretation of results was limited by the independent hypotheses with exposure-specific EWAS without multiple test adjustments across EWAS. Although the adoption of individual strategies (e.g., wearing appropriate clothing and staying in an air-conditioned place) could be an important factor to explain the variation in the effects of temperature fluctuation, their impact had not been taken into account due to the paucity of data. In addition, this study was based on blood samples and could not identify DNA methylation levels among different cell types and tissues. Although we controlled cell-type proportions that can partly control for cellular heterogeneity, findings based on peripheral tissues may not be generally applicable to other tissues, such as brain and visceral fat (Heijmans and Mill 2012). Finally, individual exposure measurement may be inaccurate in our study because we could not obtain individual movement data. Finer exposure data accounting for person-specific space–time patterns are needed to provide a more accurate association between temperature fluctuations and DNA methylation.

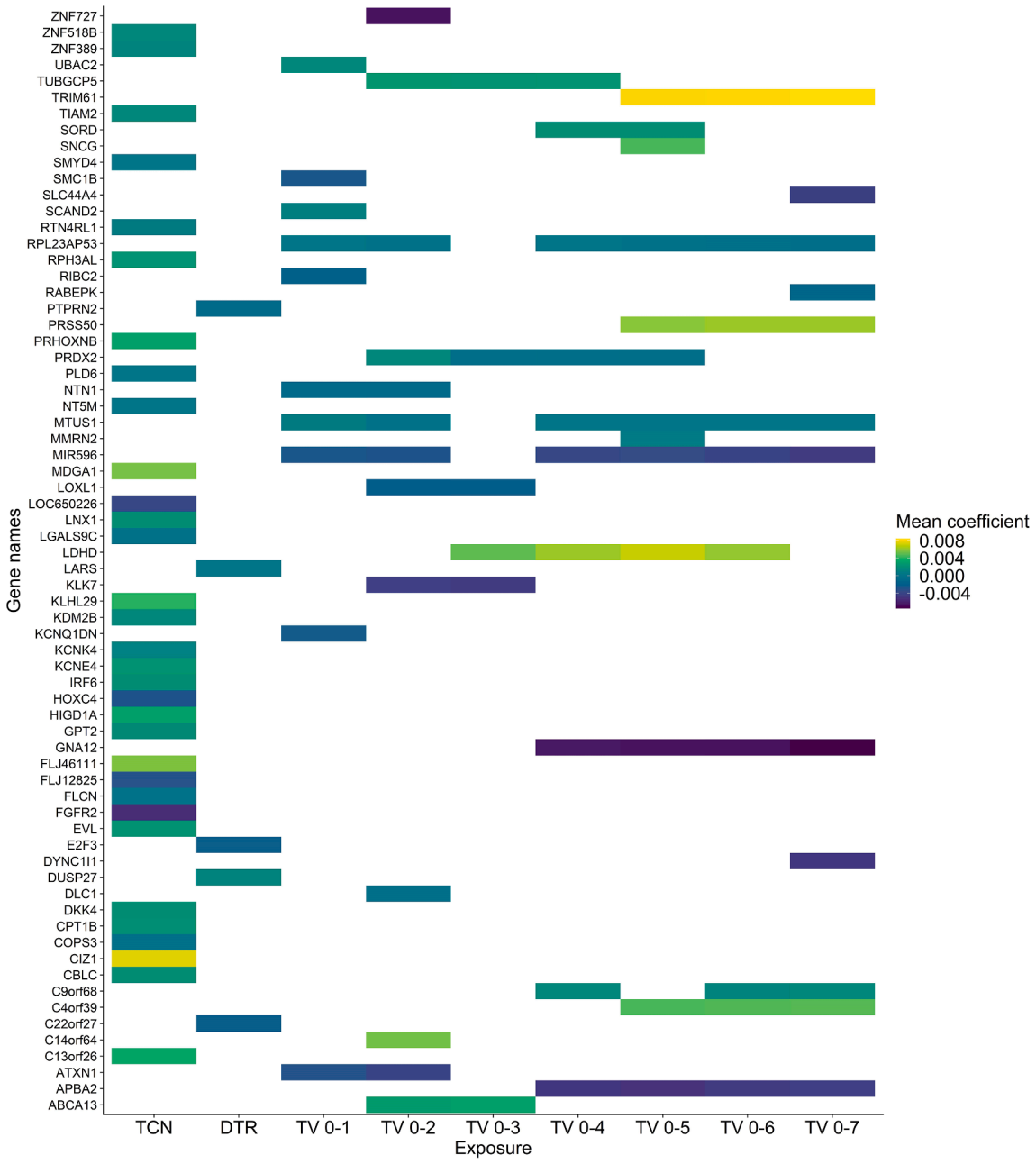

Fig. 3. Genes mapped to identified CpGs and DMRs by exposure. Notes: the mean coefficient refers to the average change in CpG-specific methylation levels associated with every 1℃ increase for all identified CpGs located in each specific gene. Definition of abbreviations: TV, temperature variability; DTR, diurnal temperature range; TCN, temperature change between the neighbouring days.

5. Conclusion

This study suggests that short-term temperature fluctuations were associated with differentiated DNA methylation levels across the human genome. Differentially methylated signals varied across different indices of temperature fluctuation. Future work is needed to confirm and expand our findings.

CRediT authorship contribution statement

Yao Wu: Software, Methodology, Writing – original draft, Visualization, Investigation. **Rongbin Xu:** Conceptualization, Data curation, Methodology, Software, Visualization, Writing – review & editing. **Shanshan Li:** Conceptualization, Writing – review & editing, Supervision. **Ee Ming Wong:** Data curation. **Melissa C. Southey:** Data curation. **John L. Hopper:** Data curation, Methodology, Software. **Michael J. Abramson:** Writing – review & editing. **Shuai Li:** Conceptualization, Data curation, Methodology, Software, Writing – review & editing. **Yuming Guo:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michael J. Abramson reports a relationship with Pfizer and Boehringer-Ingelheim that includes: funding grants. Michael J. Abramson reports a relationship with Sanofi that includes: consulting or advisory. Michael J. Abramson reports a relationship with GlaxoSmithKline that includes: speaking and lecture fees.

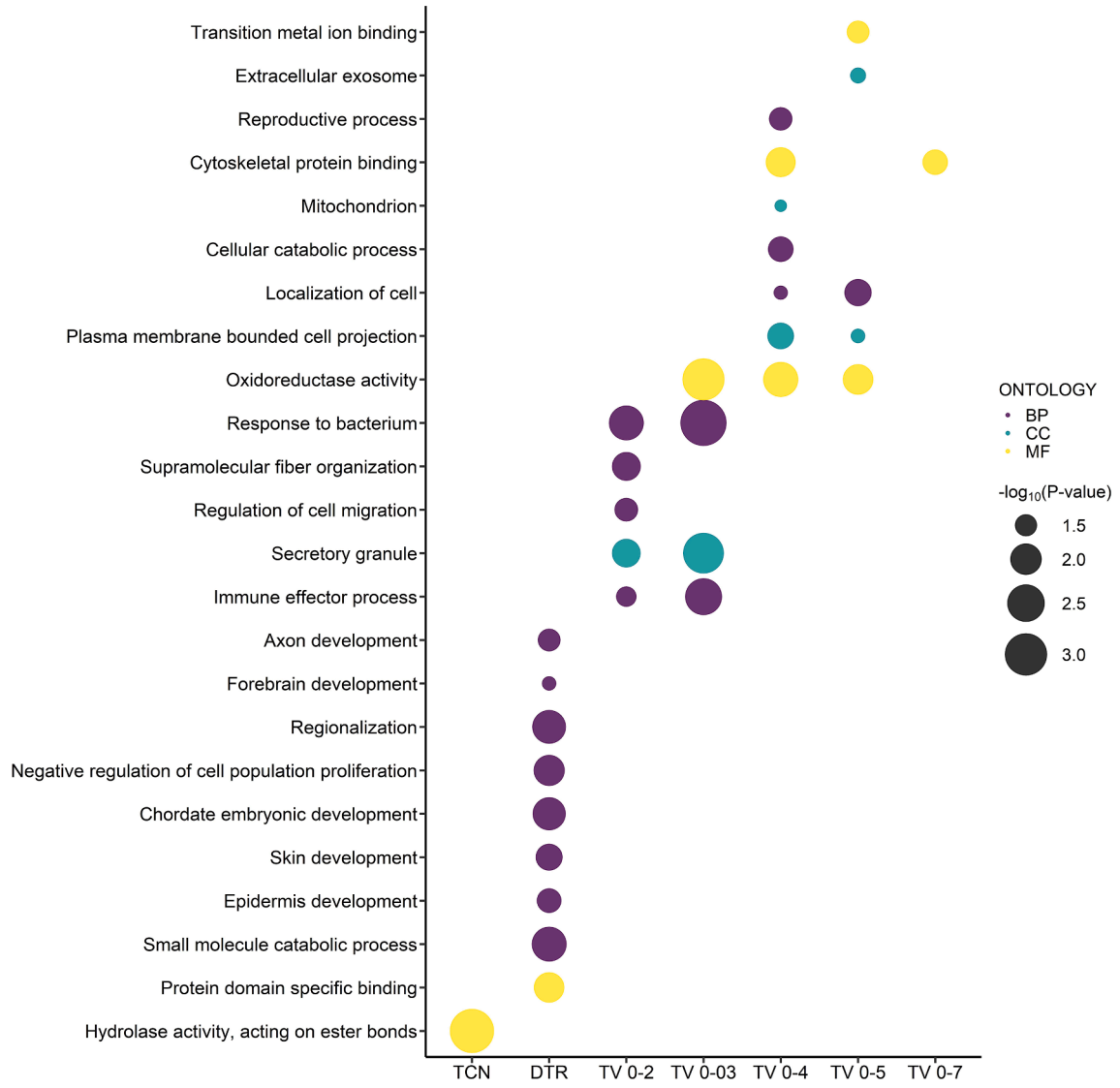

Fig. 4. Significantly enriched GO terms by exposure. Definition of abbreviations: BP, biological process; CC, cellular component; MF, molecular function; TV, temperature variability; DTR, diurnal temperature range; TCN, temperature change between the neighbouring days.

Data availability

Data will be made available on request.

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Data availability

The raw and processed DNA methylation data set are open accessed or free on Gene Expression Omnibus (accession number GSE100227). As required by the ethics approval, the other data (e.g., data on covariates) are not allowed to be open for access. The data could be accessed on reasonable request to J.L.H. (j.hopper@unimelb.edu.au).

Ethics approval and consent to participate

The AMDTSS was approved by the Human Research Ethics Committee of The University of Melbourne. Written informed consent was obtained from all participants. The present study was approved by the Monash University Human Research Ethics Committee.

Competing interests

Michael Abramson holds investigator-initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. He has undertaken an unrelated consultancy for and received assistance with conference attendance from Sanofi. He also received a speaker's fee from GSK. The other authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107655.

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