Epigenetic mechanisms underlying the association between maternal climate stress and child growth: Characterizing severe drought and its impact on a Kenyan community engaging in a climate change-sensitive livelihood

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ABSTRACT

Pastoralists in East Africa are among the world's most vulnerable communities to climate change, already living near their upper thermal limits and engaging in a climate-sensitive livelihood in a climate change global hot spot. Pregnant women and children are even more at risk. Here we report findings of a study characterizing Samburu pastoralist women's experiences of severe drought and outcomes in their children (N=213, 1.8-9.6 years). First, we examined potential DNA methylation (DNAm) differences between children exposed to severe drought in utero and same-sex unexposed siblings. Next, we performed high dimensional mediation analysis to test whether DNAm mediated associations of exposure to severe drought with body weight and adiposity. DNAm was measured using the Infinium MethylationEPIC BeadChip array. After quality control; batch, chip, and genomic inflation corrections; covariate adjustment; and multiple testing correction, 16 CpG sites were differentially methylated between exposed and unexposed children, predominantly in metabolism and immune function pathways. We found a significant indirect effect of drought exposure on child body weight through cg03771070. Our results are the first to identify biological mediators linking severe drought to child growth in a low-income global hot spot for climate change. Better understanding of the mechanisms underlying the association between drought exposure and child growth is important to increasing climate change resilience by identifying targets for intervention.

KEY POLICY HIGHLIGHTS

For pregnant women in populations engaging in climate-sensitive livelihoods, severe drought is characterized by multiple stressors, including intense, sometimes hazardous labor, food and water insecurity, and other stressors. This study found differential methylation between children exposed to severe drought in utero versus their unexposed same-sex siblings in 16 CpG sites in pathways relevant to the immune system and metabolism. Cg03771070 was found to mediate the association between severe drought exposure and child body weight. The necessary next step includes context-nuanced prospective studies to further refine our understanding of biological mechanisms for climate-associated child outcomes. This is necessary for targeted interventions to improve climate change resilience in these communities.

KEY WORDS: drought, Africa, pregnancy, child growth, DNA methylation

INTRODUCTION

Based on the 2022 Intergovernmental Panel on Climate Change, the increase in climate extremes, including heat and drought, has already pushed some human systems "beyond their ability to adapt" (p.8). Moreover, those at highest risk include people – particularly pregnant women, children, and the elderly – already living near their upper thermal limits. Pastoralists in East Africa are listed as among the most vulnerable communities, engaging in a climate-sensitive livelihood in a climate change global hot spot.[1] The health consequences of drought and high ambient temperatures in pregnancy include fetal and infant mortality, lower birth weights, and body composition changes in children that are associated with enduring cardiometabolic and other health risks.[2-6] In spite of the inclusion of pregnant women as a higher risk group for climate change and increasing evidence of the impact of heat waves and drought on pregnancy outcomes,[2] the biological pathways for these outcomes are not well understood.[7] Studies of the epigenetic mechanisms for health outcomes associated with climate extremes could help address this gap but are particularly rare.[8]

The purpose of our retrospective pregnancy cohort study was to characterize the impact of a 2008-2009 severe drought event on a pastoralist community (Samburu) in northern Kenya, a natural disaster that led to losses of over half of Samburu cattle and sheep,[9] and to examine whether DNA methylation (DNAm) mediated associations of in utero exposure to climate extremes with growth and adiposity in 213 children, ages 1.8 - 9.6 years. A unique feature of our study is its strong ethnographic foundation[10] and collaborative engagement with its Samburu study partners.

The Samburu (population 307,957)[11] are livestock herders who live primarily in Samburu County, part of Kenya's north-central semi-arid and arid lands. Poverty rates are higher, and literacy rates lower, in the county than Kenyan national averages.[12] Additionally, the county has 3 doctors per 100,000 people compared to the national average of 10 doctors; antenatal care from a skilled provider is estimated at 51-58% compared to 95-99% for Kenyan ethnic majorities;[13-14] and, in children less than 5 years, 39.9% and 37%, respectively, are 2 or more SD below WHO standards for height- and weight-for-age.[13]

Primary child outcomes for the current study are adiposity and body weight. Previous, non-DNAm studies by other researchers have reported associations between drought or high ambient heat exposure in utero and reduced body weight in newborns and children.[2, 14-16] Concerning adiposity, a non-DNAm study of climate extremes and body composition in adults reported higher adiposity in contexts of temperature volatility or conversely, lower adiposity in food insecure settings.[17] In children, adiposity has been identified as an important energy store that may buffer linear growth in adverse conditions.[18] DNAm studies of human exposure to high ambient temperatures are rare. A 2020 review identified one study by Abraham et al. (2018) that tested genome-wide DNAm associations to exposures in utero in France to moderate temperatures (5-16° Celsius). The authors found null results for temperature except that first trimester mean ambient temperature associated to the density of the methylation distribution.[19] One DNAm study of exposure in utero to an ice storm and one DNAm study of exposure in utero to famine, both in wealthy countries, have identified DNAm mediators relevant to adiposity.[20-21]

We previously hypothesized directions of effect between climate exposures (severe drought, regional climate extremes) and child outcomes that included stature and lower limb growth, body weight, and adiposity. In support of our drought hypotheses, we found lower body weight and higher adiposity in children exposed to the severe drought in utero compared to their same-sex siblings. Linear growth findings were null for severe drought except in girls in the hotter climate region.[22] Both climate region and early gestational ambient temperature exposures significantly associated to linear growth overall, and we report DNAm findings relevant to those exposures elsewhere.[23] For this current DNAm study, we examined potential differential methylation between drought-exposed and unexposed children and performed high dimensional mediation analyses to identify potential mediators of associations between exposure in utero to severe drought and the child outcomes of weight-for-age z-scores and peripheral adiposity (triceps-for-age z-score) that were found to be significant in our previous, non-DNAm study.[22]

MATERIALS AND METHODS

Sample characteristics and data collection methods. All data collection and analysis methods conformed to the principles stated in the Declaration of Helsinki and were approved by Western Michigan University Human Subjects Institutional Review Board [Protocol #17-05-09] and Kenya's National Commission for Science, Technology & Innovation. All recruitment and informed consent materials were translated and back translated by a multilingual team that included Samburu community partners. The study was explained in the Samburu vernacular at community meetings and to parents and child participants at each data collection visit, with consent (and assent for child participants) obtained at each visit. Based on our same-sex sibling

design aimed to test associations between early gestational exposure to drought and child outcomes in a community engaged in a climate-sensitive livelihood, we recruited rural Samburu women who had a child exposed to the peak months of the drought in early gestation and a subsequent child of the same sex unexposed to severe drought in utero or early childhood.

Since detailed antenatal records are not collected in Samburu County, children's medical records (documenting birth dates and vaccinations) were used to determine gestational exposure to the drought, using an estimated gestational age range of 30 to 42 weeks at birth to account for possible preterm births. Drought onset was in 2008, with emergency status beginning no later than April 2009 and continuing through December 2009.[9] Any child whose gestational age was estimated as less than 84 days during the emergency months was considered exposed. The sample includes 104 drought-exposed children and 109 unexposed same-sex siblings (some families had more than one eligible unexposed sibling). For triceps skinfold only, 4 values were missing in the unexposed group and therefore we performed the mediation analysis on a sample of 105 controls. The missing values were due to frightened children who declined skinfold measurements.

Detailed descriptions of our (2017-2019) recruitment, informed consent, and data collection methods for measuring mothers' socioeconomic status and psychosocial stress, for collecting children's saliva using Oragene-500 kits for DNA methylation assays, and for measuring child outcomes and conversion to z-scores, are described in detail elsewhere.[22, 24] Relevant to DNA data collection in brief: DNA from saliva collected in Oragene containers is reportedly stable for a minimum of five years at ambient temperatures and can even withstand degradation at

temperatures of 50º Celsius.[25-26] Regarding tissue specificity, Langie[27] and colleagues have validated a statistical method for estimating cell type proportions in saliva for epigenome-wide studies.

Outcomes

For the overall study, we measured linear growth (height-for-age and tibia-length-for-age z-score), body weight (weight-for-age z-score), and adiposity. For adiposity, we measured triceps-, subscapular-, and suprailiac-skinfold-thicknesses-for age z-scores as most appropriate in rural East African field settings in young children with low body mass index.[28-30] All measurements followed WHO protocols and Lohmann procedures and also those procedures described by Weiner and Lourie. These methods and our non-DNAm findings for all these growth and adiposity outcomes and for our study of telomere length in this sample of children are described in detail in our other papers. [22, 24] As previously noted, for this paper, we focused on significant child outcomes for drought (triceps-skinfold and body weight) based on our previous (non-DNAm) study.[22]

Exposure

Our study examines the impact of a 2008-2009 severe drought event characterized by historically low rainfall, high losses of livestock, and widespread food insecurity that necessitated international humanitarian interventions. Interventions occurred late in the drought however, which likely increased human and animal morbidities and mortality.[9] Since climate change is

increasing the number of heat events and rainfall volatility in East Africa generally, we restricted our exposure window to 2009 pregnancies when the drought was in its worst phase as noted above, in order to capture the highest contrast possible to control pregnancies.

Mediator

We used the Illumina MethylationEPIC (EPIC) BeadChip array-based platform to obtain epigenome-wide data on DNAm, which produces a molecular data set of more than 850,000 methylation marks per subject.

EPIC Methylation Array: Saliva samples were sent to the UM Epigenomics Core for DNA extraction, quality control and processing for the Illumina MethylationEPIC BeadChip array. DNA was extracted from saliva using the PureGene Cell and Tissue Kit, according to the protocol suggested for Oragene collection kits (DNA Genotek document PD-PR-00212). Samples were quantified using the Qubit high sensitivity dsDNA assay, and their high molecular weight quality assessed with the TapeStation genomic DNA kit. For each sample, 250ng were bisulfite converted with Zymo's EZ DNA Methylation kit and using the manufacturer's incubation parameters specific for Illumina MethylationEPIC arrays. Cleaned up samples were then sent to the UM DNA Sequencing core for hybridization to the Infinium MethylationEPIC BeadChip array, washing, and scanning, according to the manufacturer's instructions (Illumina EPIC Datasheet). Quality control methods are described in Supplemental Material.

Covariates

Child covariates. All models were adjusted for age, sex, and cell-type proportion effects, as described in Statistical Analyses in the next subsection.

Maternal stressors. In Steps 2 and 3, models were adjusted for the two stressors that our Samburu community partners specifically identified (husbands or male kin forcing women to work too hard during pregnancy - 'forced work'; or denying them food during pregnancy - 'denied food'). As shown in Table 1, the proportion of mothers who reported having experienced these stressors during drought pregnancies was not significantly different than the proportion of mothers who reported having experienced these stressors during same-sex sibling control pregnancies ('forced work' $p = 0.34$; 'denied food' $p = 1$). Samburu study partners specifically identified 'forced work' and 'denied food' as substantial pregnancy stressors in both drought and typical season conditions. In Samburu society, husbands or male kin (for widowed, divorced, or unmarried women) control women's labor and food access. Even in pregnancy, women may be forced to engage in herding tasks in hazardous conditions (climbing trees to obtain animal feed, herding even in hot ambient temperatures) in addition to carrying water and firewood several kilometers. Additionally, men may decline to slaughter or sell livestock or other commodities as needed to obtain food, and may obtain food for themselves without adequately provisioning the family.[22, 31]

Lifetime maternal trauma was also considered for adjustment. Methods for collecting and creating these variables and for assessing lifetime maternal trauma have been described in detail previously.[22] In brief, lifetime maternal trauma concerns events experienced before each drought

or control pregnancy based on a weighted procedure, and included, for example, parental and child deaths, direct witnessing of deaths, and war exposure with fatalities.

Demographic and maternal status covariates. Parent education, livestock wealth, and maternal status were also considered for adjustment in models. Maternal status refers to whether a woman is monogamous, a first polygynous wife, a second or later polygynous wife, or a woman who is widowed, divorced, or never married. Each of these statuses is important to a woman's social capital and resource access. Since this is a paired sibling study, demographic and maternal status covariates are the same for exposed and unexposed siblings.

Statistical Analyses

DNA methylation. We applied a single CpG-site-based mediation approach using a counterfactual framework[32-33] to investigate whether effects of maternal climate stress on child growth could be mediated by DNA methylation. This approach decomposes the estimate of total effect of maternal climate stress on child growth into estimates of (i) natural direct effects of maternal climate stress on child growth through other biological mechanism and (ii) natural indirect effects of maternal climate stress on child growth through DNA methylation. The single CpG-site-based mediation approach was implemented through a three-step algorithm as follows. In step one, we need to first identify candidate CpG mediators that have significant associations with in utero exposure to drought. More specifically, following quality control as described in Supplemental Material, we converted DNA methylation beta values to M-values, which have been shown to be statistically more robust than beta values, and we used the *ComBat* function based on an empirical Bayesian procedure to adjust for batch effects (Sentrix_ID, categorical

variable with 28 groups) and positional effects (Sentrix_Position, categorical variable with 8 groups).[34-35] A batch-group balance was evaluated with the Stuart-Maxwell test for categorical variables, and we found a balanced batch-group design for Sentrix_ID ($p = 0.2533$) or Sentrix_Position ($p = 0.1174$). Due to our same-sex sibling design, we then used the ComBat-corrected M-values, which hereafter we call M-values for simplicity, as our outcomes in linear mixed mixed-effects models with drought as exposure, and sibling identifier as a random effect. Our models were also adjusted for age, sex, and cell-types effects, the fractions of a priori known cell subtypes (Epithelial (Epi), Fibroblast (Fib) and Immune cells (ICs) as reference) calculated using R package *EpiDISH*, and seven major ICs were included: neutrophils, eosinophils, monocytes, $CD4^+$ and $CD8^+$ T cells, B cells and natural killer [NK] cells.[36-37] We used the R package *lme4* to estimate the effects, and we performed a one-degree of freedom test for a coefficient of exposure variable in the model by using t-test.[38]The corresponding p-values were computed using the Satterthwaite's (Kenward-Roger's) approximation.[39-40] Next the R package *BACON* was implemented to adjust the regression data for estimated bias and genomic inflation. All analyses accounted for multiple testing by controlling the false discovery rate (FDR) at 5% level, and we selected CpG sites showing significant association with drought ($FDR < 0.05$) as our candidate mediators.[41-42]

In step two, to make sure there exists an association between the in utero exposure to drought and weight-for-age z-scores as well as triceps-skinfold-thickness-for-age z-score, we regressed each outcome to drought, controlling for age, sex, cellular heterogeneity, and maternal stress and resource covariates identified through model selection: With drought as the exposure, we performed model selection for child body weight and triceps-skinfold-thickness-for-age z score

as dependent variables, included 'forced work' and 'denied food' maternal stressor covariates, and considered additional covariates including lifetime maternal trauma, maternal status, parents' education, and livestock wealth. Stressors were excluded if collinear with drought exposure ($r_s \geq 0.7$). The best fit of models was determined by the Akaike information criterion (AIC) and Bayesian information criterion (BIC). Maximum likelihood method was used to estimate the fixed effect. Models with significant total effect of drought proceeded to the following step.

Finally, in step three, we calculated the indirect effects of the exposure on the outcomes by fitting two linear mixed-effects models for each candidate CpG mediator identified from step 1. First, we regressed M-values of candidate CpG mediator on drought, and next, we regressed weight-for-age z-scores and triceps-skinfold-thickness-for-age z-score on drought, and candidate CpG mediator. Both regression models controlled for age and sex; corrected for cellular heterogeneity; and adjusted for maternal stressors of 'forced work' and denied food'. Additionally, based on model selection in step 2, we adjusted for lifetime maternal trauma in weight-for-age z-score models. For counterfactual approach to the causal mediation model, we adopted the sequential ignorability (identifiability) assumption, that there is no unmeasured confounding of the exposure-mediator, exposure-outcome and mediator-outcome relationships and that none of the mediator-outcome confounders are affected by the exposure.[43] If all identifiability assumptions are satisfied, the average natural direct and indirect effects are identified. The bootstrap estimations and 95% confidence intervals of direct and indirect effects were obtained from R package *mediation* with 10,000 Monte Carlo draws. Based on the number of candidate mediators identified from Step 1, results were adjusted for multiple comparisons

using FDR at 5% level. Details of models and brief discussion of mediation analysis are provided in the Supplementary Material.

We checked the sequential ignorability assumption of mediation analysis by estimating the correlations of the residuals between the mediator model and the outcome model. The correlations ranging from 9.85E-16 to 0.04 suggested that there was no violation of the sequential ignorability assumption.[44] Moreover, we examined the regression diagnostic plots to assess the assumptions of linear regression models and outliers, and all models' assumptions are met.[45]

RESULTS

Maternal Stressors. Table 1 shows descriptive statistics for lifetime maternal trauma up to each pregnancy, and of pregnancy-timed culturally-specific stressors of 'forced work' and 'denied food' for drought compared to same-sex sibling control pregnancy. Reporting did not differ substantially between drought and control pregnancies for these two culturally specific stressors.

Children's Growth Variable Descriptive Statistics. Table 2 shows descriptive statistics for child demographic characteristics and the outcomes of child body weight and peripheral adiposity (triceps skinfold thickness). There were more girls than boys. Based on the study design, unexposed same-sex siblings were conceived after the drought ended and therefore were younger than drought-exposed siblings. Children's mean weight-for-age and triceps-skinfold-thickness-for-age z-scores were at least a standard deviation below reference populations, except for triceps

skinfold-thickness-for-age z-scores in drought-unexposed siblings, which was less than one standard deviation below reference.

Maternal Demographic Descriptive Statistics. Table 3 shows demographic characteristics of mothers. Mothers had very low educational levels, with an average of less than first grade. The same percentage of mothers were wives of monogamous as those of polygynous husbands, with some widowed, never married, or divorced. Families had the equivalent of 20 cows and 2 dairy cows on average, as measured in tropical livestock units (TLU) that are each economically equivalent to one cow.

DNA Methylation Results. There were 16 CpG sites differentially methylated in children exposed to drought in early gestation compared to unexposed same-sex siblings (Table S1), most in the gene body or 5'-UTR region, with 7 CpGs of drought siblings hypermethylated and 9 hypomethylated relative to unexposed siblings. The range of difference based on the Beta-values was 0.09% to 2.89%, with a mean of 0.76%. The number of differentially methylated CpG sites (16) was too small for gene ontology analysis. Descriptions and relevant literature for associated genes can be found in Table S1 of Supplemental materials. Most genes were relevant to metabolism (for example, voltage-gated ion transport) or the immune system (for example, cytokines).

Body weight. Table 4 shows that total effect of exposure to drought was significant for weight-for-age z-score (Estimate = -0.47, p = 0.01). There was one significant CpG mediator (cg03771070) of the association between drought exposure in early gestation and weight-for-age

z-score, as shown in Table 5. The indirect effect through the mediator (Average Causal Mediated Effect/ ACME) = -0.31 and proportion mediated = 0.69. Illumina Methylation Annotation linked the mediator at A-kinase-anchoring protein 7 (AKAP 7; splice variants in gene body or 5' untranslated region). Both the direct and indirect effects were negative: drought negatively associated to child body weight and the effect through the mediator was also negative. This implies that the effects of drought on child body weight are partially mediated by the CpG mediator (cg03771070). Also, the CpG was hypermethylated in drought-exposed compared to unexposed siblings (methylation difference 0.96, Table S1).

Adiposity. Total effect of drought exposure was significant for triceps-skinfold-thickness-for-age z-score (Estimate = 0.65, $p < 0.01$) (Table 4). There was one significant CpG mediator (cg23311137) of the association between drought exposure in early gestation and triceps-skinfold-for-age z-score (ACME = -0.2, proportion mediated = -0.29), which Illumina Methylation Annotation linked at ATP2C1 (splice variants in the gene body; transcriptional start sites 1500, 200; 5' or 3' untranslated regions). The association between drought exposure and adiposity was positive but the effect through the mediator was negative. Since the direct and indirect effects have opposing signs, the results suggest that the effect of CpG mediator (cg23311137) is considered a suppressor or an inconsistent mediator. Also, the CpG was hypermethylated in drought-exposed compared to unexposed siblings (methylation difference 0.62, Table S1). However, although cg23311137 was differentially methylated between drought-exposed and unexposed (FDR < 0.01) and therefore was a candidate for mediation analysis, and was significant at the mediation step (ACME = -0.2, ACME p = 0.04, proportion mediated = -0.30), cg23311137 was non-significant after mediation-step FDR correction.

DISCUSSION

Consistent with previous (non-DNAm) studies by other researchers that have linked gestational exposure to drought with child's body weight,[2, 46-47] our study found that body weight (based on age- and sex-specific z-scores) was lower in children exposed to severe drought relative to their unexposed same-sex siblings. We also found higher adiposity in drought-exposed children, which is consistent with other studies of famine and extreme weather events. Our study is unique in its nuanced characterization of drought-timed stressors based on women's reporting and its epigenetic focus relevant to severe drought in a climate change vulnerable community – testing for differentially methylated CpG sites as potential mediators of the association between severe drought exposure in early gestation and child outcomes.

The dichotomous drought exposure variable used in this study is a proxy for multiple embedded exposures, including psychological stress and heat stress, that are methodologically challenging to distinguish and quantify.[48-49] Our retrospective pregnancy cohort study characterized the stressors of severe drought from the perspective of study participants (Table S5, Supplemental file) and confirmed through historical weather data that the drought coincided with high daytime ambient temperatures and historically low rainfall. In addition to the two culturally-specific stressors of being denied food or forced to work too hard during pregnancy by husbands or other male kin (which were not highly correlated with drought), women identified 24 additional stressors or potentially traumatic events. A cumulative count variable of these pregnancy-timed stressors was significantly higher in drought-exposed pregnancies compared to later pregnancies

(after the drought resolved) (Table S5). Notable substantial differences characterizing drought included food and water insecurity, resource loss (livestock death), hazardous work relevant to food acquisition, and physical weakness. With respect to heat, as reported in our non-DNAm study for this sample,[22] mean maximum daytime ambient temperatures for drought-exposed pregnancies were 102° Fahrenheit/39.05° Celsius in early gestation averaged across pregnancies, although the comparison temperatures for unexposed same-sex siblings were still relatively high at 95° Fahrenheit/35° Celsius. Ethnographically, participants reported subjective heat stress, particularly while engaging in resource acquisition tasks (e.g., herding, collecting water and firewood). Cumulative rainfall in early gestation drought-exposed pregnancies (79.21 mm) contrasted more sharply with unexposed pregnancies (185.49 mm).

After quality control and corrections for batch, chip position, and genomic inflation, and adjusting for sex, age, and cellular heterogeneity, we identified 16 CpGs differentially methylated in children exposed to the 2008-2009 drought in early gestation compared to their same-sex siblings conceived after the drought resolved, predominantly relevant to metabolism and the immune system (Table S1). We also performed a high dimensional mediation analysis for eligible mediators of the association between drought exposure and child outcomes. Two CpGs were identified as mediators between exposure and child outcomes. Both were hypermethylated in drought-exposed children and located in gene regions often found to suppress gene expression, although the association of DNA methylation with gene upregulation or down regulation is complex.[50-52]

Cg03771070 at AKAP7 mediated the association between exposure and child body weight. Children exposed to drought in utero had lower body weight for age compared to unexposed same-sex siblings. The AKAP proteins play a role in a number of processes, including insulin secretion and cardiac function.[53] Additionally, AKAP7 is believed to play a role in antiviral immunity, with potential relevance to coronaviruses, rotaviruses, and others.[54] A variant of AKAP7 is among PKA variants with high to moderate impact identified in a cohort of obese children with and without nonalcoholic fatty liver disease.[55]

Cg23311137 at ATP2C1 mediated the association between drought exposure and child peripheral adiposity, although it was non-significant after FDR correction. Children exposed to drought in utero had more peripheral fat (triceps-for-age-skinfold-thickness z-score) than unexposed same-sex siblings. Immune-relevant ATP2C1 is a member of the ATPase group of enzymes, which play an essential role in cell metabolism. It has been found to be hypermethylated in infants who were born small-for-gestational-age.[56]

Drought and Developmental Conditioning

Based on the developmental origins of health and disease (DOHaD) hypothesis, fetuses are developmentally conditioned in response to the maternal environment in ways that optimize an organism's survival to successfully reproduce.[57] Gestational timing of maternal stress is important to the direction of effects in offspring, possibly because early gestational timing provides a key developmental window for offspring to recalibrate their growth patterns. In a metaanalysis leveraging 719 studies based on 21 mammal species, early gestational stress

associated to accelerated growth and faster time to maturation, while later gestational stress associated to reduced offspring growth and slower maturation. Importantly, elevated maternal glucocorticoid levels were involved in altered growth patterns for all stressors (heat, nutritional, psychosocial – predation or restraint), even when artificially introduced in the absence of maternal stress. Additionally, if reduced prenatal maternal investment coincided with elevated prenatal glucocorticoid levels, the growth effects on offspring could cancel each other out. As the authors point out, not enough is known about the mechanisms for these observed effects of gestational timing of maternal stress.[58]

Postnatal environment is also critical to lasting impacts of gestational exposure to maternal stress. In DNAm studies in high income countries where the postnatal environment following famine or weather anomaly was one of adequate nutrition for example, offspring have been found to be at higher risk for obesity and adverse cardiometabolic outcomes.[20-21] Conversely, in contexts like that of our Samburu study population – the energetic demands of high pathogen burdens, extreme psychosocial stress, and intensive physical labor (often in high ambient temperatures) while experiencing food and water insecurity[13, 59] may be too high relative to the competing needs for growth, maturation, and immune response. Neither "catch-up" nor accelerated growth may be adequate to allow children to reach their expected size and overall life expectancy may be reduced.[18, 60-61]

STRENGTHS AND LIMITATIONS

To our knowledge, this is the first epigenome-wide study evaluating DNAm as a mechanism underlying associations between exposure to drought in utero and child outcomes in one of the global hot spots for climate change vulnerability. The study leveraged fine-grained ethnographic observation to fully characterize the effects of drought for our respondents and to identify and adjust for 2 culturally specific maternal pregnancy-timed stressors our respondents identified during the pilot phase. Given the challenges of parsing the effects of drought, our methodology and the stressors we have identified can be usefully leveraged in future prospective pregnancy cohort studies that assess these stressors and measure ambient temperature exposure in real time. We also took overall drought stressor timing into account and recruited based on first trimester gestational exposures. The study's same-sex sibling design reduced the potential for maternal and household level confounders. Our study met the sequential ignorability assumption of mediation analysis, suggesting there were no unobserved pre-treatment confounders, as well. However, as a retrospective study, we could not measure pregnancy-timed maternal nutrient intake, pathogen exposure, and physical activity, that might be important modifiers of the effects of drought exposure on child outcomes. Additionally, to avoid biasing our results by including same sex sibling controls who might have been exposed to the drought in early childhood, our drought-exposed siblings were older than their unexposed same-sex siblings, although we used age- and sex-specific z-scores for child outcomes and adjusted for age in all models. This also meant that maternal parity was consistently lower for drought-exposed compared to same-sex drought-unexposed siblings. (All children were prepubertal at data collection based on observed Tanner stage.) Finally, although our same sex sibling controls were unexposed to severe drought, some were nevertheless exposed to high ambient temperatures and historically low rainfall in utero because climate change is not only increasing severe drought frequency, but increasing the

number of heat waves and overall rainfall volatility in East Africa. This may have biased results towards the null. This is evidenced in our study examining climate region and gestational ambient temperature exposures, where we find differential methylation even in children unexposed to drought in utero.[23]

CONCLUSION

Our study found an association between DNAm and early gestational exposure to the severe drought in pathways relevant to metabolism and the immune system. The study also identified metabolism and immune system relevant DNAm mediators of the association between drought and child weight (at AKAP7), and possibly between drought and children's peripheral adiposity (at ATP2C1). This begins to address a need for more precision in understanding the biological mechanisms for previously observed associations between gestational exposure to climate extremes and children's body weight and other outcomes. Although the evolutionarily adaptive biological mechanisms in response to heat and psychosocial stress are similar, differing postnatal social environments pose contrasting risk, such as wasting versus obesity. Better understanding of the biological mechanisms underlying fetal responses to climate stress exposure in utero is important for evaluating the life long costs of evolutionary adaptive responses so that appropriate biomedical and public health interventions can be identified. Comparison between exposures, mediators, and outcomes in communities at risk for stunting and wasting, as in the current study, and in communities at risk for obesity, crucially enhances our overall understanding of climate-change relevant biological mechanisms.

Our study also identified maternal stressors relevant to child outcomes, some of which are generalizable to other populations (for example intercommunity and interpersonal forms of violence) and others which are culturally-specific (forms of patriarchal control over women's labor and food supply). Identifying potentially modifiable community-specific maternal resources that might ameliorate the impacts of heat stress – by, for example, reducing the intensity of, and coercion surrounding, women's physical labor pertinent to our study – is important for partnering with communities to design relevant public health interventions to enhance climate change resilience.

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DECLARATION OF INTEREST. The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT. The partners of this study are African indigenous peoples. The data that support the findings of this study are available on request from the corresponding author, subject to restrictions imposed by Kenya's National Commission for Science, Technology, and Innovation (NACOSTI). The data are not publicly available due to NACOSTI rules and privacy or ethical restrictions.

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Table 1. Descriptive Statistics for Individual Maternal Stressor Variables

Parameter	During Drought Pregnancies	During Same-sex Sibling Control	*P*-value[a]
'Lifetime maternal trauma', number of lifetime traumatic events prior to the pregnancy, range = 0 to 4 events per woman	0.721 ± 0.864	0.954 ± 0.994	<0.01*
Culturally-specific stressors identified by women as very stressful during pregnancy			
Forced to work too hard by husband or male kin	13/104 (12.5%)	9/109 (8.3%)	0.34
Denied food by husband or male kin	12/104 (11.5%)	13/109 (11.9%)	1
[a] Paired t-test for continuous stressors; McNemar's test for dichotomous (yes/no) variables.			

Table 2: Descriptive Statistics for Children's Demographic Characteristics and Growth Variables

Parameter	Drought (N = 104)	Unexposed (N = 109)	*P*-value[a]
Female	56/104 (53.8%)	59/109 (54.1%)	
Male	48/104 (46.2%)	50/109 (45.9%)	
Age in years	8.515 ± 0.336	5.006 ± 1.162	<0.01*
Weight (kg)	20.1 ± 2.54	14.4 ± 2.32	<0.01*
Weight-for-age z-score	-1.597 ± 0.516	-1.033 ± 0.656	<0.01*
Triceps skinfold thickness (mm)	5.94 ± 1.52	7.2 ±1.83	<0.01*
Triceps-for-age z-score	-1.112 ± 0.681	-0.658 ±0.864	<0.01*
Unknown/Missing (for Triceps only)	0 (0.00%)	4 (3.67%)	
[a] Paired t-test for children's growth variables.			

Table 3. Descriptive Statistics for Demographic Variables

Parameter	Mean ± SD	N (%)
Mother's highest grade	0.822 ±2.067	
TLU values (cattle equivalents)[a]	19.966 ± 32.169	
Dairy TLU values	1.864 ± 2.699	
Wife status - 1st polygynous wife		21/104 (20.2%)
Wife status - 2nd or later polygynous wife		27/104 (26.0%)
Wife status - monogamous wife		48/104 (46.2%)
Wife status - widow unmarried divorced		8/104 (7.7%)
[a]TLU is tropical livestock equivalent, as follows: 1 cow, 0.7 camel, 10 goats or sheep		

Table 4: Total Effect Models[a]

Parameter	Weight-for-age z-score (Estimate, *P*-value)	Triceps-skinfold-thickness-for-age z-score (Estimate, *P*-value)
(Intercept)	-1.03 (0.00)	-1.17 (0.00)
Drought-exposed	-0.47 (0.01)	0.65 (0.00)
Female sex	0.37 (0.00)	0.24 (0.03)
Age in years	-0.07 (0.41)	-0.64 (0.00)
Epi cell type[b]	-0.01 (0.98)	-0.33 (0.5)
Fib cell type	-7.19 (0.12)	-5.98 (0.31)
'Forced work' during pregnancy	-0.04 (0.76)	0.28 (0.11)
'Denied food' during pregnancy	0.21 (0.11)	0.18 (0.29)
# 'Lifetime maternal trauma' (up to each specific pregnancy)	-0.11 (0.01)	--

[a]Linear mixed model fit by maximum likelihood; t-tests use Satterthwaite's method; [b]cellular heterogeneity: epi=epithelial; fib = fibroblast; immune cells are reference.

Table 5: Significant Mediator of the Association between Drought Exposure and Weight-for-Age Z-Score[a]

Model	CpG	Nearest Gene	ACME[c]	ACME *P* value	ADE[d]	ADE *P* value	Proportion mediated	ACME *p*FDR[e]
zWeight[b]	cg03771070	AKAP7	-0.31	<0.01	-0.15	0.41	0.69	<0.01

[a] cg23311137 (near ATP2C1) is differentially methylated between drought exposed and unexposed siblings (*p*FDR < 0.01) and is a significant mediator for drought and zTriceps skinfold thickness (ACME -0.2, ACME $p = 0.04$, proportion mediated -0.2976). However, cg23311137 is non-significant after FDR correction at mediation step (0 of 1 CpG site for drought and triceps skinfold thickness for age are significant after FDR correction at mediation step.); [b]As shown, 1 out of 1 CpG site is significant after FDR correction at mediation step; [c]ACME is indirect effect (average causal mediated effect); [d]ADE is average direct effect. [e] P-values were corrected for 16 tests.

Supplemental file

[Table S1 is at the end of this document]

EPIC Methylation Array Quality Control Methods

Pipeline: Microarray data quality control was done using the UM Epigenomics Core pipeline built with Snakemake[i] to manage the bioinformatics workflow in a reproducible manner.

Quality Control: Raw red/green IDAT files were read into R using the minfi Bioconductor package (v1.28.3).[ii] Initial quality control based on detection p-values and signal intensity were performed using the ENmix Bioconductor package (v1.18.1).[iii] A sample with more than 5% of probes having detection p-value < 0.05 is removed from downstream analysis. There were 3 samples that were removed for this reason. A probe with detection p-value < 0.05 in more than 5% of samples is removed. There were no probes removed for this reason.

After filtering, a series of corrections and normalizations occurred. Probe intensities were background-corrected using out-of-band Infinium I intensities and dye-corrected using the RELIC algorithm.iii. Next, inter-array normalization was performed by separately quantile normalizing methylated and unmethylated intensities for Infinium I or II probes.v. Then, probe-type biases were corrected for with the beta-mixture quantile normalization method (BMIQ).[iv] Finally, any probe within 2bp of a SNP was removed, as were known cross-hybridizing probes.[v,vi] An additional 50,413 probes were removed for either their proximity to a SNP, their known cross-reactivity, or both.[v, vi]

Genomic inflation.

The genomic inflation factor λ was 2.07 before adjustment and reduced to 1.23 after adjustment. Quantile-quantile (QQ) plots of unadjusted p-values (left) and genomic inflation adjusted p-values (right) are shown in Figure 1. Unadjusted p-values and adjusted p-values are reported in Table S1 for comparison.

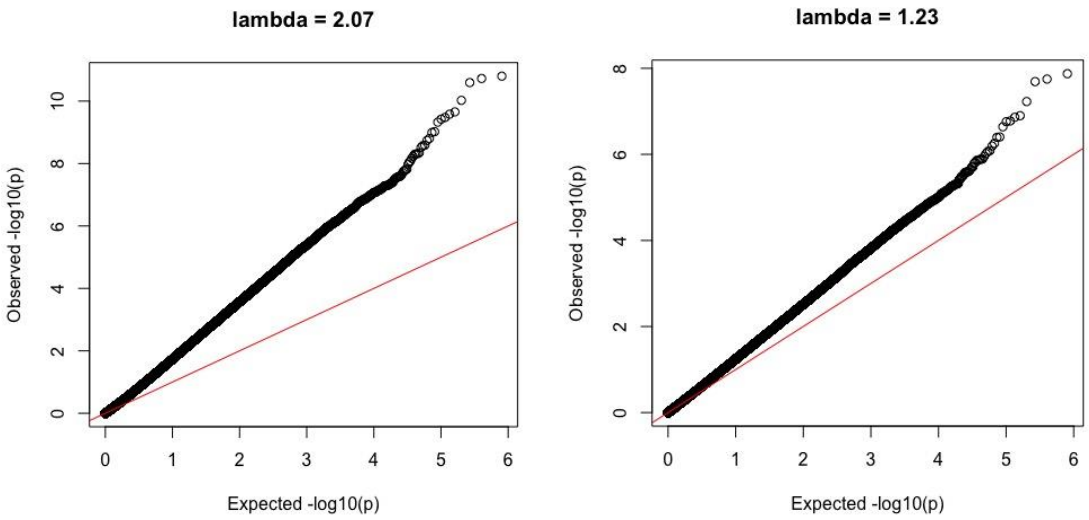

Figure 1. Quantile-quantile (QQ) plots of -log10 transformed p-values.

Differentially Methylated CpGs.

Table S1 provides detailed information for each of the 16 differentially methylated CpGs.

Mediation analysis.

We applied a single CpG-site-based mediation approach using a counterfactual framework,[vii,viii] to investigate whether the effects of maternal climate stress on child growth could be mediated by DNA methylation. This approach decomposes the estimate of total effect of maternal climate stress on child growth into estimates of (i) natural direct effects of maternal climate stress on child growth (Y) through other biological mechanism, and (ii) natural indirect effects of maternal

climate stress (T) on child growth through DNA methylation (M). Figure 2 demonstrates a graphical representation of a mediation analysis.

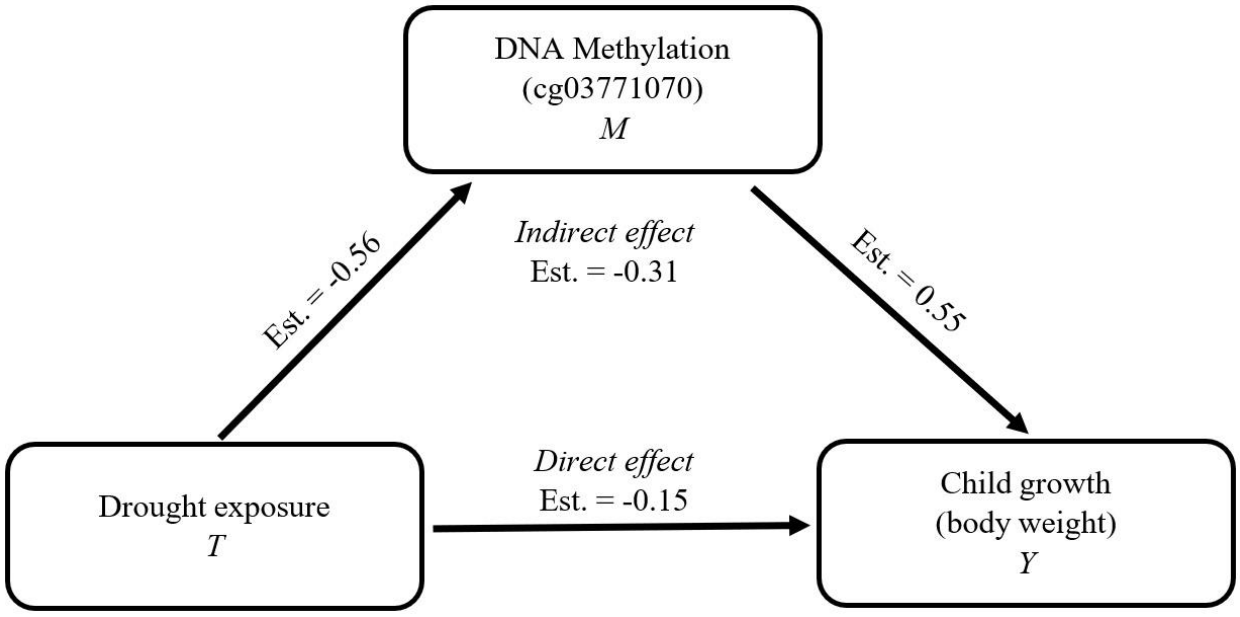

Figure 2. Illustrating mediation analysis framework for DNA methylation (cg03771070) as a mediator. The estimated indirect effect (ACME) is -0.31, which is a product of the estimated effect of drought on mediator (-0.56) and the estimated effect of mediator on child body weight-for-age z-score (0.55).

The single CpG-site-based mediation approach using a counterfactual framework can be implemented as the following system of linear equations:

$$M_i = \alpha_1 + \beta_1 T_i + \gamma_1 X_i + b_{0i} + \epsilon_{i1}, \qquad (1)$$

$$Y_i = \alpha_2 + \beta_2 T_i + \delta M_i + \gamma_2 X_i + b_{1i} + \epsilon_{i2}, \qquad (2)$$

where Y_i represents the child growth for unit i, T_i denotes the binary extreme climate exposures, M_i represents the DNA methylation as a mediator, and X_i denotes the vector of covariates. In addition, $\epsilon_{i1}, \epsilon_{i2}$ are error terms, and b_{0i}, b_{1i} are random intercepts in the models, and we assume that these parameters are normally distributed. Under sequential ignorability assumptions,[ix] the average natural direct effect is β_2, the average natural indirect effect is $\beta_1\delta$, and the average total effect is $\beta_2 + \beta_1\delta$.

We implemented a three-step algorithm for the single CpG-site-based mediation approach using a counterfactual framework. In the first step, we make sure that there is an association between the mediator and the exposure variable. The variables used in our models are summarized in Table S2. The results of step one provide candidate mediators (CpG site) which have significant association (FDR < 0.05) with exposure variable.

Outcome	DNA methylation M-values.
Exposure	In utero exposure to drought.
Predictors	Age, sex, and cellular heterogeneity (Epithelial (Epi), Fibroblast (Fib) and Immune cells (ICs) as reference).
Random Effect	Sibling identifier.

Table S2. List of variables are used in linear mixed-effect model in step one.

Since we have different types of child growth outcomes, we examined an association between the exposure and each outcome. The variables used in our models are summarized in Table S3. The results of step two provide outcomes which have significant total effect of the exposure.

Outcome	Weight-for-age z-score and triceps-skinfold-thickness-for-age-z-score.
Exposure	In utero exposure to drought.
Predictors	• Weight-for-age z-score: Age, sex, and cellular heterogeneity (Epi, Fib, and ICs as reference), forced work, denied food, lifetime maternal trauma. • Triceps-skinfold-thickness-for-age z-score: Age, sex, and cellular heterogeneity (Epi, Fib, and ICs as reference), forced work, denied food.
Random Effect	Sibling identifier.

Table S3. List of variables are used in linear mixed-effect model in step two.

Finally, in step three, we calculated the indirect effects of the exposure on the outcomes by fitting two linear fixed effects models (Equation 1 and 2) for each candidate CpG mediator at a time. The variables used in our models are summarized in Table S4.

Equation 1: $M_i = \alpha_1 + \beta_1 T_i + \gamma_1 X_i + b_{0i} + \epsilon_{i1}$	
Outcome	Candidate mediators from Step 1.
Exposure	In utero exposure to drought.
Predictors	• Weight-for-age z-score: Age, sex, and cellular heterogeneity (Epi, Fib, and ICs as reference), forced work, denied food, lifetime maternal trauma. • Triceps-skinfold-thickness-for-age-z-score: Age, sex, and cellular heterogeneity (Epi, Fib, and ICs as reference), forced work, denied food.
Random Effect	Sibling identifier.
Equation 2: $Y_i = \alpha_2 + \beta_2 T_i + \delta M_i + \gamma_2 X_i + b_{1i} + \epsilon_{i2}$	
Outcome	Weight-for-age z-score and triceps-skinfold-thickness-for-age-z-score.
Exposure	In utero exposure to drought.
Predictors	• Weight-for-age z-score: Age, sex, and cellular heterogeneity (Epi, Fib, and ICs as reference), forced work, denied food, lifetime maternal trauma. • Triceps-skinfold-thickness-for-age-z-score: Age, sex, and cellular heterogeneity (Epi, Fib, and ICs as reference), forced work, denied food.
Random Effect	Sibling identifier.

Table S4. List of variables are used in linear mixed-effect model in step three.

Characteristics of Drought

Ambient temperature. To descriptively characterize the ambient temperature exposure component of our dichotomous drought variable, we compiled historical daytime mean maximum temperature data specific to the timing and closest known location of each pregnancy

using MODIS LST Land Surface Temperature (https://modis.gsfc.nasa.gov/) available on the Famine Early Warning Systems Network (FEWS NET: https://earlywarning.usgs.gov/fews).

Maternal Stressors. In order to characterize drought-exposed compared to same-sex sibling control pregnancies, standardized instrumentation and ethnographic methods were used, as described in our non-DNAm study for this sample.[x] Through these methods, 24 stressors were identified and assessed, as reported in Table S5 below. A cumulative count variable of these stressors was higher in drought-exposed compared to control pregnancies.

Parameter	**During Drought Pregnancies**	**During Same-sex Sibling Control pregnancy**	***P*-value[a]**
Cumulative Score of Stressors Individually identified below, score range = 0 to 15	4.78 ± 2.69	1.16 ± 1.49	<0.01*
Stressors relevant to severe and less severe drought, and resource scarcity			
Food insecurity	62/104 (59.62%)	7/109 (6.42%)	<0.01*
Water insecurity	18/104 (17.31%)	2/109 (1.83%)	<0.01*
Livestock deaths	63/104 (60.58%)	5/109 (4.59%)	<0.01*
Voluntary/not forced hazardous herding or tree climbing during pregnancy	44/104 (42.31%)	7/109 (6.42%)	<0.01*
Witnessed non-fatal injury during hazardous herding work (e.g., climbing	1/104 (0.96%)	0	<0.01*
Witnessed fatality hazardous livestock work (e.g., climbing trees to cut leaves as	3/104 (2.88%)	0	<0.01*
Weak/unable to work at some point during pregnancy	16/104 (15.38%)	5/109 (4.59%)	<0.01*
Drought related pregnancy illness or complication	8/104 (7.69%)	3/109 (2.75%)	<0.01*
Interpersonal (spousal) violence and fear			
Interpersonal (spousal) violence[b]	30/103 (29.13%)	25/108 (23.15%)	<0.01*
Interpersonal (spousal) violence – severity (0/none to 3/most severe)[b]	0.92 ± 1.56	0.68 ± 1.3	0.34
Feared husband[c]	55/104 (53.4%)	44/109 (40.74%)	0.60
Forcibly returned to abusive spouse	0	1/109 (0.92%)	<0.01*
War & government-sponsored violence			
Indirect war exposure (hearing about)	8/104 (7.69%)	0	<0.01*
Direct war experience, no fatalities	8/104 (7.69%)	0	<0.01*
Direct war experience with fatalities	4/104 (3.85%)	1/109 (0.92%)	<0.01*

Experienced government destroying their property during government-sponsored	2/104 (1.92%)	0	<0.01*
Described illness from war-caused "shock"	1/104 (0.96%)	0	<0.01*
Non-drought illness, accidents, non-war deaths, witnessed sexual assault			
Pregnancy illness or complication	11/104 (10.58%)	16/109 (14.68%)	<0.01*
Experienced an accident	2/104 (1.92%)	1/109 (0.92%)	<0.01*
Witnessed accidental death	1/104 (0.96%)	0	<0.01*
Witnessed suicide completion	0	1/109 (0.92%)	<0.01*
Witnessed non-fatal accident	0	1/109 (0.92%)	<0.01*
Witnessed/experienced own child having injurious accident	1/104 (0.96%)	1/109 (0.92%)	<0.01*
Witnessed non-fatal assault	0	1/109 (0.92%)	<0.01*
Witnessed sexual assault	0	1/109 (0.92%)	<0.01*
[a]Paired t-test for continuous stressors; McNemar's test for dichotomous (yes/no) variables			

Table S5. Maternal stressors.

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[Table S1 is below, showing annotations for the 16 significant differentially methylated CpGs.]

Ilmn ID	Location	Nearest Gene	SourceSeq	Strand	UCSC_RefGene_Accession	UCSC_RefGene_Group	GencodeCompV12_Accession	GencodeCompV12_Group	Esti-mates	se	pvalue	pvalue_Inflation_Adjust	Pval-ue_FDR	mean Difference in % (+/higher or -/lower in Drought vs Control)
cg00776112	chr10 : 6290651	PFKFB3	CGGGCTGCTGCCTGTCTGTCTTGTGCATTGCACACCATCAGAGAGGTTAGT	F	NM_001145443; NM_001282630; NM_004566	Body	ENST00000379784.4;ENST00000379781.3;ENST00000444592.1	5'UTR	-0.41	0.06	2.6E-10	0.00000014	0.02	-1.3
cg01183122	chr21 : 34806218	KCNE1	TAGTATGGCTCTCCTTCGAACTCACCTAGACCCTTGCATCAAAGGACTCG	R	NM_000219	TSS1500	ENST00000489175.1;ENST00000399284.1;ENST00000337385.3;ENST00000399286.2	3'UTR;TSS1500;1stExon;5'UTR	-0.37	0.05	1.6E-11	1.3E-08	0.01	-2.89
cg01940810	chr12 : 66837922	IFNG	AAATAGCTAATGTCTACTTTCTGGAGAATAAATGCTTTGCAAGACCCTCG	R	NM_000619	Body	ENST00000536914.1	5'UTR	-0.42	0.07	3E-09	0.00000096	0.05	-0.15

cg02619315	chr11:9071007	SCUBE2;FLJ46111	CAGCTGCACTAGCCACAGCCCAAAGAGGCAGGCCCCCGTGGGCATGTTCG	F	NM_020974;NM_001170690;NR_027713	TSS1500	ENST00000450649.2;ENST00000052046 7.1;ENST00000309263.3;ENST00000457346.2;ENST00000534295.1	TSS1500;3'UTR	-0.25	0.04	2.2E-10	0.00000013	0.02	-0.09
cg03029755	chr17:73777039	AC087645.1	AGCTTTTCCAGTAAAACCACTGCAGAAATCCATACTTCCCGACAGGCACG	R			ENST00000374945.1	3'UTR	-0.24	0.03	2.5E-11	0.00000002	0.01	1.39
cg03771070	chr6:131594261	AKAP7	TTTTCACTGTTCCCTCTTCTAAAATGCTTGAAATAAATTCATCTTAATCG	F	NM_016377	Body	ENST00000535150.1	5'UTR	-0.56	0.09	3.3E-10	0.00000017	0.02	0.96
cg06397457	chr11:124173085	C11orf61	AAACTGTTTCAGCTTGATTGATTTCTGTCTGTAGAAACTTCCCAATCACG	F	NM_024631	Body			-0.45	0.07	1.8E-09	0.00000064	0.04	-0.21
cg10897631	chr3:197257629		CGGGGGGAAAACTCTCCCAAACCTGAGATGTAAACAAAGGTAG	R					-0.39	0.06	3.8E-10	0.00000017	0.02	-0.22

			GCAAACA											
cg17064161	chr2 : 120753261	RALB	CGGGAGAACTGGATCTGATGTCTATCACTTGCGAAAGGATTAAGTTGTAT	R	NM_002881	Body	ENST00000470417.1	5'UTR	-0.43	0.06	9.5E-11	5.9E-08	0.01	1.88
cg20113068	chr5 : 59982132	DEPDC1B	CGGAAGACATTTGGCAATGTCTGGAGAAATGTTTTGTTGTCACAACAGGT	R	NM_001145208;NM_018369	Body	ENST00000512452.1;ENST00000051207 8.1	3'UTR	-0.47	0.07	1.6E-09	0.00000055	0.04	-0.91
cg22624429	chr18 : 53865369	NEDD4L	CGGCAGGGTTGTTCTTCCAAGGCGTATTAAATATGGGTACACTTAGTTTG	F	NM_001144964;NM_015277;NM_001243960;NM_001144967	TSS200;Body	ENST00000456986.1;ENST00000025683 2.7	TSS200;5'UTR	-0.54	0.09	2.6E-09	0.00000085	0.05	0.44
cg23121096	chr15 : 89237142	FES	CGGTGGTCAAGCACTTCACACTGTGCCATGCAGTGTAACCTCTGTGTATG	R	NM_001143784;NM_001143783;NM_001143785;NM_002005	Body	ENST00000464684.1	3'UTR	-0.53	0.08	1E-09	0.0000004	0.03	-0.09

cg23311137	chr3 : 132131332	ATP2C1	AATTACTTATGAAGCTGCATCAAGTACGTACCTTGATTACTACTATCTCG	R	NM_001199184; NM_001199183; NM_001199181; NM_001199182; NM_001199180; NM_001199179; NM_001001485; NM_014382;NM_001199185;NM_001001486;NM_001001487	5'UTR;Body	ENST00000347421.4;ENST00000508297.1;ENST00000505330.1;ENST00000507488.2;ENST00000504948.1;ENST00000504737.1	TSS1500;TSS200;5'UTR;3'UTR	-0.57	0.08	1.9E-11	1.8E-08	0.01	0.62
cg25051885	chr15 : 34749105	C15orf41	CGTCTGGAGTGCAATCAATAATAGTAAGGTACCAGTGAGGGTTTCTTAGT	R	NM_001290233; NM_001130010; NM_032499;NM_001290232	Body	ENST00000570265.1;ENST00000565366.1	5'UTR;TSS1500	-0.39	0.06	9.5E-10	0.00000039	0.03	-0.4
cg25526789	chr1 : 225153677	ADCK3	TTGTAACCAGGTGTCCCAGCACTGTTTACTGAACAGTCTCTCCTTTCTCG	R			ENST00000366779.1;ENST00000485677.1	1stExon;TSS200;5'UTR	-0.5	0.08	4.7E-10	0.00000023	0.02	0.34

cg25780496	chr15 : 98954776	LINS	CGCAGACAGGCATGATGAAGGCACAAATATTCTATGATGAAAGGAGAGGG	R	NM_001040616	5'UTR	ENST00000560934.1;ENST00000055914 9.1;ENST00000561073.1;ENST00000560941.1;ENST0000 0559577.1;ENST00000561308.1;ENST00000056013 3.1;ENST00000559736.1;ENST00000314742.8;ENST0000 0559827.1	TSS200;3'UTR;5'UTR	-0.59	0.09	2.7E-09	0.00000082	0.05	0.23