FUNCTION-ON-FUNCTION REGRESSION FOR THE IDENTIFICATION OF EPIGENETIC REGIONS EXHIBITING WINDOWS OF SUSCEPTIBILITY TO ENVIRONMENTAL EXPOSURES

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The ability to identify time periods when individuals are most susceptible to exposures, as well as the biological mechanisms through which these exposures act, is of great public health interest. Growing evidence supports an association between prenatal exposure to air pollution and epigenetic marks, such as DNA methylation, but the timing and gene-specific effects of these epigenetic changes are not well understood. Here, we present the first study that aims to identify prenatal windows of susceptibility to air pollution exposures in cord blood DNA methylation. In particular, we propose a function-on-function regression model that leverages data from nearby DNA methylation probes to identify epigenetic regions that exhibit windows of susceptibility to ambient particulate matter less than 2.5 microns (PM$_{2.5}$). By incorporating the covariance structure among both the multivariate DNA methylation outcome and the time-varying exposure under study, this framework yields greater power to detect windows of susceptibility and greater control of false discoveries than methods that model probes independently. We compare our method to a distributed lag model approach that models DNA methylation in a probe-by-probe manner, both in simulation and by application to motivating data from the Project Viva birth cohort. We identify a window of susceptibility to PM$_{2.5}$ exposure in the middle of the third trimester of pregnancy in an epigenetic region selected based on prior studies of air pollution effects on epigenome-wide methylation.

1. Introduction. Recent epidemiological evidence supports the hypothesis that exposures during fetal development and in early life can lead to a...
variety of adverse birth and child health outcomes. The fetal in utero environment can be altered by external factors such as the mother’s diet or toxins to which she is exposed, thereby influencing the early development of a child at a time of heightened susceptibility. The National Institutes of Health, through its 2016 Environmental influences on Child Health Outcomes (ECHO) initiative, highlighted the importance of not only identifying child health outcomes associated with environmental exposures, but also of identifying sensitive developmental windows during which an exposure has increased association with a child’s health outcomes. Understanding when these windows of susceptibility occur and how they coincide with environmental exposures may shed light on the underlying biological pathways through which exposures act. Ultimately, this can lead to the development of interventions that mitigate risks due to an exposure.

One proposed biological pathway by which prenatal exposures could contribute to subsequent adverse health outcomes involves DNA methylation at cytosine-phosphate-guanine (CpG) sites. DNA methylation is an epigenetic modification that can regulate gene expression. Previous studies have found associations between DNA methylation levels and environmental exposures such as particulate air pollution (Gruzieva et al., 2019; Soberanes et al., 2012; Baccarelli et al., 2009) and lead (Bollati et al., 2010; Schneider, Kidd and Anderson, 2013). Lee et al. (2018) found evidence linking in utero PM$_{2.5}$ exposure to both hypermethylation of the $GSTP1$ gene in nasal epithelia and impaired early childhood lung function. In a cohort of elderly men in the Normative Aging Study, Lepeule et al. (2012) found an association between lower DNA methylation levels in several gene promoter regions and reduced lung function. While a large number of epigenetic studies based on adult populations have been published, due to the inherent difficulties and health risks of interrogating the epigenome of a developing child, relatively few prenatal epigenetic studies have been conducted. Thus, our understanding of which prenatal exposures affect the epigenome, which epigenetic regions are affected, and which time periods are most sensitive remains limited.

Previous work to identify windows of susceptibility during pregnancy often involved regressing the outcome of interest on trimester-average exposures (TAEs). Either separate models for each of the three TAEs were fit, or a single model that jointly estimated the associations for each TAE was constructed (Shah and Balkhair, 2011; Dadvand et al., 2013). However, Wilson et al. (2017) demonstrated that trimester-specific effect estimates obtained using separate regression models can be biased and identify incorrect windows of susceptibility. Furthermore, Wilson et al. (2017) found that while a joint model suffers less from these issues, a distributed lag model
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A DLM models the association between an outcome and a finely sampled time-varying exposure by assuming that their relationship varies smoothly over time (Schwartz, 2000; Zanobetti et al., 2000). In the context of prenatal exposure, the DLM regresses a health outcome measured after the exposure period of interest against exposure measured at frequent, regular intervals throughout pregnancy. DLMs have been used to identify windows of susceptibility during which air pollution is associated with disrupted neurodevelopment (Chiu et al., 2016), childhood asthma (Lavigne et al., 2019; Bose et al., 2017; Hsu et al., 2015), reduced lung function (Bose et al., 2018), sleep disruption (Bose et al., 2019), and low birth weight (Wu et al., 2018; Darrow et al., 2011). Warren et al. (2019) recently proposed critical window variable selection as an alternative to the DLM in the context of identifying windows during pregnancy when PM$_{2.5}$ is associated with an increased risk of preterm birth.

While DLMs and methods like critical window variable selection efficiently model the multivariate nature of exposure, further efficiency gains can be made by taking into account the dependence among multivariate outcomes. In mammalian genomes, DNA methyltransferase enzymes can co-methylate adjacent CpG sites, resulting in blocks of CpG sites with similar methylation statuses and genomic functionality (Guo et al., 2017). Lee et al. (2017) proposed a Bayesian variable selection method for multivariate methylation outcomes that, through leveraging information on the covariance structure of outcomes, increases power to detect associations while maintaining a low false discovery rate. Additionally, Lee and Morris (2016) used the wavelet-based functional mixed model approach introduced by Morris and Carroll (2006) to model DNA methylation outcomes jointly, thereby capturing correlations among neighboring probes as well as across samples. This yielded gains in efficiency and the ability to detect differentially methylated regions (DMRs).

Lee and Morris (2016) focused on detecting DMRs associated with a scalar exposure, such as cancer status. Here, we consider the functional exposure setting where our goal is not only to find DMRs, but to simultaneously identify time periods during which an exposure is associated with these DMRs. In particular, we are interested in the association between two functions: (1) cord-blood DNA methylation levels measured at birth as a function of CpG site position in the genome, and (2) maternal air pollution exposure as a function of time during pregnancy. Importantly, we view the exposure and DNA methylation measurements as two sources of functional data; that is, data where the ideal unit of observation is a function defined on a continuous
domain and the observed data is sampled on a discrete grid (Morris, 2015).

A large literature exists on functional data analysis (FDA). Ramsay and Silverman (2005) provide a foundational FDA text that includes strategies for regression analyses involving functional predictors (scalar-on-function regression), functional responses (function-on-scalar regression), and the combination of the two (function-on-function regression). Morris (2015) provides a comprehensive review of the work done in each of these subclasses of FDA. Within the function-on-function regression literature, previous work has considered both constrained regression coefficient surfaces, such as the historical functional linear model (see for example Malfait and Ramsay (2003)), and unconstrained surfaces, the latter being our target of interest. Several modelling approaches have been considered for unconstrained coefficient surfaces, including functional principal component analysis (Yao, Müller and Wang, 2005), kernel regression (Ferraty et al., 2011; Ferraty, Keilegom and Vieu, 2012), linear mixed modeling (Wang, 2014), and penalized splines (Ivanescu, 2018). Scheipl, Staicu and Greven (2015) introduced the functional additive mixed model (FAMM) framework initially designed for splines, which was then extended to handle sparse, irregularly sampled outcomes by Cederbaum et al. (2016) and further generalized by Scheipl et al. (2016). Greven and Scheipl (2017) give a review of this series of developments along with a discussion of model identifiability and software implementation options.

A Bayesian alternative to the FAMM framework is the wavelet-based functional mixed model (WFMM) proposed by Morris and Carroll (2006) and since used by many others (see for example Lee and Morris (2016); Lee et al. (2019); Zhu et al. (2018)). The Bayesian approach yields many options for inference, including posterior probabilities, pointwise and joint bands, and local and global tests of significance.

Morris (2017) contains a helpful comparison of the similarities and differences between these two general functional regression modelling frameworks. Greven and Scheipl’s FAMM framework is best-suited for smooth functional data observed on a sparse sampling grid that potentially varies across subjects, as is often the case with longitudinal data. In contrast, WFMM was designed to be used with functional data on a fine and potentially high-dimensional, common grid. Wavelets also tend to perform better than splines when modelling complex surfaces containing spikes, change points, and flat regions.

We hypothesize that the association surface for our application of interest is likely to be complex, with flat, sparse areas where genomic regions are inaccessible to the biological machinery necessary to modify DNA methylation signatures and spikes when structural changes (perhaps driven in part by
environmental shocks) allow for methylation markers to be modified. Thus, to characterize the association surface between the DNA methylation and air pollution functions, we use Bayesian function-on-function regression (FFR), a method proposed by Meyer et al. (2015) that builds upon the WFMM framework. FFR transforms both the DNA methylation and air pollution profiles to the wavelet basis space, fits a regression model in that space, and then performs an inverse transformation to present results on the original methylation scale. The basis transformation affords the ability to capture spatial and time-varying correlations within the two functions, while the Bayesian approach simultaneously allows for the smoothing of the association surface via the prior specification as well as strict control for multiple testing.

We fit the model using a Monte-Carlo Markov Chain (MCMC) procedure and then perform statistical inference while accounting for multiple testing using a Bayesian False Discovery Rate (BFDR) procedure for functional regression and a simultaneous band score (SimBaS) (Meyer et al., 2015). In a simulation study, we demonstrate the efficiency gains and better control of false discoveries attained by the functional approach relative to DLMs applied on a site-by-site basis. We then take two biologically-interesting and significant sites reported in Gruzieva et al. (2019), the largest analysis to date of particulate matter exposure and DNA methylation in infants, and perform the first analysis of prenatal windows of susceptibility driving these associations. We perform the analysis using DNA methylation data from 412 mother-child pairs enrolled in the Project Viva birth cohort and daily PM$_{2.5}$ measurements recorded over the third trimester of pregnancy, a critical period in fetal somatic growth, as well as in neural, lung, endocrine, and immune system development (Hill, 2019). We identify one window of susceptibility halfway through the third trimester for CpG probes in the FAM13A gene region.

While we present the functional model in the context of a specific outcome measure (DNA methylation) and exposure (air pollution), the method is flexible enough to analyze other types of outcome and predictor functions that vary spatially and/or temporally.

2. Methylation and Exposure Data in Project Viva. In this section we briefly describe the pre-birth cohort, DNA methylation data, and air pollution data sets to which we apply our method.

2.1. Description of Project Viva. Project Viva is a longitudinal study designed to examine the effect of maternal diet and other lifestyle factors during pregnancy on the mother’s and child’s health. Pregnant women were
enrolled at their initial obstetric visit at Harvard Vanguard Medical Associates in Massachusetts from 1999-2002. Of the 2,128 mother-child pairs enrolled in the cohort, 485 had cord blood DNA methylation measurements that passed quality control. For a more detailed description of the Project Viva cohort see Oken et al. (2015).

2.2. DNA methylation data. Umbilical vein cord blood DNA was extracted using the Qiagen Puregene Kit (Valencia, CA) and bisulfite converted using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA). Samples were randomly allocated to chips and plates and analyzed using Infinium HumanMethylation450 BeadChip arrays (Illumina, San Diego, CA) that probe approximately 485,000 CpG sites at a single nucleotide resolution.

We adjusted for sample plate as technical batch by including the batch number as a categorical covariate in the FFR and DLM regression models. We modelled the logit-transformed percentage DNA methylation value, or M-values, where the percentage methylation value for an individual CpG site is the percentage of methylated cytosines over the sum of methylated and un-methylated cytosines at the 5C position for that probe (Du et al., 2010).

We focused our analysis on two regions encompassing CpG sites identified in closely related work by Gruzieva et al. (2019). From their meta-analysis of the associations between prenatal exposure to particulate matter and DNA methylation in nine birth cohorts, one of which was Project Viva, Gruzieva et al. (2019) identified 20 CpGs that were significantly associated with either prenatal PM$_{2.5}$ or PM$_{10}$ exposure. Two of these CpGs mapped to FAM13A and NOTCH4, genes previously associated with COPD and asthma, respectively (Hobbs et al., 2017; Hancock et al., 2009; Li et al., 2013). We selected CpG probes annotated to these two genes for our analysis.

2.3. PM$_{2.5}$ data. Particulate matter with diameter less than 2.5 µm (PM$_{2.5}$) is released by vehicles and other industrial processes via the combustion of solid and liquid fuels. Estimated daily ambient PM$_{2.5}$ levels at the home addresses of the mothers enrolled in Project Viva were obtained using a hybrid satellite-based model that integrated remote sensing data and spatio-temporal land-use and meteorology data (Kloog et al., 2011, 2014). Previous work in Project Viva has demonstrated associations of estimated residential third trimester PM$_{2.5}$ or its black carbon component with subsequent reduced fetal growth measures at birth (Fleisch et al., 2015); childhood executive function and behavior (Harris et al., 2016); and allergen sensitization (Sordillo et al., 2019). Since recruiting for Project Viva began in 1999,
but the satellite technology necessary to make daily predictions only became available in 2000, we do not have complete daily 1-by-1 km-resolved PM$_{2.5}$ exposure estimates throughout pregnancy for all mother-child pairs. Because of the known importance of the third trimester in fetal development, and in the interest of retaining a large number of subjects, we limited analysis to the 412 mothers for whom we had daily PM$_{2.5}$ measurements at their residential addresses for the last 90 days prior to delivery. While analyzing the entirety of gestation would be preferable, exploring the last trimester of pregnancy does not preclude us from finding biologically meaningful windows of susceptibility; previous studies have found associations between prenatal air pollution in the third trimester and newborn health outcomes such as systolic blood pressure (van Rossem et al., 2015) and fetal growth (Lamichhane et al., 2018).

3. Methods.

3.1. Function-on-Function regression model. Here, we describe the FFR model introduced by Meyer et al. (2015) in the context of identifying regions of the genome that exhibit windows of susceptibility to an exposure of interest. Suppose for each of $i = 1, \ldots, n$ individuals we observe two functions: (1) the DNA methylation profile $y_i(s)$ on a common grid of CpG sites $s = 1, \ldots, S$, and (2) the air pollution exposure profile over time, $x_i(t)$, $t = 1, \ldots, T$. For our application, $x_i(t)$ represents daily ambient PM$_{2.5}$ levels at each mother’s residence.

A FFR model to regress $y_i(s)$ on the functional predictor $x_i(t)$ is given by

\begin{equation}
 y_i(s) = \alpha(s) + \int_{t \in T} x_i(t) \beta(t, s) dt + e_i(s),
\end{equation}

where we assume observation-specific Gaussian process errors $e_i(s) \sim \mathcal{GP}(0, \Sigma_e)$. The target of interest in Model (3.1) is the two-dimensional surface $\beta(t, s)$ that characterizes the association between exposure at any given time and DNA methylation at any given CpG site.

If we stack row vectors by subject, then $Y$ and $X$ represent $n \times S$ and $n \times T$ matrices of observed DNA methylation and exposure profiles respectively. We can then represent Model (3.1) in matrix form as

\begin{equation}
 Y = X\beta + E,
\end{equation}

where $\beta$ is a $T \times S$ matrix of functional effects and $E$ is a $n \times S$ matrix of model errors. The intercept $\alpha(s)$ can be incorporated into $\beta$, but in practice
and without loss of generality, we center and scale $y_i(s)$ and $x_i(t)$ (each time point $t$ has mean 0 and variance 1 and each CpG site $s$ has mean 0 and variance 1) such that $\alpha(s)$ is zero in Model (3.1).

One possible approach to fitting Model (3.2) is to fit each column of $Y$ independently using a DLM. This approach regresses DNA methylation at a particular CpG site against lagged exposure values over time for each site $s$ separately:

\begin{equation}
    y_{i,s} = \alpha + \int_{t \in T} x_i(t) \beta(t) dt + e_{i,s}.
\end{equation}

The DLM falls into a large class of scalar-on-function regression methods involving a scalar outcome and functional predictor. A large body of literature exists on scalar-on-function regression (see for example Ramsay and Dalzell (1991); Malloy et al. (2010); Goldsmith et al. (2011) or the comprehensive review provided by Reiss et al. (2017)). However, the site-by-site DLM approach fails to borrow information across nearby, correlated CpG sites, likely reducing the efficiency of the method relative to a joint approach. Instead, we fit Model (3.2) jointly by using a basis function transform approach (Lee and Morris, 2016; Meyer et al., 2015; Morris and Carroll, 2006). This involves first transforming $y(s)$ and $x(t)$ from the data space into a basis space. We fit the model in the basis space and then transform the parameter estimates back to the data space to conduct inference.

3.2. Discrete Wavelet Transform in Functional Regression. While a number of basis functions could be used to represent the observed functions, including splines, principal components (PCs), or Fourier series, we use wavelets as the transformation for both the DNA methylation and air pollution datasets. Wavelets have previously been used in genomic settings for the purposes of denoising high-throughput DNA copy number data (Hsu et al., 2005), performing transcriptome analysis with tiling arrays (Clement et al., 2012), detecting histone modification enrichments (Mitra and Song, 2012), and identifying nucleosome position (Nguyen, Vo and Won, 2014; Zhang et al., 2008). For equally spaced data, such as daily air pollution measurements, the discrete wavelet transform (DWT) maps the data to the wavelet space in linear time. For unequally spaced data, like CpG site positions across chromosomes, we have a choice for how to perform the wavelet basis transform. We choose to perform the transformation treating the positions as if they were equally spaced for two reasons. First, Morris and Carroll (2006) showed that the wavelet-based functional mixed model can flexibly estimate a complex covariance structure such as one that might arise from
unequally spaced measurements. Second, Sardy et al. (1999) compared four different approaches for handling unequally spaced data and found that the method that treats data as if it were evenly spaced performed as well as more computationally-expensive methods that account for unequal spacing. Therefore, we proceed by treating CpG sites as if they were equally spaced as others have previously done for CpG site (Fernández et al., 2020; Lee and Morris, 2016) and DNA copy number data (Hsu et al., 2015).

First, we apply the discrete wavelet transform (DWT) to each row of \( Y \), giving an \( n \times S^\ast \) matrix of wavelet basis coefficients, \( Y^\ast \), which represents the methylation data in the wavelet space. Each wavelet coefficient is double-indexed by \((j,k)\) with frequencies indexed by \( j \) and locations indexed by \( k \). For the exposure data we similarly perform the DWT on each row of \( X \), giving an \( n \times T^\ast \) matrix \( X^\ast \). Applying the DWT to \( Y \) is equivalent to post-multiplication by the \( S \times S^\ast \) wavelet transform matrix \( \Omega' \), \( Y^\ast = Y \Omega' \), where \( \Omega' \) contains the wavelet basis functions evaluated on the grid \( S \). Similarly, if the \( T \times T^\ast \) matrix \( \Phi' \) contains the wavelet basis functions evaluated on the grid \( T \), then applying the DWT to \( X \) is equivalent to \( X^\ast = X \Phi' \).

After transforming both the response and exposure data, we arrive at our model in the wavelet space:

\[
Y^\ast = X^\ast \beta^\ast + E^\ast,
\]

where \( E^\ast \sim MN(0, I, C^\ast) \) and \( I \) is the appropriately sized identity matrix. The whitening property of the wavelet transform, discussed in Johnstone and Silverman (1997), allows us to assume that wavelet coefficients within a given curve are independent across \( j \) and \( k \). Thus, we assume a diagonal structure for \( C^\ast \), the between-column covariance of the methylation data in the wavelet space (Morris and Carroll, 2006). Importantly, this assumed independence in the wavelet space does not imply independence in the data space; in fact, heterogeneous variances across the wavelet scales and locations \((j,k)\) induce correlations in the data space (Morris and Carroll, 2006).

The independence assumption in the wavelet space allows us to view Model (3.4) as \( S^\ast \) separate models, one for each column of \( Y^\ast \). Thus, the model for each column (equivalently, \( Y \)-space wavelet coefficient) is

\[
y^\ast_{(j,k)} = X^\ast \beta^\ast_{(j,k)} + e^\ast_{(j,k)},
\]

where \( y^\ast_{(j,k)} \) and \( e^\ast_{(j,k)} \) are \( n \times 1 \), \( X^\ast \) is \( n \times T^\ast \), and \( \beta^\ast_{(j,k)} \) is \( T^\ast \times 1 \). The separability of the model in the wavelet space enables the method to scale to large \( S \), especially if parallel computing resources are available. Calculations are linear in \( S^\ast \), but quadratic in \( T^\ast \). This is well-suited for genomic applications, such as ours, where \( S \) can be very large. For applications with
large $T$, researchers may wish to perform PC compression on the wavelet-
space exposure data before fitting the model, as previous implementations
have done (Meyer et al., 2015). We forego the computational advantage of
compressing the exposure data since $T$ is relatively small for our setting.

We fit Model (3.5) in a similar fashion to Meyer et al. (2015). We place a
spike-and-slab prior on each $\beta^*_{(p,j,k)}$, where $p$ indexes the wavelet coefficients
of the transformed exposure data, $p = 1, \ldots, T^*$:

$$(3.6) \quad \beta^*_{(p,j,k)} \sim \gamma_{(p,j,k)} N(0, \tau_{p,j}) + (1 - \gamma_{(p,j,k)}) d_0, \quad \gamma_{(p,j,k)} \sim \text{Bern}(\pi_{p,j}).$$

This prior is a mixture of a normal distribution and a point-mass at zero,
$d_0$, with regularization parameters $\tau_{p,j}$ and $\pi_{p,j}$ estimated using an Empir-
ical Bayes-type approach. This prior specification is consistent with other
wavelet-based functional models (Morris and Carroll, 2006; Malloy et al.,
2010).

We generate posterior samples for the coefficient surface $\beta^*$ in the wavelet
space and then project back to the data space through two inverse discrete
wavelet transforms, $\beta = \Phi^T \beta^* \Omega$, which ultimately yields posterior samples
of the discretized coefficient surface $\beta$. We then perform inference on $\beta$ as
described in Section 2.4. We perform computations in MATLAB (version
2017a). Code for running the above model along with simulated data can be
found at https://github.com/MorrisStatLab/BayesFMM.

3.3. Incorporation of Scalar Covariates. Model (3.1) can be extended
to include scalar covariates, scalar-by-function interactions, and subject-
specific random effect functions (Meyer et al., 2015). In the Viva analysis,
we adjust for scalar covariates $\{w_a, a = 1, \ldots, q\}$ using

$$(3.7) \quad y_i(s) = \alpha(s) + \sum_{a=1}^{q} w_a \gamma_a(s) + \int_{t \in T} x_i(t) \beta(t, s) dt + \epsilon_i(s),$$

where $\gamma_a(s)$ are functional coefficients for scalar predictors. These coeffi-
cients are typically of less interest than the coefficient surface $\beta(t, s)$. Be-
cause they are not functional data, we do not transform scalar covariates
into the wavelet space prior to fitting the model.

3.4. Posterior Functional Inference. In order to account for the multiple
comparisons that occur when testing coefficients corresponding to all sites
and exposure times within an analysis, we use two posterior functional infer-
ence procedures. The first is the Bayesian false discovery rate (BFDR), which
was originally proposed by Müller et al. (2006) and subsequently extended
to the functional regression setting (Morris et al., 2008; Malloy et al., 2010; Meyer et al., 2015). For details on the BFDR procedure, see the Appendix.

The second approach uses joint credible bands to detect significantly differentially methylated loci while controlling the experiment-wise error rate (Meyer et al., 2015). Suppose one constructs joint credible bands for a range of different values of \( \alpha \) and finds for each location \((t,s)\) the minimum level \( \alpha \) for which the \((1-\alpha)\% \) joint credible band excludes zero. Meyer et al. (2015) refers to this minimum \( \alpha \) level, \( p_{\text{SimBaS}}(t,s) = \min\{\alpha : 0 \notin I_\alpha(t,s)\} \), as the simultaneous band score (SimBaS) for each location \((t,s)\). For any specific \( \alpha \), we flag all \((t,s)\) for which \( p_{\text{SimBaS}}(t,s) < \alpha \) as significant, meaning that the \((1-\alpha)\% \) joint credible interval at those locations does not include zero. For details regarding the construction of joint credible bands, see the Appendix.

4. Simulation. We performed two simulation studies to compare the operating characteristics of the FFR and site-by-site DLM approaches in the context of pre-birth studies of air pollution and DNA methylation. For the first study, we set the true surface of association, \( \beta \), over a \( T = 90 \) by \( S = 100 \) grid to be \( \beta = 0.2 \) in the region \( T \times S = \{(T, S) : T \in \{40, \ldots, 44\}, S \in \{1, \ldots, 100\}\} \) and \( \beta = 0 \) everywhere else. This corresponds to a “vertical band” of association (Figure 1) representing a biologically plausible association between air pollution exposure and DNA methylation in which changes in air pollution halfway through the study period are associated with changes in DNA methylation in a genomic region spanning 100 CpG sites. Next, we randomly sampled PM\(_{2.5}\) pollutant profiles from individuals in the Project Viva cohort and used these randomly sampled profiles as the exposure curves \( x_i \). Motivated by the 412 mother-child pairs for whom we had data in the Project Viva cohort, we used a sample size of \( N = 400 \) subjects for the simulations. Using measured exposure data, instead of simulating exposure curves, allowed us to capture a realistic correlation structure among daily pollutant exposures during pregnancy. (See the Appendix for a sample of the observed PM\(_{2.5}\) exposure curves used in the simulation study and data application.) We then generated response curves \( y_i \) using \( y_i = x_i \beta + e_i \), where \( y_i \) is a row vector of length 100, \( x_i \) is a row vector of length 90, \( \beta \) is the \( 90 \times 100 \) matrix described above, and \( e_i \) is a row vector of length 100. For the model errors \( e_i \), we generated data using Gaussian Processes with auto-regressive 1 covariance structures which were then scaled by factors of \( \sigma^2_e \in \{4, 16, 64\} \) to create three scenarios of varying noise levels. We defined the signal-to-noise ratio, STNR, as the pointwise effect in the non-zero region of the surface (\( \beta = 0.2 \)) expressed as a proportion of \( \sigma_e \). Using this
definition, the three STNRs we considered were STNR ∈ \{0.10, 0.05, 0.025\}. For each scenario, we generated 100 simulated data sets and fit the FFR to obtain 100 \( \hat{\beta}_{FFR} \) estimates of the association surface. To fit the models, we transformed the data to the wavelet space using Daubechies wavelets with six levels of decomposition, four vanishing moments, and zero-padding. We drew 2000 posterior samples and discarded the first 1000 samples.

Fitting the site-by-site DLM to the simulated data sets required additional steps. Recall that the FFR takes a functional response and functional exposure as inputs and estimates a two-dimensional surface of association, \( \beta(t, s) \), whereas the DLM takes a scalar response and functional exposure as inputs and estimates a curve of association \( \beta(t) \) for each site. To compare the FFR and DLM approaches, we fit separate DLMs for each of the \( S = 1, \ldots, 100 \) probes and concatenated the results, in essence stacking the DLM-estimated curves one behind the other to create a surface, \( \hat{\beta}_{DLM} \), analogous to the FFR-estimated surface, \( \hat{\beta}_{FFR} \). We used the \texttt{regimes} R package to fit the DLMs (Wilson et al., 2017). Note that the key difference between \( \hat{\beta}_{FFR} \) and \( \hat{\beta}_{DLM} \) is that we fit \( \hat{\beta}_{FFR} \) using information from all sites simultaneously, whereas we constructed \( \hat{\beta}_{DLM} \) using a separate model fit for each site. An additional difference between the two methods is that \texttt{regimes} uses the principal components of the covariance matrix of \( x(t) \) as the basis to represent \( x(t) \), whereas our FFR implementation uses wavelets to represent \( x(t) \) (Wilson et al., 2017).

Figure 2 displays heat maps of the estimated association surfaces for both methods across the three STNR scenarios. Results for the estimated surfaces
Figure 2 shows that both methods estimated the true surface relatively well even in situations where the magnitude of the noise was 10-40 times larger than that of $\beta$ in the signal region. However, visually we see that FFR produced sharper estimates of the association surface. In particular, the edges of the vertical band are clearly delineated and the null region is more accurately estimated in the FFR heat maps than in the DLM heat maps.

Figure 3 shows the root mean square error (RMSE) for all scenarios. The site-by-site DLM estimates had higher RMSE throughout the surface at each STNR level. Relative to the site-by-site DLM analysis, the FFR method reduced the sum of the RMSE over the entire surface by 68%, 63%, and 65% for the STNR = 0.10, 0.05, and 0.025 scenarios, respectively. The null regions saw the largest gains in efficiency from the joint approach, while the top and bottom edges of the region of interest had the smallest gains. These differences in efficiency gains are due to the fact that spatial smoothing is less effective for sites at the boundary of the signal region. Sites in the interior of the region borrow information from a greater number of sites and therefore gain more from a joint-modeling approach.

We also compared the performance of the BFDR and SimBaS inferential procedures for the two methods, using $\alpha = 0.05$ to select significant locations for both procedures. For the BFDR, we used $\delta$-intensity changes of
Fig 3. Heat maps displaying the RMSE averaged over 100 simulations for the FFR method (top panel) and DLM method (bottom panel).

0.15, 0.10, and 0.05 corresponding to 75%, 50% and 25% of the true signal in the vertical band. We performed BFDR and SimBaS procedures on each estimated surface and then averaged over simulations. The heat maps in Figure 4 are shaded according to the proportion of simulations in which each location was flagged as significant by the BFDR procedure. Figure 4 shows heat maps for the STNR = 0.10 scenario across the three $\delta$ levels. Locations that were flagged as significant by all simulations are white, those that were never flagged are black, and locations that were occasionally flagged vary from red to yellow shading. The accompanying Table 1 displays the sensitivity and false discovery rate, FDR, for both methods at varying $\delta$ levels. Across $\delta$ levels, FFR performed well, flagging regions with a true signal as significant in all estimated surfaces, while maintaining a FDR below 5%. This does not hold true for the DLM method. At $\delta = 0.15$, BFDR only flagged 66% of the vertical band as significant, while at $\delta = 0.05$, the method flagged too many locations neighboring the true band as significant, pushing the FDR to 30%.

Figure 5 shows heat maps obtained by averaging the SimBaS for each location over all simulations and then flagging locations with scores $\leq 0.05$ in white. Similar to the BFDR results, at STNR = 0.10, FFR outperformed DLM by maintaining high sensitivity and low FDR, while the DLM had a high FDR of 29%. However, as the STNR decreases, SimBaS for FFR was more conservative than SimBaS for the DLM; when STNR = 0.025, the FFR
Fig 4. Heat maps of BFDR results at the STNR = 0.10 level for the FFR (top panel) and DLM (bottom panel) methods averaged over 100 simulations. Left: $\delta = 0.15$ (75% of true signal). Center: $\delta = 0.10$ (50% of true signal). Right: $\delta = 0.05$ (25% of true signal).

Table 1

| Measure | Method | $\delta = 0.15$ | $\delta = 0.10$ | $\delta = 0.05$ |
|---------|--------|----------------|----------------|----------------|
| Sensitivity | FFR    | 100.0%         | 100.0%         | 100.0%         |
|          | DLM    | 65.9%          | 100.0%         | 100.0%         |
| FDR     | FFR    | 0.0%           | 0.0%           | 4.7%           |
|         | DLM    | 0.0%           | 4.8%           | 29.9%          |
sensitivity dropped to just 13% while the DLM maintained 60% sensitivity (Table 2).

We also considered a second set of simulations for which the true association surface was a narrow, horizontal band with $\beta = 0.2$ at probe $s = 50$ for $T \in \{1, \ldots, 45\}$ and $\beta = 0$ everywhere else (Figure 1; right panel). In contrast to the vertical band setting, this association surface represented a sustained window of susceptibility at a single CpG site rather than across a genomic region. Figure 6 shows the estimated surfaces, BFDR, and SimBaS heat maps for the FFR and DLM approaches at the STNR = 0.10 level. In this setting with the signal confined to a single site, the shrinkage employed by the FFR hinders its ability to identify the probe affected by exposure. The magnitude of $\hat{\beta}_{FFR}$ in the signal region is about half the true value and the window fails to appear on either the BFDR or SimBaS plots. The DLM, however, successfully detects the true window of susceptibility for the site whose methylation is affected by exposure.
These simulations highlight the relative strengths and weaknesses of the FFR and DLM methods. For the global exposure effect setting in which an effect of exposure is shared across probes within a region of interest, FFR consistently outperforms DLM in terms of RMSE across the surface, as well as sensitivity and FDR for both the BFDR and SimBaS inferential procedures. On the other hand, for very small effect sizes (STNR = 0.025 scenario), both methods had low power, but the DLM site-by-site approach is more powerful than the FFR joint approach.

For the situation in which the window of susceptibility is localized to a single probe, the DLM site-by-site approach is more powerful than the FFR joint approach. Thus, while the FFR’s ability to borrow strength across probes is beneficial when there are shared windows of susceptibility across a genomic region, the corresponding smoothing across the surface can be detrimental when the signal is sparsely distributed across probes or when the strength of the signal is low. These findings are unsurprising since bias-variance trade-offs are typical of shrinkage methods, particularly nonlinear shrinkage, like that used by the Bayesian FFR approach.

5. Results. Using data from Project Viva, our goal was to characterize the time- and position-varying association between DNA methylation levels and air pollution exposure within the last trimester of gestation. To this end, we fit Model (3.7) using DNA methylation level $y(s)$, a function of CpG site
position \( s \) (relative to other CpG sites on the same chromosome) as the outcome function. The exposure function was daily maternal PM\(_{2.5}\) exposure during the 90 days prior to delivery. We included the following scalar covariates in the model: maternal BMI, race, smoking status, education level, household income, child’s race, child’s sex, gestational age, season of birth, sample plate (as a categorical variable), as well as estimated cell proportions of leukocytes (CD8+, CD4+, natural killer cells, B-lymphocytes, monocytes, granulocytes, and nucleated red blood cells).

We used Debauchies wavelets with four vanishing moments, six levels of decomposition, and zero-padding for both the outcome and predictor functions. We ran a total of 12,000 MCMC samples and discarded the first 3000 as burnin. We then thinned every third sample from the remaining 9000 samples resulting in 3000 samples to use for posterior inference. We assessed convergence of the estimated surface coefficients using (1) the Geweke convergence diagnostic which generates a Gaussian Z-score to test the equality of the first 10% and the last 50% of each chain (Geweke, 1992), and (2) the first order autocorrelation coefficient for each chain. Additionally, we used the \texttt{mcmcse} R package to calculate the Monte Carlo standard error of the estimated \( \beta \) coefficients and verify that the simulated standard error was sufficiently small to estimate the BFDR reliably. These diagnostics and their summary statistics can be found in the Appendix.

As discussed in Section 2.2, we applied the FFR method to two regions encompassing CpG sites previously identified by Gruzieva et al. (2019) where DNA methylation levels in cord blood were both significantly associated with PM exposure as well as implicated in respiratory-related outcomes: \( FAM13A \) and \( NOTCH4 \). Figure 7 shows the FFR- and DLM-estimated association surface for the 23 CpG probes annotated to \( FAM13A \) on chromosome 4. These probes span 348,819 base pairs and are labeled on the heat map according to their position relative to other CpG probes on chromosome 4 (e.g. the first CpG probe on chromosome 4 corresponds to position 1, the next CpG probe corresponds to position 2, etc.). We set \( \alpha = 0.05 \) as the global BFDR bound and used \( \delta = 0.01 \) as the minimum practical effect size in the BFDR calculation. The areas flagged as significant in the BFDR analysis correspond to probes in \( FAM13A \) coding regions and fall near the beginning of the third trimester, 78-79 days before delivery, as well as halfway through the third trimester for four probes (CpG positions 9409 - 9412 corresponding to \( \text{cg17769793, cg06884401, cg25779483, cg04536922} \)). We include additional plots with cross sections of the estimated surface for select probes in the Appendix. The CpG identified by Gruzieva et al. (2019), \( \text{cg00905156} \), corresponds to position 9402 in Figure 7. No windows
of susceptibility were detected for this probe. In sensitivity analyses performed using Debauchies wavelets with varying levels of decomposition and vanishing moments, the window of susceptibility halfway through the third trimester remained flagged, indicating that this window is the more robust finding (see Appendix).

The direction of the effect can be assessed using two complementary strategies, one graphical and one inferential. First, one can visually inspect the sign of the estimated associations in the regions identified as significant after control for multiple testing via BFDR or SimBaS. Second, our Bayesian approach to model-fitting permits straightforward calculation of the posterior mean and 95% credible interval of the integrated surface effect. This corresponds to a cumulative association between methylation within the region of interest and exposure over the time period of interest. The posterior means and credible intervals of the integrated surface effects were negative across the sensitivity analyses (see Appendix).

When we performed the analogous analysis using a DLM approach, the BFDR procedure did not flag any areas of the surface, suggesting that the power gains observed in the simulation study manifested in the analysis of this genomic region as well. We note, however, that in addition to the joint versus site-by-site approach, the difference in basis expansion used by the FFR and DLM methods could partially account for the discrepancy between the findings of the two approaches.

Little is currently known about what constitutes a biologically meaningful change in methylation level, but small changes in DNA methylation in some genomic regions have been shown to have a strong effect on transcriptional activity (Breton et al., 2017); we note that in sensitivity analyses with levels of $\delta > 0.03$, all of the regions flagged in Figure 7 disappear. The region did not appear significant when we used the SimBaS procedure to control the experiment-wise error rate.

Figure 8 shows the FFR- and DLM-estimated association surfaces for the 137 CpG probes annotated to $\text{NOTCH4}$ on chromosome 6. These probes span 28,306 base pairs and are labeled according to their position relative to other CpG probes on chromosome 6. For this region, we see a potential band of association across much of the NOTCH4 gene 67-72 days before delivery on the estimated surface, but none of these regions appear significant after applying the BFDR and SimBaS inferential procedures. The CpG identified by Gruzieva et al. (2019), cg06849931, corresponds to position 11295 in Figure 8 and does not exhibit a window of susceptibility on the BFDR heat map. No significant regions were identified in sensitivity analyses using different levels of decomposition or vanishing moments for the Debauchies
Fig 7. FFR analysis (top panel) and DLM analysis (bottom panel) for a region on chromosome 4 encompassing 23 CpG probes annotated to the FAM13A gene. Position numbers for each probe correspond to their position relative to other CpG probes on chromosome 4. Right panel: BFDR results with $\delta = 0.01$ and $\alpha = 0.05$. 
Fig 8. FFR analysis (top) and DLM analysis (bottom) for a region on chromosome 6 encompassing 137 CpG probes annotated to the NOTCH4 gene. Position numbers for each probe correspond to their position relative to other CpG probes on chromosome 6. Right panel: BFDR results with $\delta = 0.01$ and $\alpha = 0.05$.

wavelets. The corresponding BFDR image for the DLM approach also does not flag any regions as significant.

5.1. Discussion. Functional regression is a powerful method for analyzing and visualizing associations between different sources of functional data. While a number of methods already exist for identifying differentially methylated regions and a separate body of literature addresses identifying windows of susceptibility, here we accomplish both objectives within a unified modeling framework. By enabling us to analyze associations between a spatially-varying outcome function and a time-varying exposure, functional regression provides a means of identifying differentially methylated regions exhibiting windows of susceptibility to exposures measured at a fine temporal resolution. More generally, the flexible framework presented here could be useful in a variety of high-throughput genomic applications pertaining to the tran-
In our setting, exposure was indexed by time, but this need not be the case.

In simulation, we demonstrated that by jointly modeling epigenetic sites, FFR had greater power to identify regions associated with windows of susceptibility than the DLM approach that models sites independently. The FFR approach also maintained high sensitivity and low FDR under all but the lowest STNR scenario. In very low signal settings, the FFR lost power due to shrinkage across sites within the region of interest. This shrinkage also rendered the FFR approach less effective than the DLM at identifying windows of susceptibility when signal was confined to a single site. Overall, the vertical and horizontal band simulation studies suggest that FFR is more effective at identifying sustained temporal effects across a genomic region, whereas a DLM is more effective at pinpointing sustained temporal effects at a spatially-localized site. In the Project Viva data analysis, FFR showed a greater ability to highlight differentially methylated regions associated with PM$_{2.5}$ exposure during the last trimester of pregnancy than did the DLM.

Because both sustained temporal effects across genomic regions and sustained temporal effects at individual sites could be biologically significant, we recommend running both DLM and FFR analyses in a staged analytic plan. Similar to the way in which site-by-site epigenome-wide association studies are often followed by region-finding methods like DMRcate or Bumphunter, when interest focuses on finding windows of susceptibility to an exposure, we suggest performing a site-by-site DLM analysis followed by the multivariate FFR approach.

There are several limitations of our work. First, the PM$_{2.5}$ daily measurements we used were estimated based on where the mothers enrolled in Project Viva lived, rather than by direct personal monitoring. We do not account for the prediction error from the air pollution exposure model in our inferential procedures. A worthwhile direction for future research would be the development of methods for function-on-function data that account for exposure measurement error. Second, we used wavelet basis functions in our FFR implementation since simulations showed that these worked well for the simulated surfaces that we used, but it is possible that wavelets are not the optimal basis expansion. Future work could explore whether a different basis expansion is preferable for modeling methylation profiles and air pollution exposures. In particular, a basis that is better suited for unequally-spaced data may improve the model fit. It is also possible that a different approach to implementing the wavelet transformation, such as semiparametric regression with penalized wavelets may lead to better model performance (Wand et al., 2011). An additional limitation is that we restricted our investiga-
tion to CpG probes annotated to two genes. Our aim here was to perform the first analysis of prenatal windows of susceptibility driving the two most noteworthy associations reported by Gruzieva et al. (2019). Future work will involve a more comprehensive exploration of the genome and additional air pollutants. Identifying additional windows of susceptibility can direct attention to specific biologic mechanisms underlying associations and ultimately inform interventions to improve children’s health. This work suggests function-on-function regression is a valuable tool to achieve these objectives.

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APPENDIX A: POSTERIOR FUNCTIONAL INFERENCE

Below we include details about the two posterior functional inference procedures we use to account for multiple comparisons across the coefficient surface.

A.1. Bayesian FDR. Interest focuses on detecting a biologically meaningful effect size of at least $\delta$. For $M$ MCMC samples, let $\beta^{(m)}(t, s)$ be one posterior sample of the coefficient surface for sample $m$, $m = 1, \ldots, M$. We can compute the point-wise posterior probability of a $\delta$-sized intensity change on the $T \times S$ grid determined by each time-point $t = 1, \ldots, T$ and genomic loci $s = 1, \ldots, S$ via

$$p(t, s) = Pr\{|\beta(t, s)| > \delta|Y\} \approx \frac{1}{M} \sum_{m=1}^{M} I\{|\beta^{(m)}(t, s)| > \delta\},$$

where $I$ is the indicator function. High values of $p(t, s)$ provide stronger evidence for a true discovery at a specific point on the surface; correspondingly, the quantity $1 - p(t, s)$ can be interpreted as a local false discovery rate at a given location (Morris et al., 2008). A location on the coefficient surface is flagged as significant if $p(t, s) > \nu_\alpha$, where $\nu_\alpha$ is a threshold and $\alpha$ is a pre-specified global FDR-bound. The threshold $\nu_\alpha$ is constructed such that on average we expect at most $\alpha$ percent of flagged sites to be false positives. We do this by first sorting $\{p(t, s), t = 1, \ldots, T, s = 1, \ldots, S\}$ in descending order across all locations to obtain $\{p(r), r = 1, \ldots, R\}$, where $R = TS$. 

We then define $\lambda = \max \{ r^* : \frac{1}{r} \sum_{r=1}^{r^*} (1 - p(r)) \leq \alpha \}$ and set the cutoff for flagging significant coefficients as $\nu_\alpha = p(\lambda)$.

### A.2. Joint Credible Bands and Simultaneous Band Scores.

In applications where there is not consensus on a biologically meaningful value for $\delta$, it is useful to consider constructing joint credible bands. Joint credible bands have the benefit of not requiring specification of $\delta$, while still providing a means of controlling the experiment-wise error rate. A $100(1 - \alpha)\%$ credible band of $\beta(t, s)$ must satisfy

\begin{equation}
Pr\{L(t, s) \leq \beta(t, s) \leq U(t, s) \forall t \in T, s \in S\} \geq 1 - \alpha,
\end{equation}

where $L(t, s)$ and $U(t, s)$ are the lower and upper bounds of the band respectively (Ruppert, Wand and Carroll, 2003). If we assume approximate normality of the posterior samples, an interval satisfying this constraint is

\begin{equation}
I_\alpha(t, s) = \hat{\beta}(t, s) \pm q(1 - \alpha)[SD\{\hat{\beta}(t, s)\}].
\end{equation}

Here, $\hat{\beta}(t, s)$ is the mean for a given position $(t, s)$ taken over all $M$ MCMC samples and $SD\{\hat{\beta}(t, s)\}$ is the standard deviation over all $M$ MCMC samples divided by the factor $A(M, \rho_{MCMC})$, where $\rho_{MCMC}$ is an estimate of the lag autocorrelation in the samples, and $A$ is the bias correction factor described in Anderson (1971) (Meyer et al., 2015; Lee and Morris, 2016). The variable $q_{(1-\alpha)}$ is the $(1 - \alpha)$ sample quantile taken over $M$ samples of

\begin{equation}
Z^{(m)} = \max_{t \in T, s \in S} \left| \frac{\beta^{(m)}(t, s) - \hat{\beta}(t, s)}{SD\{\hat{\beta}(t, s)\}} \right|.
\end{equation}

Suppose one constructs joint credible bands for a range of different values of $\alpha$ and finds for each location $(t, s)$ the minimum level $\alpha$ for which the $(1 - \alpha)\%$ joint credible band excludes zero. Meyer et al. (2015) refers to this minimum $\alpha$ level, $p_{SimBaS}(t, s) = \min\{\alpha : 0 \notin I_\alpha(t, s)\}$, as the simultaneous band score (SimBaS) for each location $(t, s)$. For any specific $\alpha$, we flag all $(t, s)$ for which $p_{SimBaS}(t, s) < \alpha$ as significant, meaning that the $(1 - \alpha)\%$ joint credible interval at those locations does not include zero.
APPENDIX B: ADDITIONAL SIMULATION RESULTS

Fig 9. Heat maps of the estimated surface averaged over 100 datasets. Top panel: FFR estimates. Bottom panel: DLM estimates concatenated across the surface.

APPENDIX C: METHYLATION AND AIR POLLUTION EXPOSURE LEVELS

Fig 10. NOTCH4 methylation levels (left) and PM$_{2.5}$ measurements (right) for a randomly selected subject in Project Viva (black) and nine additional subjects (light grey).

APPENDIX D: BAYESIAN DIAGNOSTICS

We assess convergence of the estimated surface coefficients using (1) the Geweke convergence diagnostic which generates a Gaussian Z-score to test
the equality of the first 10% and the last 50% of each chain (Geweke, 1992), and (2) the first order autocorrelation coefficient for each chain. These diagnostics and their summary statistics are shown below for both gene regions used in the data application. For both gene regions, the mean values of the Geweke Z-scores are near zero and the middle 95% of Z-scores fall within roughly 2 standard deviations of zero. The distributions of the first order autocorrelation functions for each gene region are concentrated near zero.

Additionally, we used the \texttt{mcmcse} R package to calculate the Monte Carlo standard error of the estimated $\beta$ coefficients. The median Monte Carlo standard error for the FAM13A analysis was 0.00022 and the maximum was 0.00114. These values are 1-2 orders of magnitude smaller than the $\beta$ coefficient values flagged as significant by the BFDR in that analysis and are sufficiently precise estimates of the local discovery rate for our purposes.

Together, these summary statistics give us confidence that, overall, the MCMC algorithms used in both analyses have converged and that the empirical standard errors were sufficiently small to estimate the BFDR reliably.

| Gene region | Geweke Mean | 2.5%-ile | 97.5%-ile | First Order ACF Mean | 2.5%-ile | 97.5%-ile |
|-------------|-------------|---------|---------|---------------------|---------|---------|
| FAM13A      | -0.030      | -2.328  | 2.310   | 0.072               | -0.008  | 0.213   |
| NOTCH4      | 0.013       | -2.072  | 2.120   | 0.039               | -0.012  | 0.214   |

\textbf{Table 3}

\textit{Geweke convergence diagnostics and first order autocorrelation coefficient summary statistics for both gene regions considered in the data application.}

| Gene region | Median | Mean   | Max   |
|-------------|--------|--------|-------|
| FAM13A      | 0.00022| 0.00025| 0.00114|
| NOTCH4      | 0.00027| 0.00029| 0.00133|

\textbf{Table 4}

\textit{Summary statistics for Monte Carlo standard error of $\beta$ across the coefficient surface obtained using the \texttt{mcmcse} R package.}
Fig 11. Histograms of convergence diagnostics. The left panel contains Geweke Z-scores for the two gene regions calculated as in Geweke (1992), testing equality of the first 10% and last 50% of each chain. The right panel contains first order autocorrelation coefficients for the two gene regions considered in the data application.
APPENDIX E: CROSS-SECTION OF ESTIMATED ASSOCIATION SURFACE FOR FAM13A GENE REGION

Fig 12. Cross-sections of the FAM13A estimated association surface for two fixed CpG sites, \( s = 9404 \) (left) and \( s = 9411 \) (right). CpG site 9411 was included in the window of susceptibility flagged using the Bayesian False Discovery Rate inferential procedure.

APPENDIX F: SENSITIVITY ANALYSIS

Here, we include the results of sensitivity analyses performed for the FAM13A gene region. For these analyses, we varied the levels of decomposition (LD) and number of vanishing moments (VM) of the Daubechies wavelet. Additionally, we calculated the posterior mean and 95% credible interval of the integrated surface effect for each analysis. In low-signal settings, such as the one studied here, we anticipate noise and oscillation in the estimated surface, but each of the positive and negative spikes are not of the same magnitude. By calculating an integrated effect over the BFDR-identified windows of susceptibility, we can learn the overall direction of the effect. Comparing the direction of these integrated effects after varying wavelet specifications then allows us to understand how robust these findings are.

| Analysis setting | Posterior mean of integrated effect | 95% credible interval |
|------------------|------------------------------------|-----------------------|
| 4 VM, 3 LD       | -0.138                             | (-0.233, -0.033)      |
| 4 VM, 4 LD       | -0.061                             | (-0.138, -0.013)      |
| 4 VM, 6 LD       | -0.071                             | (-0.151, -0.013)      |
| 5 VM, 6 LD       | -0.052                             | (-0.127, -0.012)      |

Table 5
Summary across analysis settings of the posterior means and 95% credible intervals of the integrated effect within the areas of the surface identified as significant by the BFDR inferential procedure. VM: vanishing moments. LD: levels of decomposition.
Daubechies wavelet family with 4 vanishing moments and 3 levels of decomposition:

\[ \text{Fig 13. The posterior mean of the integrated effect over areas of the surface flagged by the BFDR as significant is -0.138 [95\% credible interval (-0.233, -0.033)].} \]

Daubechies wavelet family with 4 vanishing moments and 4 levels of decomposition:

\[ \text{Fig 14. The posterior mean of the integrated effect over areas of the surface flagged by the BFDR as significant is -0.061 [95\% credible interval (-0.138, -0.013)].} \]

Daubechies wavelet family with 4 vanishing moments and 6 levels of decomposition:
The posterior mean of the integrated effect over areas of the surface flagged by the BFDR as significant is -0.071 [95% credible interval (-0.151, -0.013)].

Daubechies wavelet family with 5 vanishing moments and 6 levels of decomposition:

The posterior mean of the integrated effect over areas of the surface flagged by the BFDR as significant is -0.052 [95% credible interval (-0.127, -0.012)].

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