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DNA methylation of food sensitization in a French-Canadian population

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Abstract

Background Food allergy (FA) is a great public health concern with an increased prevalence in the last decades. The underlying development mechanisms of FA and food sensitization (FS), which represents the first stage of development of FA, are influenced by environmental, epigenetic, and genetic factors. DNA methylation is an important epigenetic mediator of gene–environment interactions and key to understanding these mechanisms. Studies have linked whole-genome DNA methylation profile to FA and FS, but they all use methylation arrays. Methylation sequencing captures target regions of methylome with an extensive coverage. Thus, our objective was to identify CpG sites in genome-wide immune regulatory regions associated with FS and test their association with genetic variants using methylation quantitative trait loci (mQTL) analysis in French-Canadian individuals.

Results In 114 individuals from the Saguenay–Lac-Saint-Jean asthma family cohort, a total of 10 CpG sites out of 5,233,004 CpG sites were associated with the FS status ($P < 1 \times 10^{-8}$). CpG sites were located in 10 genes (*ARRDC1*, *B9D1*, *CFL1*, *CROCC*, *DHX36*, *DND1*, *PMS1*, *RASSF1*, *TOP2A*, and *USP21*) all of which were associated with immune response and allergic diseases (allergy, asthma, atopic dermatitis, and allergic rhinitis) in the literature. Almost all individuals with FS clustered based on the methylation levels of these CpG sites which reinforces their importance and relevance to discriminate individuals with and without FS in our cohort. Among the top 10 most significant CpG sites, five were associated with 478 genetic variants in *trans*-mQTL (FDR $< 1 \times 10^{-8}$).

Conclusions To our knowledge, this is a unique association study between FS and DNA methylation using targeted bisulfite sequencing across the genome. This approach provides high-resolution assessment of genome-wide functional methylome that yields valuable understandings to this field of research. The results reveal potential relationships between FS, CpG sites, and genetic variants located in genes involved in allergic diseases. This provides potential insights on the underlying effects of DNA methylation and genetic variants on FS and possibly the pathogenesis of FA. Further epigenome-wide studies on larger samples combined with genome-wide genotyping are needed to validate the results and verify the biological potential of these CpG sites.

Keywords Aeroallergen, DNA methylation, Food allergy, Food sensitization, Methylation quantitative trait loci, Targeted bisulfite sequencing, Whole blood

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Background

The prevalence of food allergies (FA) has increased considerably over the last decades to reach 6–10% in children and 3–4% in adults in developed countries [1]. Moreover, as many as 40% of children with FA are allergic to more than one food [2]. The most common food allergies in children are cow's milk, hen's egg, peanut, tree nut, fish, shellfish, wheat, and soy [3]. In addition

to physical manifestations ranging from mild symptoms to potentially fatal anaphylaxis, FA also affect the general quality of life [4], notably because FA management implies constant vigilance, increased anxiety and limitation of social activities [5, 6]. Also, societal compromises have to be made to provide safe environments for allergic children [7].

Food sensitization (FS) represents the first stage of development of FA and is defined by the presence of allergen specific immunoglobulin E (IgE) [8]. While most FA are IgE-mediated, FS may be present with or without clinical FA manifestations [9]. In a sensitized individual, an oral food challenge is required to diagnose a FA [10]. The FS prevalence trend follows that of FA in many countries [11]. The prevalence of sensitization to one or more foods reaches 16.2% in European countries [12] and varies between 13 to 15.9% in USA depending on age [13]. FS and FA are intrinsically linked and are part of the atopic march, a pattern of progression of allergic diseases that begins with atopic dermatitis in early childhood and progresses with FA, asthma, and allergic rhinitis later in life [14]. The inflamed and disrupted skin barrier of infants with atopic dermatitis allows the penetration of food allergens, leading to FS, the first step in becoming allergic. The underlying development mechanisms of FA and FS are a complex interplay of environmental, epigenetic, and genetic factors [3]. Several genetic variants have been associated with FA and FS, among which many have also been linked to atopic dermatitis, asthma, and allergic rhinitis thus reinforcing the hypothesis of the underlying mechanisms of the atopic march [15].

DNA methylation is a major epigenetic mediator of gene–environment interactions and key to understanding these mechanisms [16]. DNA methylation plays an important role in the development of FS and FA, as demonstrated by candidate genes and epigenome-wide association studies (EWAS). DNA methylation of several candidate genes such as interferon gamma (*IFNG*), interleukin-4 (*IL4*) and 5 (*IL5*), and forkhead box P3 (*FOXP3*) has been associated with FS and FA [17, 18]. EWAS have also linked several CpG sites to FA and FS though all were using methylation arrays [17–24]. Methylation sequencing captures target regions of methylome with a more extensive coverage [25]. Only one study used targeted bisulfite sequencing to evaluate DNA methylation levels in peanut allergy but only in 125 genomic regions containing 602 CpG sites for 70 immune-related gene regions [26]. To the best of our knowledge, no study has evaluated the association between FS and genome-wide DNA methylation using targeted bisulfite sequencing. High-resolution assessment of genome-wide functional methylome is needed to identify potentially novel CpG sites associated with FS. So, our objective was to identify CpG sites in genome-wide immune regulatory regions associated with FS and test their associations with genetic variants using methylation quantitative trait loci (mQTL) analysis in French-Canadian individuals.

Methods

Patients and design

A total of 114 individuals were considered from the Saguenay–Lac-Saint-Jean (SLSJ) asthma family cohort which is characterized by a multigenerational design, a 20-year follow-up and abundant data (clinical, environmental, genealogical, genomic, epigenomic, and transcriptomic). Individuals were recruited to study the genetic determinants and predisposition to asthma and related phenotypes. The complete description of recruitment of the cohort is presented in Laprise et al. [27]. In brief, the first phase of recruitment was from 1997 to 2007. Recruitment was conducted through probands and then included all family members who wanted to participate. A total of 1,394 individuals from 271 independent families were recruited in the SLSJ region in Quebec, Canada. Informed consent was obtained from all participants and/or their legal guardians at recruitment. The experimental protocol was approved by the ethics committee of the *Centre intégré universitaire de santé et de services sociaux* (CIUSSS) *du SLSJ* (project #0002-001).

Diagnosis of food sensitization

A total of 27 allergens were tested: food allergens (rye, oat, barley flours, peanuts, whole wheat, and egg whites), common aeroallergens from animals (cat, dog, cow, and horse dander and bird feathers), indoor aeroallergens (*Dermatophagoides farinae* and *D. pteronyssinus*), and outdoor aeroallergens (ryegrass, weeds, ambrosia, timothy hay, grasses, tree mix, maple, birch, oak, elm, *Cladosporium/Hormodendrum*, *Alternaria*, *Aspergillus*, and *Penicillium*). FS was defined as a positive skin prick test (wheal diameter ≥ 3 mm of the negative control after 10 min) result to any of the six food allergens [28]. Skin prick test results were available for a subsample of 136 individuals from the 20-year follow-up, and among them, 128 individuals also had DNA methylation data. FA was not defined in this cohort as we only have total IgE levels and not specific IgE and oral food challenge results.

DNA methylation data

Genomic DNA was extracted from whole blood using the Blood and Cell Culture DNA Midi Kit (Qiagen, Valencia, CA, USA). Bisulfite conversion and quantitative DNA methylation analysis were processed using Illumina HiSeq 4000 System at the McGill University and Genome Quebec Innovation Center (Montreal, Canada). Whole blood DNA methylation levels were

measured using a custom sequencing panel targeting immune regulatory regions as previously described [25, 29]. This panel permits high-resolution assessment of functional methylomes by targeting multiples regions (regulatory regions in immune cells, hypomethylated footprints from immune cells, Illumina 450K methylation assay included regions, and autoimmune SNPs from GWAS catalog) [25]. Libraries were prepared using the KAPA High Throughput Library Preparation Kit according to the manufacturer's protocol (Roche/KAPA Biosystems, Wilmington, MA, USA). AMPure beads were validated on Bioanalyzer High Sensitivity DNA Chips (Agilent, Santa Clara, CA, USA) and quantified by PicoGreen (Thermo Fisher, Waltham, MA, USA). The final library was purified using these AMPure beads. Targeted bisulfite sequencing reads were aligned using the Epigenetics Pipeline (DRAGEN EP v2.6.3) available from the DRAGEN Bio-IT Platform (Edico Genomics/Illumina, San Diego, CA, USA). Details on the sequencing are presented in Madore et al. [30]. Briefly, sequence reads were demultiplexed into FASTQ files with bcl2Fastq2 Conversion Software v2.19.1 (Illumina) and trimmed for quality (phred33 $\geq$ 20) using Trimgalore v.0.4.2. Reads were aligned to a bisulfite-converted reference genome with DRAGEN EP v2.6.3 or later in paired-end mode using the directional (Lister) methylation protocol presets. The methylation levels were calculated as the ratio of total non-converted C reads (forward and reverse) and total reads (forward and reverse). After quality control steps (read coverage > 5 and < 99^{e} percentile) and exclusion of CpG sites located on SNPs (dbSNP 151), in the ENCODE blacklist [31] and out of the custom panel (considered as each region ± 100 bp), 5,233,004 CpG sites were considered in the analysis. Since methylation levels were obtained from sequencing, CpG sites were annotated by their chromosomal positions on the genome (Genome Build 37).

Genotype data

Genotypes were obtained for all 114 individuals using the Illumina Human610-Quad BeadChip. Imputation was performed in the data that followed specific criteria: minor allele frequency $\geq$ 1%, *P* value for Hardy–Weinberg equilibrium $\geq 1 \times 10^{-5}$, genotype and individual call rates $\geq$ 95%. The prephasing step for the imputation process was performed with the Shapeit2 Software using the duoHMM method [32]. Impute 2 Software [33] was used for imputation with two reference samples (1000 Genome Project database (phase 3) [34] and the UK10K). Only imputed data meeting the following criteria were included: minor allele frequency $\geq$ 1%, *P* value for Hardy–Weinberg equilibrium $\geq 1 \times 10^{-5}$, and genotype and individual call rates $\geq$ 95%. Details of the quality filtering are presented in Madore et al. [30]. A total of 7,829,249 genetic variants were considered in the mQTL analysis.

Statistical analysis

R software v3.5.1 (R Foundation for Statistical Computing; http://www.r-project.org) [35] was used to compute linear models between normalized DNA methylation levels of all 5,233,004 CpG sites and FS status (defined as a positive skin prick test result for at least one of the six food allergens measured) adjusted for age, sex, smoking status, cell count proportions, and surrogate variables using R function "glm" in "stats" package. Using the R package "sva" [36], surrogate variables were calculated to take into account relatedness between samples and hidden confounders. All cofactors were considered, and the first five surrogate variables were used in the linear models. White blood cell count proportions (eosinophils, lymphocytes, neutrophils, monocytes, and basophils) were measured using a blood differential test, and the proportions of the first four cell types were included in the statistical models. A total of 14 individuals had missing values (13 for cell counts and 1 for smoking status); thus, linear models were computed using 114 individuals (10 individuals with FS and 104 without FS). Methylation levels were all transformed using arcsine square root. A significant *P*-value was defined as $< 1 \times 10^{-8}$. Considering the small sample size, two methods were used to assess the robustness of the results. First, bootstrapping (10, 000 iterations) was performed to evaluate the accuracy of the regression coefficients. In addition, 10,000 permutations of the food allergy status were performed to assess the significance of the results. To test whether white blood cell count proportions mediated the association between FS and FS-associated CpG sites, a mediation analysis was performed using the R package "mediation" [37]. The same covariates as the main model were used.

In addition to the main model, two other secondary models were tested to better understand the methylation profile associated with FS. First, aeroallergen sensitization was added to the list of cofactors for all 114 individuals. Second, the main model was performed only in individuals with allergic sensitization (9 with food and aeroallergen sensitization and 57 with aeroallergen sensitization only). Moreover, mQTL analysis was performed using the "MatrixEQTL" package in R [38] in FS-associated CpG sites. A significant *P*-value was defined as FDR < 0.05 for *cis* associations ($< 1 \times 10^{6}$ bp) and FDR $< 1 \times 10^{-8}$ for *trans* associations ($> 1 \times 10^{6}$ bp). More severe threshold values were chosen to limit the analysis to a reasonable number of significant associations. Regarding the figures, R package "qqman" was used for the Manhattan plot and the qqplot (https://github.com/stephenturner/qqman, accessed June 12, 2023). The

"qqplot2" R package was used to make box plots [39]. Heatmaps were made using the "ComplexHeatmap" R package using default settings (Euclidean distance) [40]. GeneMANIA (http://www.genemania.org) was used to generate interactive functional association networks using a very large set of functional association data including protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity [41]. Networks were weighted to maximize connectivity between all input genes using linear regressions [41]. Enrichment analysis was performed using g:Profiler (g:SCS threshold and only annotated genes) [42]. All positions were from the Genome Build 37.

Results

Individuals' characteristics

FS, DNA methylation, and clinical data were available for 114 individuals in the SLSJ asthma family cohort of French-Canadian origin. A total of 10 individuals had clinically confirmed FS and 104 had no FS (Table 1). Individuals were sensitized to peanut ($n=2$), whole wheat ($n=1$), egg white ($n=3$), rye ($n=2$), oat ($n=2$), and barley ($n=4$) flours allergies. Two individuals were sensitized to more than one food allergen (four and two allergens, respectively). Age was significantly different ($P=0.01$) between groups (mean age of 41 and 55 for individuals with and without FA, respectively). Aeroallergen sensitization was also significantly different ($P=0.04$) between groups. There were no significant differences in the male/female ratio, body mass index, number of individuals with asthma, eczema, rhinitis or who smoke, and immunoglobulin (Ig) E levels.

Table 1 Characteristic of the study individuals ($n=114$)

Characteristics	Food-sensitized (n = 10)	Non-food-sensitized (n = 104)	*P*
Age, mean (range)	41 (26–64)	55 (19–83)	0.01
Sex, M:F	5:5	49:55	1.00
BMI, kg/m^2 (SD)	27.9 (7.0)	26.8 (4.7)	0.98
Asthma diagnosis[a], *n* (%)	6 (67)	59 (58)	0.74
PH eczema, *n* (%)	2 (20)	32 (31)	0.72
PH rhinitis[b], *n* (%)	1 (10)	21 (20)	0.71
Smoker, *n* (%)	5 (50)	60 (58)	0.74
IgE[c], µg/L[d] (SD)	175.7 (3.9)	102.1 (4.2)	0.24
Aeroallergen sensitization, *n* (%)	57 (55)	9 (90)	0.043

Differences between food-sensitized and non-sensitized groups were performed with a Wilcoxon test for continuous variables and a Fisher's exact test for categorical variables. [a] Asthma diagnosis was available for nine food-sensitized individuals and 101 non-food-sensitized individuals. [b] Personal history of allergic rhinitis was available for all food-sensitized and 103 non-food-sensitized individuals. [c] Values of the geometric means and SD. [d] IgE conversion factor to IU/ml = 0.4167. Abbreviations: Body mass index (BMI), immunoglobulin (Ig) E, personal history (PH), standard deviation (SD)

Association between DNA methylation and food sensitization

DNA methylation levels of 5,233,004 CpGs were obtained from a custom sequencing panel targeting immune regulatory regions [25]. Associations between DNA methylation levels and FS status adjusted for age, sex, smoking status, cell counts, and surrogate variables were tested using linear models. A total of 12 CpG sites were associated with the FS status ($P<1\times10^{-8}$ and P from permutations <0.05) (Table 2), and their chromosomic positions are represented in the Manhattan plot (Fig. 1). The QQ plot is showing observed versus expected $-\log10P$ values for association at all loci (Supplementary Fig. 1, Additional file 2). The lambda value is 1.05. Among the top 12 CpG sites, two located in ArfGAP with SH3 domain, ankyrin repeat, and PH domain 3 (*ASAP3*) and zinc finger and BTB domain containing 7A (*ZBTB7A*) had missing values for more than 40% of individuals (Supplementary Table 1, Additional file 1). One of them also had a non-significant 95% confidence interval after bootstrapping. Both were therefore excluded from further analyses. Measured white blood cell count proportions (eosinophils, lymphocytes, neutrophils, monocytes, and basophils) did not mediate the observed association between these 10 CpG sites and FS status (data not shown). The position of the top 10 CpG sites in genes and their relationship with regulatory elements (CpG islands and transcription factor binding sites) are detailed in Supplementary Table 2, Additional file 1. A total of six CpG sites were located in CpG islands and all but one CpG sites were located in known transcription factor binding sites.

Methylations levels for the top 10 CpG sites in individuals with and without FS are represented in the box plots (Supplementary Fig. 2, Additional file 3). Individuals with FS tend to have more variation and higher methylation levels for these CpG sites compared to individuals without FS. As depicted in the heatmap of methylation levels of these top CpG sites, eight out of 10 individuals with FS clearly clustered (Fig. 2). CpG sites were located in 10 genes among which nine (DND microRNA-mediated repression inhibitor 1 (*DND1*), cofilin-1 (*CFL1*), PMS1 homolog 1, mismatch repair system component (*PMS1*), arrestin domain containing 1 (*ARRDC1*), ubiquitin specific peptidase 21 (*USP21*), DNA topoisomerase II alpha (*TOP2A*), ciliary rootlet coiled-coil, rootletin (*CROCC*), DEAH-box helicase 36 (*DHX36*), and B9 domain containing 1 (*B9D1*)) were previously associated in the literature with immune response and allergic diseases (allergy, asthma, atopic dermatitis, and allergic rhinitis).

Table 2 CpG sites associated with food sensitization

CpG location	Gene	Coeff (CI95)*	SE	*P*	*P*perm*	Adjusted *P***
Chr5:140,052,766	*DND1*	−0.52 (−0.73,−0.35)	0.066	4.63×10^{-12}	1.00×10^{-4}	2.56×10^{-12}
Chr11:65,624,379[†]	*CFL1*	0.14 (0.06,0.24)	0.020	5.43×10^{-11}	1.00×10^{-4}	8.57×10^{-11}
Chr2:190,719,381[†]	*PMS1*	−0.40 (−0.53,−0.24)	0.054	1.28×10^{-10}	1.00×10^{-4}	1.08×10^{-10}
Chr9:140,499,814	*ARRDC1*	0.17 (0.00,0.32)	0.021	2.03×10^{-10}	1.00×10^{-4}	3.33×10^{-10}
Chr1:23,811,026[††]	*ASAP3*	0.30 (−0.02,0.43)	0.040	5.33×10^{-10}	1.00×10^{-4}	8.82×10^{-10}
Chr3:50,378,007	*RASSF1*	0.086 (0.03,0.14)	0.013	9.63×10^{-10}	1.00×10^{-4}	2.11×10^{-9}
Chr1:161,129,510	*USP21*	0.16 (0.05,0.28)	0.024	1.16×10^{-9}	1.00×10^{-4}	1.68×10^{-9}
Chr17:38,573,679	*TOP2A*	0.12 (0.06,0.17)	0.019	2.48×10^{-9}	1.00×10^{-4}	3.30×10^{-9}
Chr1:17,067,332	*CROCC*	0.15 (0.00,0.31)	0.022	3.57×10^{-9}	2.00×10^{-4}	6.83×10^{-9}
Chr3:154,042,088	*DHX36*	0.24 (0.15,0.30)	0.037	5.22×10^{-9}	2.00×10^{-4}	8.84×10^{-9}
Chr17:19,266,369	*B9D1*	0.15 (0.05,0.25)	0.024	6.98×10^{-9}	1.00×10^{-4}	8.57×10^{-9}
Chr19:4,066,274[††]	*ZBTB7A*	0.16 (0.00,0.39)	0.024	8.43×10^{-9}	5.00×10^{-4}	1.13×10^{-8}

Linear models between DNA methylation levels and FS status adjusted for age, sex, smoking status, cell counts, and surrogate variables were computed using R software. *Ninety-five percent confidence intervals calculated from 10,000 bootstraps and *P*-value calculated from 10,000 permutations. ** Adjusted *P*: Association between DNA methylation and FS with the additional adjustment for aeroallergy. [†]CpG significantly associated ($P\leq1\times10^{-8}$) with FS in the secondary model in a subset of 66 individuals with allergic sensitization (food and aeroallergen). [††] CpG with missing values for more than 40% of individuals. All positions of CpG sites are from the Genome Build 37. Abbreviations: Coefficient (Coeff); 95% confidence intervals (CI95); food sensitization (FS); standard error (SE)

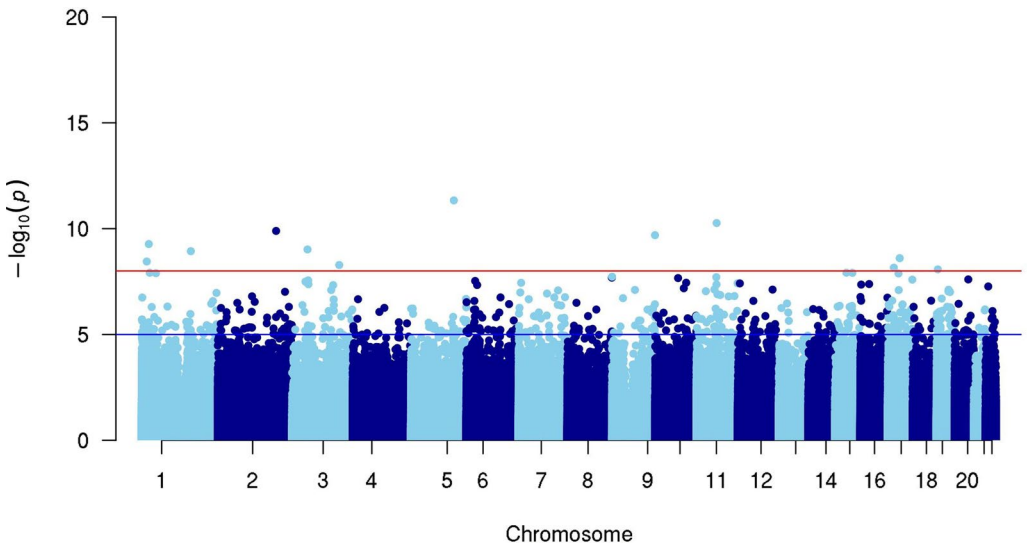

Fig. 1 Manhattan plot for the association of genome-wide DNA methylation with food sensitization. Manhattan plot showing chromosomal locations of −log10P values for association of each CpG site with food sensitization. The blue and red lines illustrate thresholds of $P<1\times10^{-5}$ and $P<1\times10^{-8}$, respectively. All positions are from the Genome Build 37

All 10 genes were represented in a functional association network (Fig. 3) to illustrate their connectivity with each other and with other genes with similar functions. A total of 6 out of 10 genes, in bold type above, had connections in the same network.

Considering the potential confounding effect of aeroallergens on the association between DNA methylation and FS, we performed the same linear model while adding clinically confirmed allergic sensitization to aeroallergens as a cofactor. A total of 11 CpG sites out of 12 kept a significant association with FS ($P<1\times10^{-8}$) (Table 2). To isolate as much as possible the association between FS and DNA methylation, a linear model in a subsample of 66 individuals with allergic sensitization only (nine individuals with food and aeroallergen sensitization and 57 with aeroallergen sensitization only) was used. A total of nine CpG sites were associated ($P<1\times10^{-8}$) with FS (Supplemental Table 3, Additional file 1), and two (located in *CFL1* and *PMS1*) out of the top 12 CpG sites from the main model were also significantly associated

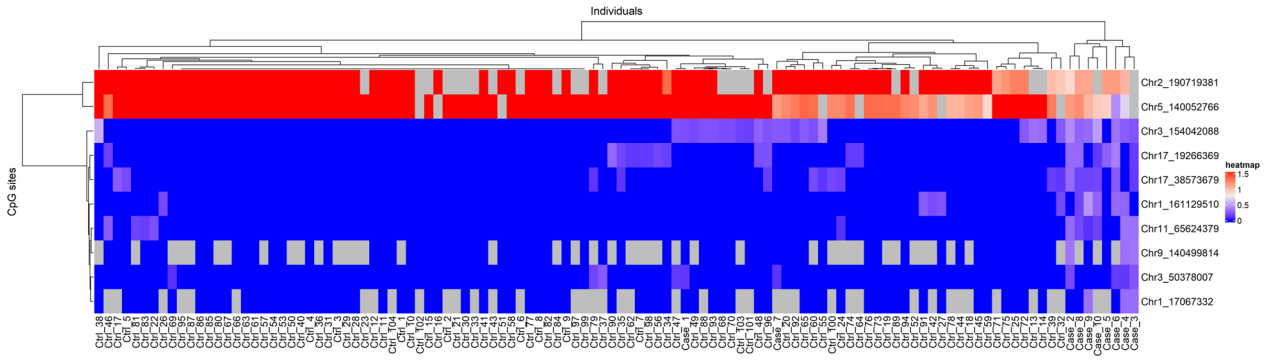

Fig. 2 Heatmap depicting methylation levels of the top 10 CpG sites in individuals with food sensitization (cases) and without food sensitization (controls). Missing values are illustrated in gray

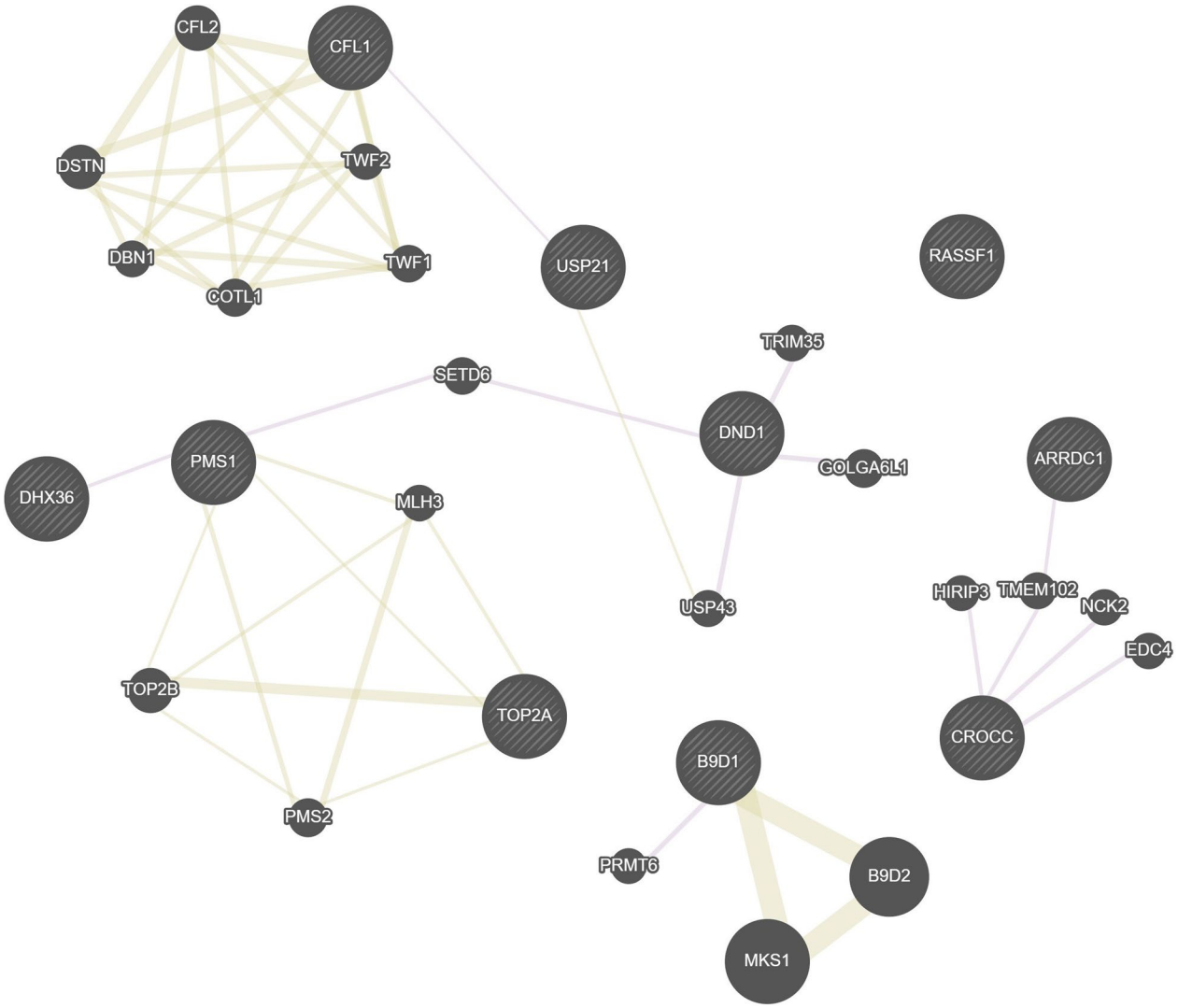

Fig. 3 Functional association network of the top 10 genes associated with food sensitization. Top 10 genes are represented by hatched black circles while other genes with similar functions are represented by solid black circles. Lilac lines represent co-expression interactions while pale green lines represent shared protein domains interactions

with the FS status (Table 2). Six CpG sites out of the same 12 CpG sites were also associated with *P*-values between 1×10^{-5} and the genome-wide cutoff of $P<1\times10^{-8}$. They were located in *DND1*, *ASAP3*, *TOP2A*, *CROCC*, *DHX36*, and *ZBTB7A*. In summary, top CpG sites remained strongly associated with FS even with the adjustment for aeroallergy and in the subset of individuals with allergic sensitization.

Methylation quantitative trait loci analysis

Exploratory analyses were performed to better understand biological mechanisms underlying the associations between CpG sites and food sensitization. Regarding *cis*-mQTL, none of the top 12 CpG sites was associated with proximal genetic variants ($<1\times10^{6}$ base pairs) among the 7,829,249 available for analyses after the imputation process using an FDR < 0.05. In *trans*-mQTL, five CpGs located in *ARRDC1, CFL1, CROCC, RASSF1,* and *USP21* genes were associated (FDR $<1\times10^{-8}$) with 478 genetic variants located in 85 unique genes (Supplementary Table 4, Additional file 1). Enrichment analysis revealed that only the Gene Ontology (GO) molecular function adenosine 5'-monophosphoramidase activity (*FHIT* and *HINT1* genes) was overrepresented ($P=0.03$) among these 85 genes. The 478 variants were not associated with allergic diseases or immune response in previous genome-wide association studies (GWAS) but other variants within 24 out of the 85 genes were associated with asthma, atopic dermatitis, allergic rhinitis, IgE levels, eosinophilic esophagitis, interleukin levels, and leukocyte type counts in the literature. These 24 genes are also represented in a functional association network (Fig. 4). Only one gene (*UBE2E2*) had no connection with the others. This could represent a potential mechanism through which DNA methylation affects FS.

Discussion

To begin with, the present study aimed at testing the association between FS and genome-wide DNA methylation using a custom sequencing panel targeting immune regulatory regions. To the best of our knowledge, this is the first study that considers the association between FS and genome-wide DNA methylation using targeted bisulfite sequencing. Among 5,233,004 CpG sites considered, 12 were significantly associated with FS ($P<1\times10^{-8}$). Two CpG sites had missing values for more than 40% of individuals, and thus, only the remaining ten CpG sites will be considered in the following literature review. Almost all individuals with FS clustered based on the methylation levels of these CpG sites and seemed to have a distinct methylation profile compared to individuals without FS. Thus, this reinforces the importance and relevance of these top CpG sites that discriminate almost all individuals with and without FS in our population study. Moreover, these CpG sites were located in 10 genes, the majority of which were interconnected based on the functional association network.

The top 10 CpG sites were located in 10 genes (*ARRDC1*, *B9D1*, *CFL1*, *CROCC*, *DHX36*, *DND1*, *PMS1*, *RASSF1*, *TOP2A*, and *USP21*) previously associated in the literature with immune response, allergy, asthma, atopic dermatitis, and allergic rhinitis. Given the strong interrelationship between allergic diseases from the atopic march, genes are often associated with more than one of them. This also strengthens the biological relevance of these CpG sites. Interestingly, an EWAS examined the relationship between DNA methylation levels in children and the development of IgE sensitization to airborne and food allergens [17]. Differentially methylated CpG sites were associated with FS in cord blood, at two years, and five years of age [17]. Some of these CpG sites were located in the same genes (*ASAP3*, *B9D1*, *DHX36*, *PMS1*, *TOP2A*, *USP21*, *ZBTB7A*) as our top 10 CpG sites. Moreover, eight out of our 10 key genes (*ARRDC1*, *B9D1*, *CFL1*, *CROCC*, *PMS1*, *RASSF1*, *TOP2A*, *USP21*) were also associated with asthma [43] and atopy [44] in other EWAS. This reinforces that DNA methylation of CpG sites within these key genes may play a role in the pathogenesis of FS.

First, three CpG sites were located in genes involved in the immune response. CpG located at Chr5:140052766 is in the body (intron) of *DND1* gene. Expression of *DND1* was highly correlated to interleukin 33 (IL-33) expression in multiple sclerosis patients [45]. IL-33 plays an important role in innate and adaptative immune responses and is an emerging key factor in the development of allergic diseases, mainly by inducing T-helper 2 immune responses [46]. Moreover, CpG located at Chr1:161129510 is in the body (intron) of the *USP21* gene. USP21 prevents the generation of T-helper-1-like Treg cells that play a key role in the maintenance of immune homeostasis and tolerance [47]. Expression of *USP21* was significantly increased in Treg cells of asthma patients [48]. Finally, CpG located at Chr3:154042088 is in the body (exon) of the *DHX36* gene. It is involved in multiple processes, such as transcription, mRNA processing, translation regulation and RNA degradation, innate immunity, and immune activation [49]. Indeed, *DHX36* can activate interferon regulatory factor 7 that mediates the release of interferon alpha [50], both of which are involved in atopic diseases such as allergic asthma [51].

Second, two CpG sites were in genes associated with allergic sensitization and allergy. Indeed, CpG located at Chr11:65624379 is in the body (intron) of the *CFL1* gene. In the literature, a CpG site (cg05072552) [52] and a

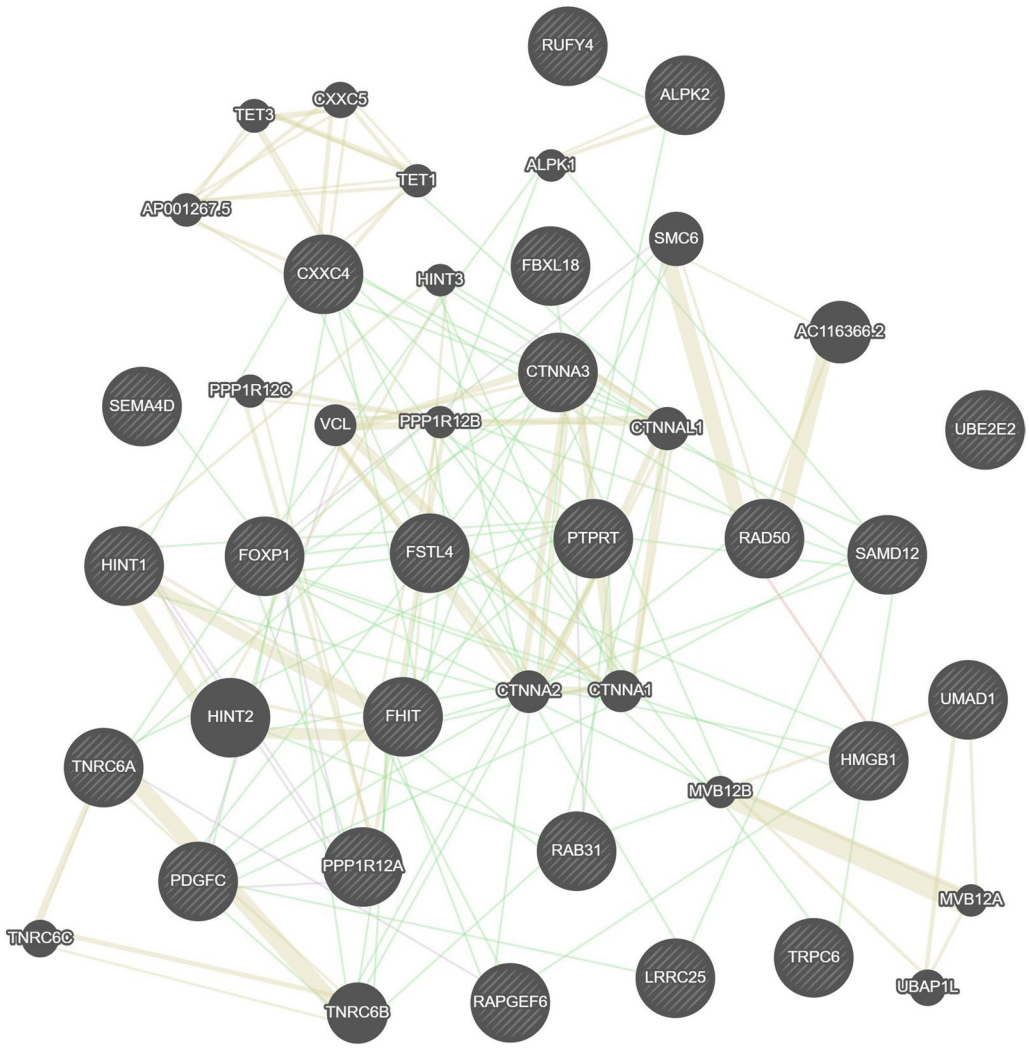

Fig. 4 Functional association network of the 24 genes identified by *trans*-mQTL which are linked to allergic diseases in the literature. The 24 genes are represented by hatched black circles while other genes with similar functions are represented by solid black circles. Lilac lines represent co-expression interactions, pale green lines represent shared protein domains interactions, pink lines represent protein–protein interactions, and green lines genetic interactions

single nucleotide polymorphism (SNP) (rs11602769) [53] were associated with allergic sensitization assessed by a skin prick test. Another CpG located at Chr17:19266369 is in the body (intron) of the *B9D1* gene. A CpG site (Chr17: 19177387) in this gene was associated with allergic diseases (IgE-mediated allergic diseases, asthma, allergic rhinitis, or atopic dermatitis) across childhood and with allergic disease symptoms only (without considering IgE levels) in eight replication cohorts [54].

Third, two CpG sites were in genes associated with asthma. CpG located at Chr9:140499814 is within 1500 base pairs (bp) upstream of the transcriptional start site (TSS1500) of the gene *ARRDC1*. The SNP rs117137535 in *ARRDC1* was associated with asthma [55] and atopic dermatitis [56]. Interestingly, ARRDC1 could be transferred between cells in microvesicles called arrestin domain containing protein 1-mediated microvesicles that represent highly versatile vehicles for therapeutic delivery [57]. These microvesicles contain Notch receptors, and it has been demonstrated that Notch signaling plays a key role in IgE-mediated FA [58]. Another CpG located at Chr17:38573679 is in the body (intron) of the *TOP2A* gene. The SNP rs11650680 in *TOP2A* is strongly associated with asthma, atopy, and total IgE which suggests that *TOP2A* may be an additional candidate gene for asthma and serum IgE levels [59]. *TOP2A* was upregulated in bronchial epithelium of asthmatic individuals compared to healthy ones [60]. Its expression is also

highly upregulated in mice with eosinophilic esophagitis [61].

Fourth, two CpG sites were located in genes associated with atopic dermatitis. CpG located at Chr2:190719381 is in the body (exon) of the *PMS1* gene. This gene has been mainly associated with several types of cancer [62]. The SNP rs1233255, located in *PMS1*, had an impact on the occurrence of atopic dermatitis in patients with rectal cancer [63]. Moreover, a GWAS used a gene-based analysis to identify 87 IgE-associated genes including *PMS1* [64]. Moreover, cofilin-1 encoded by *CFL1* is also considered to be a novel biomarker of atopic dermatitis [65], which is interesting considering that FS results from cutaneous exposure to food [66].

Fifth, a CpG site was located in a gene previously associated with allergic rhinitis. CpG located at Chr1:17067332 is in the body (intron) of the gene *CROCC*. SNP rs6586513 in *CROCC* is associated with seasonal allergic rhinitis [67]. Bet v 1, the major birch pollen allergen, is associated with nasal epithelial CROCC in the allergic, but not in the healthy individuals [68].

Finally, the last CpG was located at Chr3:50378007 in the body (exon) of the gene *RASSF1*. Expression of this gene has been principaly associated with the pathogenesis of several cancers [69], but one CpG in its intronic region (cg05989693) was also associated with atopy in a previous EWAS of a Puerto Rican cohort [44]. In summary, all CpG sites associated with FS were located in genes involved in immune response and allergic diseases from the atopic march.

The other part of this study consisted in performing mQTL analysis to test the association between FS-associated CpG sites and genetic variants. This analysis was carried out to better understand the underlying mechanisms or biological links with allergic diseases. None of the top 10 CpG sites was associated with genetic variants in *cis*-mQTL but five CpG sites were associated with 478 genetic variants in *trans*-mQTL. Moreover, among the genes identified with the EWAS, five (*CFL1*, *PMS1*, *ARRDC1*, *TOP2A*, and *CROCC*) contained SNPs previously associated with allergic diseases in the literature. We thus verify if these SNPs were also associated with top FS-associated CpG sites in the present study. We have data for only two SNPs: rs11650680 in *TOP2A* and rs11602769 in *CFL1,* but they were not significantly associated with the top CpG sites ($P = 0.019$/FDR = 0.84 and $P = 0.018$/FDR = 0.83, respectively). Even though genetic variants from *trans*-mQTL were not associated with allergic diseases in previous GWAS, other variants located in these same genes were of interest. Indeed, genetic variations within 24 out of the 85 genes identified that way were associated with asthma (*CTNNA3*, *FOXP1*, *RAD50*, *RAPGEF6*, *SEMA4D*, and *UBE2E2*), atopic dermatitis (*RAD50* and *UBE2E2*), allergic rhinitis (*RAD50*), IgE levels (*RAD50*), eosinophilic esophagitis (*FHIT* and *RAD50*), interleukin levels (*CTNNA3*, *FSTL4*, *HINT1*, *PTPRT*, and *RAPGEF6*), and leukocytes types counts (*ALPK2*, *CXXC4*, *CXXC4-AS1*, *FBXL18*, *FOXP1*, *HINT1*, *HMGB1*, *LRRC25*, *PDGFC*, *PPP1R12A*, *RAB31*, *RAD50*, *RAPGEF6*, *RUFY4*, *SAMD12*, *TNRC6A*, *TRPC6*, and *UMAD1*) in the literature. A total of 23 out of 24 genes were connected based on the functional association network. Although these analyses are exploratory, they suggest that five out of the 10 FS-associated CpG sites may be implicated in FS, at least in part, through genetic variations in these genes.

The present study has strengths but also some limitations. The study's main limitation resides in the relatively small sample size which limits the statistical power required to detect significant associations between CpG sites and FS. Nevertheless, our sample size surpasses the majority of sample sizes typically found in EWAS related to FS, FA, and IgE levels reported in the literature. Moreover, the proportion of individuals with FS in our study is also representative of the proportion in the general population. One possible caveat of EWAS using sequencing data is the number of missing values for each CpG. We took this into account by excluding two CpG sites with missing values for more than 40% of individuals in one or both phenotypic groups. These two CpG sites (located in *ASAP3* and *ZBTB7A*) must therefore be considered with caution. Finally, our study did not account for environmental factors such as diet in the association between DNA methylation and FS. It would have been interesting to test the effect of dietary patterns or specific nutrients considering that several dietary components such as vitamin D, butyrate, and folic acid can regulate epigenetic mechanisms and therefore the etiology of FA [70]. On the other hand, the main strength results from the study of genome-wide DNA methylation levels using a custom sequencing panel enriched in immune regulatory regions. Few studies, if any, have explored the relationship between FS and DNA methylation using targeted bisulfite sequencing across the genome, which highlights the novelty of our research approach. This also provides a novel extensive coverage of highly informative regions of methylome to identify novel CpG sites associated with FS which yields valuable understandings to this field of research. Moreover, mQTL analysis allows to better understand potential underlying genetic and epigenetic mechanisms of FS. Since FS and FA are intrinsically interrelated, a better understanding of its mechanisms may lead to a better understanding of the pathogenesis of FA. Finally, models were also adjusted for several covariates including

surrogate variables to account for batch effects, relatedness between samples and unmeasured biological variation.

Conclusions

In a sample of 114 French Canadians, 10 CpG sites were associated with FS at the genome-wide level. Almost all individuals with FS clustered based on the methylation levels of these CpG sites which reinforces their importance and relevance to discriminate individuals with and without FS in our cohort. In addition, mQTL analysis identified several genes associated with immune response and allergic diseases from the atopic march. To the best of our knowledge, this is the first study that considers the association between FS and genome-wide DNA methylation using targeted bisulfite sequencing. This approach provides high-resolution assessment of genome-wide functional methylome that yields valuable understandings to this field of research. These results reveal potential relationships between FS, CpG sites, and genetic variants located in genes involved in allergic diseases. This provides potential insights on the underlying effects of DNA methylation and genetic variants on FS and possibly the pathogenesis of FA. Further EWAS on larger samples combined with genome-wide genotyping are needed to validate the results and verify the biological potential of these CpG sites.

Abbreviations

CpG	Cytosine-phosphate-guanine
FA	Food allergy
FS	Food sensitization
mQTL	Methylation quantitative trait loci

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01951-8.

Additional file1 (XLSX 47 kb)

Additional file2 (JPEG 89 kb)

Additional file3 (JPEG 292 kb)

Additional file4 (DOCX 13 kb)

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Author contributions

BLT performed analyses, interpreted data, and wrote the manuscript. AMM validated the statistical analyses and contributed to the manuscript. CL designed, built, and manages the SLSJ asthma family cohort. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Canadian Dataverse Repository Borealis upon request; https://doi.org/10.5683/SP3/QEDOPE.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all participants and/or their legal guardians at recruitment. The experimental protocol was approved by the ethics committee of the *Centre intégré universitaire de santé et de services sociaux* (CIUSSS) *du SLSJ* (project #0002-001).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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