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CLINICAL EPIGENETICS

LETTER TO THE EDITOR

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DNA methylation signature of interleukin 1 receptor type II in asthma

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Abstract

Interleukin 1 and its receptors are associated with allergic diseases such as asthma. In the present study, we measured DNA methylation at the *IL1R1* and *IL1R2* gene loci and assessed for associations with asthma-related phenotypes and gene expressions. We found that asthmatic and atopic individuals have higher *IL1R2* promoter DNA methylation than control subjects. Additionally, we observed a negative correlation between DNA methylation at the *IL1R2* promoter and *IL1R2* mRNA expression. These results suggest for the first time that *IL1R2* promoter DNA methylation is associated with its gene repression in allergic diseases such as asthma.

Keywords: Epigenetics, Methylation, IL1, IL1R1, IL1R2, Asthma, Atopy

Introduction

Interleukin 1 (IL1) plays a key role in the inflammatory process of asthma [1]. We reported the association of polymorphisms within the IL1 receptors type I (*IL1R1*) and type II (*IL1R2*) gene loci with asthma and atopy in the French Canadian Saguenay–Lac-Saint-Jean (SLSJ) asthma study [2, 3]. The *IL1R2* gene expression signature in allergic asthma has also been described [4–6]. Epigenetics has received tremendous attention, and variations in DNA methylation (DNA-Me) in candidate genes have been reported associated with asthma and allergic related disorders [7–12]. These findings underline the relevance of genetic and epigenetic profiling to identify pathways associated with allergic diseases. Such a combined approach will facilitate the understanding of the functional impacts of genetic and epigenetic variations on transcription and molecular mechanisms involved in allergic diseases. In this study, we hypothesized that DNA-Me in the promoters of *IL1R1* and *IL1R2* is associated with asthma and/or atopy.

Clinical characteristics and methods

Clinical characteristics of the 93 individuals (21 non-atopic asthmatic, 26 atopic asthmatic and 21 atopic individuals, and 25 non-asthmatic non-atopic controls) from the Saguenay–Lac-Saint-Jean asthma familial collection [13] and included in the analysis are shown in Table 1. Ethics committee approved the study, and all subjects gave informed consent. Based on previous genetic [14] and epigenetic analyses [15], methylation at 1 CpG in promoter and 3 CpGs in exon 1 of *IL1R1* (Additional file 1: Figure S1) and 5 CpGs in promoter of *IL1R2* (Fig. 1a) was measured. DNA-Me differences ($\Delta\beta$) between affected (individuals with asthma, atopy, or both) and non-affected individuals were assessed and DNA-Me was correlated with gene expression for each CpG. DNA-Me was measured on DNA extracted from blood (Blood and Cell Culture DNA Midi Kit, Qiagen, Canada) using bis-pyrosequencing (EpiTech Bisulfite Kits, Pyromark PCR Kit, Pyromark Gold Q24 Reagents, Qiagen, Canada). PCR primers were designed using PyroMark Assay Design software (v2.0.1.15) (Qiagen,Canada). Total RNA was extracted from whole blood (RNeasy Plus Mini Kit, Qiagen, Canada) using a subset of affected and non-affected individuals ($n = 30$). For each sample, RNA was converted into cDNA (qScript™ cDNA SuperMix, Quanta Biosciences, USA), and mRNA quantification was determined (PerfeCTa® qPCR FastMix®, Quanta Biosciences, USA) using the two standard curves method with *RPLP0* as a reference gene [16].

The association between *IL1R1* and *IL1R2* DNA-Me levels and asthma and/or atopy at each CpG was

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Table 1 Clinical characteristics of individuals from the Saguenay–Lac-Saint-Jean asthma familial collection

Characteristics	All individuals ($n = 93$)	Controls ($n = 25$)	Asthmatics[a] and/or atopics[b] ($n = 68$)
Sex ratio (M:F)	1:1.2	1:1.8	1:1
Mean age, year (range)	15 (3–46)	14 (3–44)	15 (4–46)
<16 years old, *n* (%)	64 (69)	18 (72)	46 (68)
FEV_1, % predicted (SD)[c]	62 (40)	63 (40)	62 (40)
PC_{20}, mg/ml (SD)[d]	8.2 (4.3)	15.3 (3.4)	6.8 (4.4)
Serum IgE, μg/l (SD)[e]	109 (5)	36 (4)	157 (4)
Asthma, *n* (%)[a]	47 (51)	NA	47 (69)
Atopy, *n* (%)[b]	47 (51)	NA	47 (69)
With asthma, *n* (%)[a]	26 (28)	NA	26 (38)

[a]Present asthma or past documented clinical history of asthma. Data available for all individuals
[b]Defined as having at least one positive response on the skin prick test (wheal diameter ≥3 mm at 10 min). Data available for all individuals
[c]FEV_1 = mean and standard deviation (SD) calculated for forced expiratory volume in 1 s for 67 individuals (16 controls, 51 asthmatic and/or atopic individuals)
[d]PC_{20} = geometric mean and SD of provocative methacholine concentration inducing 20 % decline in FEV_1 calculated for 58 individuals (14 controls, 44 asthmatic and/or atopic individuals)
[e]IgE = geometric mean and SD of serum immunoglobulin (Ig) E level concentration calculated for 80 individuals (20 controls, 60 asthmatic and/or atopic individuals)

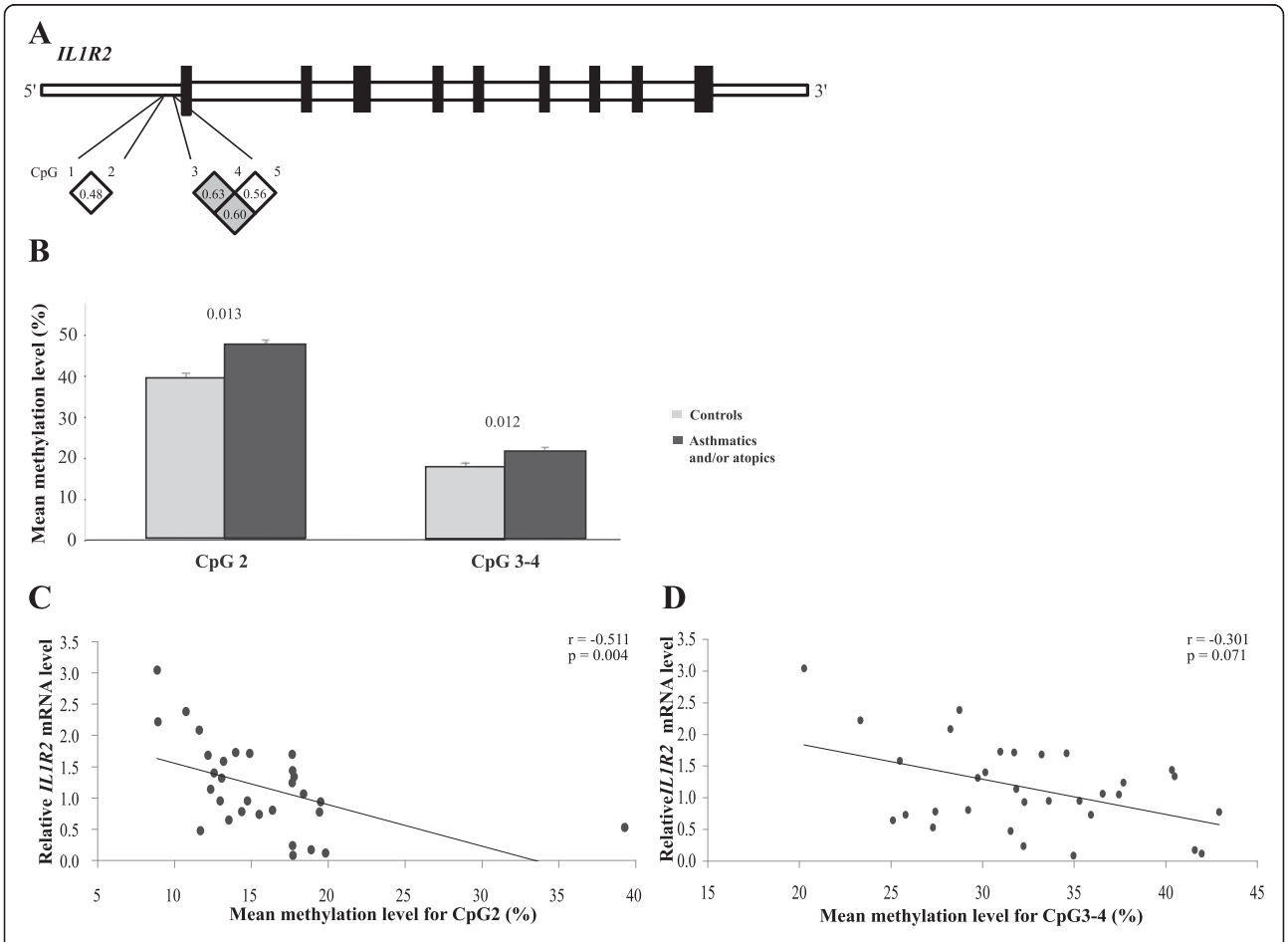

Fig. 1 Association between CpGs' DNA methylation levels for *IL1R2* and gene expression in asthma and atopy. **a** Schematic representation of *IL1R2*, location of epigenotyped CpG sites, and pairwise correlations between CpG sites. **b** Mean DNA-Me levels for CpG2 and CpG3-4 of *IL1R2* in control and affected subjects (individuals with asthma, atopy, or both). **c** Correlation between DNA-Me level of *IL1R2*-CpG2 and mRNA level. **d** Correlation between mean DNA-Me level of *IL1R2*-CpG3 and 4 and mRNA level

Table 2 Summary of DNA methylation analysis on promoter of two interleukin 1 receptors in whole blood samples from Saguenay–Lac-Saint-Jean asthma familial collection

Gene	CpG	$\Delta\beta^a$	*p* value
IL1R1 promoter and exon 1	1	1.19	0.113
	2–4	0.22	0.892
IL1R2 promoter	1	−0.59	0.569
	2	8.02	*0.013*
	3–4	3.72	*0.012*
	5	−0.50	0.564

Significant *p* values are shown in italics
[a]$\Delta\beta$ are calculated with mean methylation ratio for asthmatic and/or atopic individuals on control individuals

analyzed by logistic regression considering age and sex as covariates [9]. Gene expression analysis by phenotype was not performed as control group sample size was insufficient ($n = 4$). The association between DNA-Me and mRNA levels was assessed by Spearman correlation. CpG dinucleotides with $r > 0.6$ were combined before they were tested for associations with asthma and/or atopy and for correlation with gene expressions. $\Delta\beta$ with p value < 0.05 was considered statistically significant. Statistical analyses were conducted using the statistical software SPSS (v11.5.0, IBM, USA).

Results

In this study, we detected higher levels of DNA-Me at *IL1R2* among affected individuals (i.e., with asthma, atopy, or both) as compared to non-affected controls ($\Delta\beta = 8.02$ %, p value = 0.013, and $\Delta\beta = 3.72$ %, p value = 0.012 for *IL1R2*-CpG2 and the mean for CpG3 and CpG4, respectively (Table 2, Fig. 1b)). Atopic and non-atopic asthma were associated with DNA-Me at *IL1R2* but not atopy alone (data not shown). We also observed that DNA-Me at *IL1R2*-CpG2 was negatively correlated with its mRNA levels ($r = -0.511$, p value = 0.004) (Fig. 1c), but it was not correlated for CpG3 and CpG4 (Fig. 1d).

Discussion

An epigenetic signature has also been identified for *IL1R2* promoter in systemic lupus erythematosus (SLE) [15]. The risk of allergic disorders was significantly increased in SLE patients, which suggests that these conditions share some common biomarkers [17]. The negative correlation we observed between DNA-Me and gene expression levels for *IL1R2* may be due to stoichiometry. Methylation may limit access of a transcription factor to DNA and hinders transcriptions [18]. We identified potential binding sites for transcription factors relevant to asthma near the CpG dinucleotide sites of *IL1R2* analyzed (Additional file 2: Figure S2) which could explain the inverse correlation between methylation and gene expression [19]. Noteworthy is the potential binding site for nuclear factor kappa B/c-rel (NFKB) at the *IL1R2* promoter; it is involved in inflammation through several pathways, including IL1 signalization [20]. Given that IL1R2 acts as a decoy receptor to antagonize the bound ligand [21], our data prompted the speculation that hypermethylation of *IL1R2* in asthma and atopy negatively regulates *IL1R2* expression and less decoy receptors are available to reduce the downstream pro-inflammatory response of IL1 in the presence of unchanged IL1R1 level [22, 23]. Unlike IL1R1, IL1R2 does not have an intracellular domain and the formation of IL1-IL1R2 complex inactivates the IL1 downstream signaling cascade; hence, silences the role of IL1 in inflammation. Functional study will be needed to investigate the impact of observed epi-variations on the production of expressed receptors. This hypothesis could be attributed to both asthma and atopy as IL1R2 non-signaling receptor is suspected to influence Th2 imbalance [24], and both disorders are driven by Th2 allergic lung inflammation [25, 26].

To our knowledge, this is the first report of (1) a hypermethylation signature of *IL1R2* promoter in asthma with or without atopy and (2) an inverse correlation between methylation at *IL1R2* promoter and its gene expression. Together, they underline the relevance of IL1R2 as a potential biomarker of asthma and atopy. Further work is needed to understand the interactions between environmental exposures and epigenetic modifications like the ones identified in this study. Such understanding will aid the discovery of disease mechanisms associated and development of more effective therapies.

Additional files

Additional file 1: Figure S1. Schematic representation of *IL1R1* and location of epigenotyped CpG sites. This figure illustrates a simplified schematic representation of *IL1R1* and location of selected CpG dinucleotide sites and pairwise correlations between each CpG.

Additional file 2: Figure S2. Potential binding sites for transcription factors in IL1R1 and IL1R2. This figure shows a segment of the primary sequences of *IL1R1* and *IL1R2*. Both sequences lie in the respective gene promoter (UCSC Genome Browser assembly GRCh38/hg38). Epigenotyped CpG sites are shown in blue and are numbered from the distal part of the promoter. Exons are shown in red. Potential binding sites for transcription factors in differentially methylated loci are shown by underlined sequences, and the name of the transcription factors are indicated underneath.

Abbreviations

CpG: cytosine-phosphate-guanine; DNA-Me: DNA methylation; IL1: interleukin 1; IL1R1: interleukin 1 receptor type 1; IL1R2: interleukin 1 receptor type 2; RPLP0: ribosomal protein, large, P0; SLE: systemic lupus erythematosus; SLSJ: Saguenay–Lac-Saint-Jean; $\Delta\beta$: difference of methylation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions
VGO carried out the pyrosequencing and gene expression studies, performed the statistical analysis, and drafted the manuscript. SPG carried out the pyrosequencing primers design. AMBL participated in the pyrosequencing process. LB participated in the study design and revised the manuscript. CL led each step of the current study including the asthma familial collection build and management, study design, laboratory works, statistical analysis, as well as manuscript redaction and revision. All authors read and approved the final manuscript.

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