Effects of rs3744262, rs3744263 and DNA Methylation on Symptoms in Participants with Allergic Rhinitis During Grass Pollen Exposure in the Environmental Exposure Unit (EEU)

Michelle L. North[1,2], Sarah Mah[3], Andrew G. Day[4], Michael S. Kobor[3,5], and Anne K. Ellis[1,2]

1) Department of Biomedical & Molecular Sciences and Division of Allergy & Immunology, Department of Medicine, Queen's University, Kingston, Ontario, Canada. 2) Allergy Research Unit, Kingston General Hospital. 3) Child & Family Research Institute and Centre for Molecular Medicine & Therapeutics, Vancouver, British Columbia, Canada. 4) Clinical Research Centre, Kingston General Hospital 5) Department of Medical Genetics, University of British Columbia.

Abstract

Rationale: Genotype can affect the epigenetic code, as SNPs can alter the existence of certain CpG sites. Surrounding CpG sites may also be affected, but genetic-epigenetic interactions are not well understood. rs3744262 and rs3744263 are CpG-SNPs in the ephrin-B3 gene. Ephrin-B3 is a transmembrane ligand for receptor tyrosine kinases, involved in bidirectional signaling. Ephrin-B3 is crucial in epithelia and enhances the T cell response, thus it may be important in allergic rhinitis. However, the effects of these CpG-SNPs on nearby methylation sites and effects on symptoms during allergen challenge in an Environmental Exposure Unit (EEU) have not been examined.
Methods: 38 participants with allergic rhinitis, and 8 non-allergic controls, were exposed to grass pollen for 3 hours on two consecutive days. All participants recorded their total systemic symptom scores (TSSS), total nasal symptom scores (TNSS) and peak nasal inspiratory flow (PNIF) at baseline and 30 minute intervals. DNA was isolated from blood drawn at baseline, 3H and 27H. All participants were genotyped at rs3744262, rs3744263 and pyrosequencing was performed on the two CpG-SNPs plus three surrounding DNA methylation sites. 2 way ANOVA and Spearman correlations were used to examine genetic and epigenetic data, respectively.
Results: rs3744262 and rs3744263 were associated with DNA methylation at those sites (p<0.0001). Participants with the A->G genotype at rs3744262 exhibited lower TSSS after 3H of grass pollen exposure (p<0.05). Participants with the C->T genotype at rs3744263 exhibited lower TSSS at 3H and 27H (p<0.01 and p<0.05, respectively). Increased methylation at position 1 was associated with lower TNSS and TSSS at baseline (p<0.05 and p<0.01, respectively). Increased methylation at position 2 was associated with lower PNIF at 27H (p<0.05). Genotype significantly affected DNA methylation at position 4 only (p<0.05).
Conclusion: While DNA methylation and SNPs in EFNB3 both affect allergic rhinitis symptoms, the effects appear to occur at different time points, baseline and post pollen-exposure, respectively. DNA methylation was the only factor found to be associated with nasal airflow post-exposure. Effects of genotype on DNA methylation were noted, but were found to occur at the same site as the SNP, and the most proximal next site, and were not associated with effects on symptoms.

Background

• Allergic rhinitis is a symptomatic allergic nasal disorder involving upper-airway inflammation, rhinorrhea, sneezing, congestion, and aggravation of comorbid asthma. Allergic rhinitis affects 10% - 25% of the population worldwide [1].
• While some genetic associations have been established, genomic DNA sequence cannot fully explain why some individuals develop allergic disease and others do not, pointing to gene-environment interactions [2,3].
• The emergent field of epigenetics has begun to play a key role in explaining gene-environment interactions. Epigenetics refers to genomic modifications, such as DNA methylation, that often lead to changes in gene expression, but do not involve changes to the genetic code [4]. DNA methylation occurs when DNA methyltransferase adds a methyl group to the cytosine residue of a cytosine-guanine (CpG) dinucleotide (**Figure 1A**). The effect of DNA methylation is generally transcriptional repression [5], and methylation often works in concert with other epigenetic mechanisms such as histone modifications (**Figure 1B**).

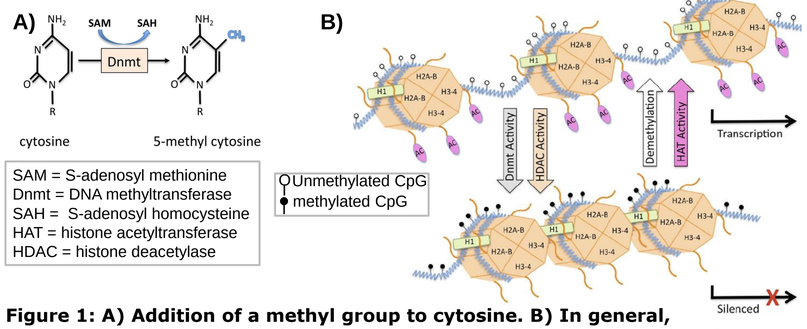

Figure 1: A) Addition of a methyl group to cytosine. B) In general, unmethylated DNA and acetylated histones promote loosening of the DNA strand, allowing transcription to take place.

Background

• Ephrin-B3 is a transmembrane ligand for receptor tyrosine kinases that is crucial in epithelia and enhances the T cell response.
• Genotype alters the epigenetic code, as SNPs affect the existence of CpG sites.
• rs3744262 and rs3744263 are CpG-SNPs in the ephrin-B3 gene. They are part of 5 possible DNA methylation sites in the DNA region (**Figure 2**), and they lie in the 3-UTR of ephrin-B3 mRNA.

Figure 2: Location of SNP and CpG sites in theephrin-B3 gene.

Objective: To characterize the effects of genotype and epigenetics in the EFNB3 gene on allergic rhinitis symptoms and nasal air flow upon allergen exposure.

Methods

• All study procedures were approved by the Queen's University and Affiliated Teaching Hospitals Health Sciences Human Research Ethics Board. Written informed consent was obtained prior to the performance of any study-specific procedures.
• 38 participants with a positive skin prick test to rye grass and symptoms during the preceding two grass seasons, and 8 non-allergic skin test negative controls were exposed concurrently to 2500-3500 grains/m^3 rye grass pollen for 3 hours on two consecutive days in the Environmental Exposure Unit (EEU), a specific, and reproducible methodology for allergen challenge [6] (**Figure 3**).
• All participants recorded their symptoms on a 0-3 scale at baseline and 30 minute intervals. Nasal symptoms included congestion, sneezing, nasal itch, and rhinorrhea, which were added to give the total nasal symptom score (TNSS). Non-nasal symptoms included teary eyes, itchy eyes and itchy throat/palate, which were added together with the nasal symptoms to give the total systemic symptom score (TSSS).
• Participants also measured their peak nasal inspiratory flow (PNIF), an objective indicator of nasal congestion
• DNA was isolated from lymphocyte-enriched blood samples at baseline, 3H and 27H.

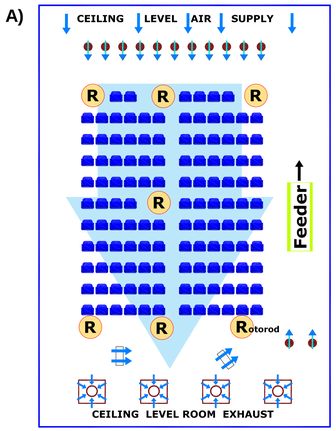

Figure 3: A) Diagram, and B) photograph of the Environmental Exposure Unit (EEU), showing the placement of the pollen distribution system, directional fans, Rotorod® pollen samplers, ventilation and participant seating.

Results

• As expected, SNPs were associated with DNA methylation at those sites (p<0.0001).
• Participants with the A->G genotype at rs3744262 exhibited lower TSSS after 3H of grass pollen exposure (p<0.05). Participants with the C->T genotype at rs3744263 exhibited lower TSSS at 3H and 27H (p<0.01 and p<0.05, respectively) (**Figure 4**).

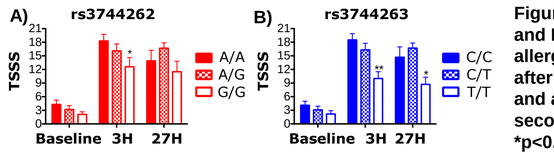

Figure 4: A) Effect of rs3744262 and B) rs3744263 on TSSS in allergic participants at baseline, after 3H of grass pollen exposure and after 3H of exposure on the second consecutive day (27H). *p<0.05, **p<0.01 to A/A or C/C.

• Increased DNA methylation at position 1 was associated with lower TNSS and TSSS at baseline (p<0.05 and p<0.01, respectively) (**Figure 5A and B**).
• Increased methylation at position 2 was associated with PNIF at 27H (**Figure 5C**).

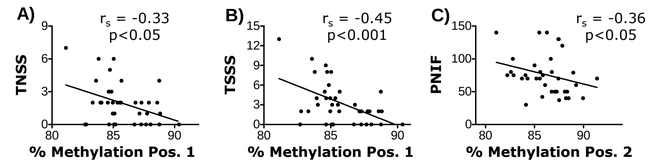

Figure 5: Spearman correlations of DNA methylation at position 1 vs. A) TNSS and B) TSSS. C) Spearman correlation of DNA methylation at position 2 vs. PNIF.

• Genotype significantly affected DNA methylation at position 4 only, while positions 1 and 2 were not affected (**Figure 6**).

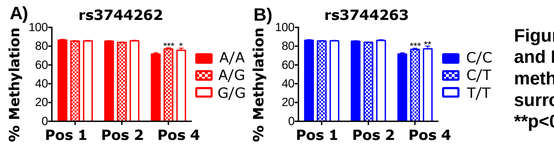

Figure 6: A) Effect of rs3744262 and B) rs3744263 on DNA methylation at baseline at surrounding CpG sites. *p<0.05, **p<0.01, ***p<0.001 to A/A or C/C.

Conclusions

In this study we found that both DNA methylation and SNPs in EFNB3 affect allergic rhinitis symptoms. However, the effects appear to occur at different time points, baseline and post pollen-exposure, respectively. DNA methylation was also associated with nasal airflow after allergen exposure. Effects of genotype on DNA methylation were noted, but did not occur at CpG sites that were associated with symptoms, suggesting that in the case of this gene, genotype and epigenetics exert independent effects.

References

[1] Bousquet *et al*. J Allergy Clin Immunol 2001; 108:S147-334.[2] Los *et al*. Twin Res 2001; 4:81-93.
[3] Miller *et al*. Am J Respir Crit Care Med 2008; 177(6):567-73.[4] Martino *et al*. Allergy 2010; 65(1):7-15.
[5] Jaenisch *et al*. Nat Genet 2003; 33 Suppl:245-54. [6] Ellis *et al*. Ann Aller Asth Immun 2013;111(5):323-8.

This study was funded via the KGH Allergy Research Trust and the Department of Medicine, Queen's University