DNA Methylation of Immune Genes is Associated with Chronic Pain in Children

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INTRODUCTION

- Chronic pain is frequently comorbid with physiological health symptoms such as problems with immune function, inflammation, stress, and sleep.[1] Chronic pain is also associated with psychiatric symptoms such as depression and anxiety.[2]
- Chronic stress and illness can lead to a heightened immune response for a long period of time.[3]
- Cytokines that are released during the immune response cause inflammation and activate pain pathways. Chronic inflammation is a risk factor for most chronic and degenerative diseases, as it has a deleterious effect on the body.[4]
- Epigenetics are heavily influenced by psychosocial and environmental factors, such as stress during crucial developmental periods in children.[5]
- Stress, either physical or mental, triggers epigenetic changes like DNA methylation, and has been shown to be strongly associated with chronic inflammation.[6]
- Few studies have investigated how potential epigenetic processes may be involved in childhood chronic pain; more specifically in how methylation of immune genes may be of influence.

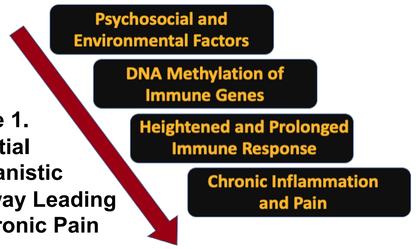


Figure 1. Potential Mechanistic Pathway Leading to Chronic Pain

AIM

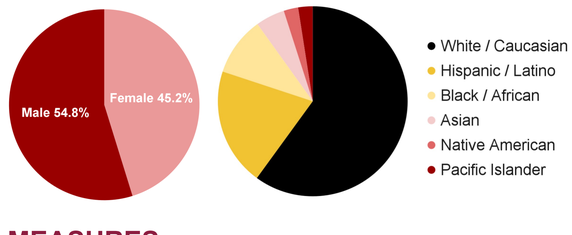
We are investigating if chronic pain in children is associated with epigenetic modifications of three key inflammatory immune genes, *TNF*, *IL-6*, and *CRP*. We hypothesize that variance in DNA methylation of these immune genes is associated with levels of chronic pain in children.

METHODS

SAMPLE

Participants consisted of 31 pairs of twin children (n = 62) recruited from the longitudinal study, The Arizona Twin Project. We compared measures from ages 8 (M_{age}= 8.46, *SD* = 0.50) and 11 (M_{age}= 11.53, *SD* = 0.53).

Figure 2. Gender and Racial Distributions



MEASURES

At age 8 buccal cell samples were collected from the children via mailed collection kits. DNA methylation values, or beta values, were then acquired from our candidate genes, *TNF*, *IL-6*, and *CRP,* using the Infinium MethylationEPIC BeadChip run on an Illumina iScanSystem.

Yearly longitudinal reports of pain were collected until age 11. Twins self-reported the total number of body sites with 1) constant or recurring pain, 2) not caused by an injury or illness, and 3) have lasted more than one month.

STATISTICAL ANALYSIS

Percent methylation was calculated for each CpG site of the candidate genes. We then used principal components analysis (PCA) to reduce data dimensionality and number of statistical tests, which had 18-52 CpG sites per gene. The first principal component, PC1, was then extracted and used for the rest of the analyses. The PC1 values represent the maximum explained variance in beta values across all of the CpG sites for the gene.

Mixed model multiple linear regression was used to control for twin pairs (IV: PC1, DV: the sum of total pain sites). Age, sex, and cell count were controlled for during these analyses. We used multivariate regression for individual CpG sites, only using one twin from each pair, with the same DV and covariates.

RESULTS

Table 1. Relationships Between Variance of Immune Genes DNA Methylation and Chronic Pain in Children

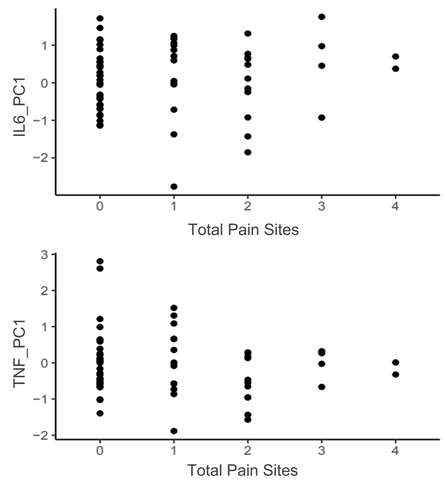
	CRP			TNFa			IL6		
Age	B (estimate)	df	p	B (estimate)	df	p	B (estimate)	df	p
8	-0.051	72.731	0.709	-0.256	71.516	0.056	-0.277	52.376	0.069
11	0.097	53.304	0.464	-0.279	54.091	**0.05***	-0.299	35.319	**0.033***

Our results indicate that DNA methylation of two immune genes, *TNF* (B = -0.279, p = 0.05) and *IL-6* (B = -0.299, p = 0.033), at age 8, significantly predict chronic pain levels of our sample at age 11, three years later. *CRP* did not significantly predict any pain levels at either age.

Table 2. Significant CpG Sites for DNA Methylation of Immune Genes at Age 11

CpG Site	B (estimate)	p
IL6cg02554338	26.408	0.0002
TNFacg08553327	-11.353	0.0152
IL6cg03310594	13.030	0.0199
CRPcg01365152	23.487	0.0250
TNFacg10650821	-14.686	0.0363

Figure 3. Scatter Plots Depicting Levels of Chronic Pain and Variance of Immune Genes



Total Pain Sites is the number of pain sites reported, lasting longer than a month, not caused by injury or illness at age 11. *IL6_PC1* and *TNF_PC1* are the PC1 Values for the corresponding genes.

CONCLUSIONS

- This study identified DNA methylation of two key immune genes, *TNF* and *IL-6*, as factors associated with chronic pain in children. Variance in DNA methylation was associated with levels of chronic pain in our sample.
- Epigenetic regulation of immune genes potentially influences chronic pain in children.
- Future research:
  - Obtain a larger sample size to further assess this mechanistic pathway.
  - Investigate specific psychosocial and environmental factors that may affect DNA methylation of immune genes.
  - Explore factors that downregulate the immune response as a potential therapeutic intervention.
  - Obtain longitudinal epigenetic data to assess modifications over time.

ACKNOWLEDGEMENTS

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