Psychoneuroendocrinology 95 (2018) 170–178

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Macrophage migration inhibitory factor (MIF) gene is associated with adolescents' cortisol reactivity and anxiety

Rebecca Lipschutz[a], Johanna Bick[a,*], Victoria Nguyen[b], Maria Lee[b], Lin Leng[c], Elena Grigorenko[a,d], Richard Bucala[c], Linda C. Mayes[b], Michael J. Crowley[b]

[a] Department of Psychology, University of Houston, Houston, TX, United States
[b] Child Study Center, Yale School of Medicine, New Haven, CT, United States
[c] Department of Internal Medicine, Rheumatology, Yale School of Medicine, New Haven, CT, United States
[d] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States

ARTICLE INFO

Keywords:
Stress reactivity
HPA-axis
Cortisol
Adolescents
Stress response
MIF
Cytokine
Anxiety

ABSTRACT

Emerging evidence points to interactions between inflammatory markers and stress reactivity in predicting mental health risk, but underlying mechanisms are not well understood. Macrophage Migration Inhibitory Factor (MIF) is a pleiotropic cytokine involved in inflammatory signaling and Hypothalamus Pituitary Adrenal (HPA) axis stress-response, and has recently been identified as a candidate biomarker for depression and anxiety risk. We examined polymorphic variations of the *MIF* gene in association with baseline MIF levels, HPA axis reactivity, and self-reported anxiety responses to a social stressor in 74 adolescents, ages 10–14 years. Genotyping was performed for two polymorphisms, the -794 CATT5-8 tetranucleotide repeat and the -173*G/C single nucleotide polymorphism (SNP). Youth carrying the *MIF*-173*C and CATT7 alleles displayed attenuated cortisol reactivity when compared with non-carriers. Children with the CATT7-173*C haplotype displayed lower cortisol reactivity to the stressor compared to those without this haplotype. Additionally, the CATT5-173*C and CATT6-173*C haplotypes were associated with lower self-reported anxiety ratings across the stressor. Results extend prior work pointing to the influence of *MIF* signaling on neuroendocrine response to stress and suggest a potential pathophysiological pathway underlying risk for stress-related physical and mental health disorders. To our knowledge, these are the first data showing associations between the *MIF* gene, HPA axis reactivity, and anxiety symptoms during adolescence.

1. Introduction

Adolescence is marked by drastic shifts in neuroendocrine activity and stress physiology which may confer risk for affective disorders (Gunnar et al., 2009; Romeo, 2013; Stroud et al., 2009). The Hypothalamus Pituitary Adrenal (HPA) axis, part of the neuroendocrine system, regulates stress response during acute or chronic stress. Identifying factors that contribute to its dysregulation during adolescence may have important clinical implications. Macrophage Migration Inhibitory Factor (MIF) is a well-established inflammatory marker and regulator of the HPA axis (Dunn, 2000; Turnbull and Rivier, 1995). Emerging evidence in adults implicates MIF in stress-related psychiatric disorders and HPA axis dysregulation. Little is known on MIF's role in stress variability in adolescence, when affective disorders often first emerge. We explore connections between polymorphic variability in the *MIF* gene, HPA axis reactivity and anxiety reports in a normative sample of adolescents.

MIF is one of the first discovered cytokines, originally named for its ability to recruit macrophages to sites of inflammation (Bloom and Bennett, 1966; David, 1966), however its effects are now known to be pleiotropic. MIF is widely expressed throughout the body. High expression is found in the endocrine system, especially the HPA axis (Calandra and Roger, 2003; Fingerle-Rowson et al., 2003; Matsunaga et al., 1999) and throughout the brain, including limbic regions implicated in stress, depression, and anxiety (Bloom and Al-Abed, 2014; Conboy et al., 2011). Among its immune-related functions, MIF acts as a regulator of innate immune- and inflammatory-signaling (Bernhagen et al., 2007; Calandra and Roger, 2003; Mitchell et al., 1999; Savaskan et al., 2012), induces pro-inflammatory cytokines (Calandra and Roger, 2003; Calandra et al., 1994), and counteracts the effects of glucocorticoid signaling (Baugh et al., 2002; Fingerle-Rowson et al., 2003; Flaster et al., 2007).

MIF is also intricately associated with the HPA axis (Bacher et al., 1997; Tampanaru-Sarmesiu et al., 1997; Baugh and Donnelly, 2003) a

* Corresponding author at: University of Houston, Department of Psychology, 4849 Calhoun Road, room 482, Houston, TX, 77024, United States.
E-mail address: jrbick@uh.edu (J. Bick).

https://doi.org/10.1016/j.psyneuen.2018.05.033
Received 1 April 2018; Received in revised form 23 May 2018; Accepted 24 May 2018

key neuroendocrinological stress response system. MIF is released by the same cells in the pituitary as those that release adrenocorticotropin hormone (ACTH) (Bernhagen et al., 1995; Nishino et al., 1995). Plasma MIF levels follow similar circadian rhythms as plasma cortisol, the end product of the HPA axis (Petrovsky et al., 2003). Interaction between MIF and the HPA axis are well-established, with most work involving in-vitro experiments or pathological conditions involving inflammatory diseases (Calandra and Roger, 2003; Nishino et al., 1995; Tierney et al., 2005). Fewer studies have investigated associations under normative, non-pathological conditions in humans. However, a recent study showed that elevated MIF levels were related to decreased HPA axis response during an acute stressor in healthy adults (Edwards et al., 2010), pointing to a stress-related neuroendocrine and immune interaction involving MIF and the HPA axis.

MIF has recently been investigated as a biomarker of depression, anxiety, and psychological stress. In animal work, *Mif*-knock-out mice showed stress-related increases in anxiety- and depression-like behavior and impairments in hippocampal dependent memory (Conboy et al., 2011). MIF has also been shown to have a role in rodents' increased neurogenesis during fluoxetine treatment, a selective serotonin reuptake inhibitory (SSRI) commonly administered as treatment for depression (Conboy et al., 2011) and was recently found to mediate the anti-depressant effects of voluntary exercise (Moon et al., 2012). Together, these data point to a potential protective effect of MIF in the development of anxiety or stress-related disorders.

In humans, higher baseline serum levels of MIF have been observed in young adults who reported mild to moderate depression symptoms (Hawkley et al., 2006; Edwards et al., 2010), although other studies have not replicated this association (Katsuura et al., 2011). Higher serum MIF levels have also been registered in pregnant women with symptoms of depression after an immune challenge, when compared with pregnant women with no depression (Christian et al., 2010). In clinical settings, depressed patients showed higher baseline MIF levels prior to starting a pharmacological treatment, yet MIF levels did not change over the course of treatment (Musil et al., 2011). Clinically depressed patients who responded to pharmacological treatment have also shown higher MIF mRNA prior to treatment onset, compared to non-responders. MIF declined over the treatment, yet the magnitude of decline was not associated with treatment response (Cattaneo et al., 2013). Recently, circulating MIF was examined in adults with anxiety, depression, stress, and/or adjustment disorders who participated in a randomized controlled trial of mindfulness, a psychological treatment for mental health problems. MIF levels were shown to significantly decrease in the treatment group, when compared to controls, yet the reduction was not associated with symptom improvement (Wang et al., 2018).

In summary, a growing body of evidence implicates MIF as a potential modulator of HPA axis functioning and as a biomarker for anxiety and depression symptoms in healthy adults. However, we are aware of no studies that have investigated such links in earlier developmental periods, such as adolescence, when affective disorders are likely to emerge. Also, no studies have examined whether genetic factors that regulate MIF expression, such as functional polymorphisms of the *MIF* gene, explain inter-individual differences in HPA axis function and behavioral signs of stress in normative adolescents.

To further this line of work, we examined associations between genetic variability in the *MIF* gene, peripheral levels of MIF, HPA axis and self-reported anxiety responses to stress, in a sample of healthy adolescents. *MIF* polymorphisms of interest included the -794CATT5-8 tetranucleotide repeats and the -173*G/C single nucleotide polymorphism (SNP), and haplotypes known to be related to MIF expression (Baugh et al., 2002; Radstake et al., 2005). We expected that adolescents who carried the C allele or higher number of CATT repeats (i.e. CATT 7 or 8) of the *MIF* gene would show reductions in HPA axis reactivity and that this would be associated with lower reports of subjective anxiety. As an exploratory step, we quantified peripheral MIF levels from saliva samples collected prior to the stressor. We expected that individuals with higher expressing alleles would show higher peripheral MIF, and that MIF levels might predict HPA axis reactivity during the stressors.

2. Material and methods

2.1. Participants

The original sample of participants included 104 children (54 female), recruited from a mass mailing list provided from Experian, a credit card company. Families living in New Haven, CT, USA and surrounding towns were targeted for recruitment. Children were considered ineligible for the study if their caregiver, typically their mother, reported that the child in question was being treated for, or carried a diagnosis of psychosis, autism, or bipolar disorder.

The final sample consisted of 74 youth (39 female) from the larger study who provided salivary samples for DNA analyses. Youth in this study ranged from 10 to 17 years of age (M = 13.93, SD = 2.33). In this sample, 64.9% reported their racial/ethnic background as Caucasian (n = 48), 9.5% African American (n = 7), 13.5% Hispanic/Latino (n = 10), 8.1% Asian (n = 6), and 4.1% "Other" (n = 3). In terms of family income, 51.5% (n = 38) reported an annual income of greater than $75,000 and 75.7% ($n$ = 56) of the sample had a parent with a college degree. Most children were living with their biological mothers, 97.3% (n = 72). At the time of the study, 79.8% (n = 59) mothers reported their marital status as "married or in a committed relationship," and 10.8% (n = 8) reported their status is "separated or divorced." Sample characteristics are displayed in Table 1.

2.2. Procedure

Families were initially recruited over the phone. If interested, families were scheduled for a series of laboratory visits. At the first visit, IRB-approved parental permission and child assent were obtained as well as parent report and self-report instruments.

In a second visit, approximately 1–2 weeks later, youth participated in a laboratory assessment that lasted approximately 1 h. Youth were asked to refrain from alcohol or drug use on the day of the laboratory visit, to avoid potential influences on HPA axis or stress reactivity. On the day of the assessment, youth were tested for alcohol and drug intake with a breathalyzer, urine test and self-reported medication use. No participants had evidence of alcohol, and one tested positive for

Table 1
Descriptive Statistics.

Variable	M (SD) or n (%), Total Sample (N = 74)
Gender	
Female	39 (52.7)
Child Age	13.93 (2.3)
Maternal Age	45.90 (6.2)
Maternal Education	3.53 (1.5)
Ethnic/Racial Background	
African American (not of Hispanic origin)	7 (13.5)
Asian	6 (8.1)
Hispanic or Latino	10 (13.5)
White (not of Hispanic origin)	48 (64.9)
Other	3 (4.1)
MIF (ng/ml)	0.62 (1.6)
Baseline cortisol (ug/nl)	0.36 (.1)
Baseline anxiety	1.19 (.8)
Puberty status	2.50 (.6)

Note. Maternal education (1–5, with 1 being HS degree and 5 being graduate degree). Gender and Ethnic/Racial Backgrounds reported as frequencies, all other variables reported as means and standard deviations.