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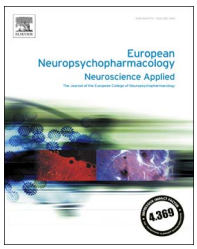

Serotonin transporter gene promoter methylation in peripheral cells in healthy adults: Neural correlates and tissue specificity

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Abstract
Early adversity can influence gene expression via epigenetic mechanisms, including DNA methylation. Peripheral tissues are essential in psychiatric epigenetics, as methylation generally cannot be assessed in the living human brain. Several magnetic resonance imaging (MRI) studies show associations of peripheral serotonin transporter gene (SLC6A4) methylation with function and/or structure of frontal-limbic circuits and brain's resting-state. Commonly used samples are derived from blood, saliva or buccal cells. However, little is known regarding which peripheral tissue is most strongly associated with human brain processes. The aim of the current study was to compare the extent of the association between peripheral SLC6A4 promoter methylation and frontal-limbic function, structure and resting-state in healthy individuals across peripheral tissues. Forty healthy prospectively-followed adults underwent anatomical, resting-state and functional MRI. Saliva-, blood- and buccal-derived DNA methylation was assessed by pyrosequencing. Blood-derived SLC6A4 methylation was positively associated with superior frontal gray matter (GM) volume and with right lateral parietal area (RLP)-frontal pole regional resting-state functional connectivity (rsFC). Saliva-derived SLC6A4

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methylation was positively associated with superior frontal GM volume. Buccal-derived SLC6A4 methylation was positively associated with superior and inferior frontal and anterior cingulate cortical (ACC) GM volumes, and with RLP-ACC, frontal pole and medial prefrontal regional rsFC. Current results confirmed the relevance of peripheral methylation for frontal-limbic processes in humans. Buccal cells may be the most sensitive cell type when studying SLC6A4 promoter methylation and its associated risk for neural vulnerability and resilience for psychopathologies in which serotonin is implicated. These data should be further validated in clinical populations.

1. Introduction

Early adverse environment has been associated with an increased risk for various mental health disorders later in life (Kessler et al., 2010). Albeit the exact mechanism underpinning this link is still unclear, a widely accepted hypothesis is that early adversity disrupts developmental trajectories and outcomes by interfering with individual neurobiological framework, a process known as biological embedding (Nelson, 2013). Various models, including the biopsychosocial pathway model, diathesis-stress model and epigenetic memory hypothesis advocate DNA methylation in genes essential for regulating neuronal function as a pivotal molecular mechanism underlying this process (Booij et al., 2015a; Levesque et al., 2016; Lutz et al., 2015). Among the genes associated with early-life adversity, brain development and mental health, those pertaining to the serotonin (5-hydroxytryptomaine; 5-HT) system appear to be particularly compelling since 5-HT system (dys)functioning has been associated with the pathogenesis of different psychiatric disorders as well as with brain development (Booij et al., 2015a).

Among the different serotonergic genes, perhaps the most studied one is the serotonin transporter gene (SLC6A4). Following the findings of the role of SLC6A4 genetic variation in interaction with stressful life events in predicting risk for depression (Caspi et al., 2003, 2010), a number of studies have examined the role of DNA methylation in SLC6A4 gene and mental health. These studies rely on measuring DNA methylation in surrogate tissues, such as whole-blood, buccal cells or saliva, as DNA methylation generally cannot be assessed directly in the human brain. A number of studies have found positive associations or trends between peripheral SLC6A4 promoter methylation and major depressive disorder (MDD) symptoms (Kang et al., 2013; Philibert et al., 2008; van der Knaap et al., 2015; Zhao et al., 2013) or symptom severity (Okada et al., 2014), as well as stress sensitivity (Kang et al., 2013) and childhood abuse (Beach et al., 2010, 2011; Booij et al., 2015b; Kang et al., 2013; van IJzendoorn et al., 2010). In addition, neuroimaging studies conducted in a sample of depressed adult patients and healthy controls showed that higher whole-blood SLC6A4 promoter methylation was associated with smaller hippocampal volume (Booij et al., 2015b) and an increased insula response to emotional stimuli (Frodl et al., 2015). Greater whole-blood SLC6A4 methylation has also been associated with an increased amygdala response to threat-related stimuli in adolescents with depressive symptoms (Swartz et al., 2017) and in adolescents with various mental health states (Nikolova et al., 2014). A positive association between amygdala reactivity in response to threat and peripheral SLC6A4 methylation has also been observed in young adults with various levels of mental health states when using saliva as the surrogate tissue (Nikolova et al., 2014). Furthermore, a brain volumetric study conducted in healthy adults carefully screened for absence of lifetime psychopathology showed that increased SLC6A4 methylation derived from venous blood was associated with greater amygdala, hippocampal and insula volumes (Dannlowski et al., 2014). Results of a resting-state functional connectivity (rsFC) study, that investigated the link between whole-blood SLC6A4 methylation and rsFC in healthy young adults, showed that increased peripheral SLC6A4 promoter methylation was associated with greater rsFC between amygdala and insula, as well as between amygdala and ACC (Muehlhan et al., 2015). These findings together support the relevance of SLC6A4 promoter methylation studies in peripheral tissues for the function and structure of human brain processes that play a role in emotion (dys)regulation, and may indicate a possible future use of peripheral SLC6A4 methylation levels in predicting diagnosis and treatment outcome for individuals with emotion dysregulation problems.

Although it is clear that DNA methylation is tissue-specific (Szyf, 2011), since studies that examined peripheral DNA methylation-brain processes relationships differ in terms of analytical approaches, utilized cell types and participant characteristics, it is presently unclear which peripheral tissue's methylation level correlates best with human frontal-limbic brain processes; i.e. is most sensitive to detect individual differences in frontal-limbic brain function. Therefore, the primary aim of the current study is to examine the association between human frontal-limbic brain processes and peripheral SLC6A4 promoter DNA methylation derived from commonly used surrogate tissues collected from the same healthy adults (blood, saliva, buccal cells). These associations were examined in a longitudinal prospective cohort of individuals followed for 30 years. Based on previous studies (Booij et al., 2015b; Frodl et al., 2015; Muehlhan et al., 2015; Nikolova et al. 2015), we hypothesized that blood-, saliva- and buccal-derived SLC6A4 methylation levels were associated with frontal-limbic volume and neural responses to negative stimuli in frontal-limbic regions (prefrontal cortex [PFC], anterior cingulate cortex [ACC], insula, hippocampus and amygdala), as well as with the resting-state functional connectivity in the Default-Mode Network. We compared the extent of each

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