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Full-length Article

Chronic stress is associated with reduced circulating hematopoietic progenitor cell number: A maternal caregiving model

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ABSTRACT

Background: Chronic psychological stress is a risk factor for cardiovascular disease and mortality. Circulating hematopoietic progenitor cells (CPCs) maintain vascular homeostasis, correlate with preclinical atherosclerosis, and prospectively predict cardiovascular events. We hypothesize that (1) chronic caregiving stress is related to reduced CPC number, and (2) this may be explained in part by negative interactions within the family.

Methods: We investigated levels of stress and CPCs in 68 healthy mothers – 31 of these had children with an autism spectrum disorder (M-ASD) and 37 had neurotypical children (M-NT). Participants provided fasting blood samples, and $CD45^+CD34^+KDR^+$ and $CD45^+CD133^+KDR^+$ CPCs were assayed by flow cytometry. We averaged the blom-transformed scores of both CPCs to create one index. Participants completed the perceived stress scale (PSS), the inventory for depressive symptoms (IDS), and reported on daily interactions with their children and partners, averaged over 7 nights.

Results: M-ASD exhibited lower CPCs than M-NT (Cohen's $d = 0.83$; $p \leqslant 0.01$), controlling for age, BMI, and physical activity. Across the whole sample, positive interactions were related to higher CPCs, and negative interactions to lower CPCs (*allp's* < 0.05). The adverse effects of group on CPCs were significantly mediated through negative interactions with the child (indirect $\beta = -0.24$, $p \leqslant 0.01$). In the full model, greater age ($\beta = -0.19$, $p = 0.04$), BMI ($\beta = -0.18$, $p = 0.04$), and negative interactions with the child ($\beta = -0.33$, $p < 0.01$) were independently associated with lower CPCs. M-ASD had a less healthy lipid profile (total cholesterol/HDL), which in turn, was associated with lower CPCs.

Conclusions: Chronic stress adversely impacts CPC number, an early-stage biomarker that predicts subclinical atherosclerosis and future CVD events, independent of traditional cardiovascular risk factors and inflammatory factors. Among maternal caregivers, child-related interpersonal stress appears to be a key psychological predictor of stress-related CVD risk.

1. Introduction

Psychological stress is associated with a heightened risk of cardiovascular disease (CVD) Yusuf, 2004. This risk may arise not only because markers of damage (e.g., inflammation) are elevated, but also because the body's endogenous mechanisms for repair are impaired. Hematopoietic progenitor cells, which are derived from bone marrow, promote tissue repair and regeneration (Kawamoto, 2001; Kang, 2012). Circulating hematopoietic progenitor cells (CPCs) can be mobilized into circulation and identified by combinations of cell surface markers: $CD45^+CD34^+$ KDR^+ and $CD45^+CD133^+$ KDR^+. CPCs (previously termed endothelial progenitor cells, or EPCs) play a role in vascular repair, vascular aging

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(Thum, 2006), and axonal or white matter protection (Kijima, 2009), placing them at the intersection of neurovascular health.

Caring for an ill family member is one of the best-established human models of chronic stress. Caregiving is associated with higher risk of endothelial dysfunction (Mausbach, 2010), procoagulant and pro-inflammatory activity (Aschbacher, 2006, 2008), metabolic dysregulation (Aschbacher, 2014), and cardiovascular disease (Lee et al., 2003a,b). One reason that caregiving is a potent stressor may be because it encapsulates the experience of having one's closest interpersonal attachments disrupted for years upon end. However, most self-report measures quantify stress as a quality of an *individual*, not of a family system – i.e., as the daily stressful interactions with family members.

The current study's model of chronic stress contrasts mothers of children with an autism spectrum disorder (M-ASD) with demographically similar mothers of healthy, neurotypical children (M-NT). Other studies show M-ASD endorse significantly higher stress levels and poorer mental health than M-NT (Montes and Halterman, 2007). While most parents experience parenting stressors on a daily basis, there are differences in the types and severity of the stressors for children with developmental disorders. Children with autism can engage in unpredictable aggression, self-injury, oppositional behavior, and unresponsiveness. In some cases, autistic children also express less affection, contributing to fewer positive interactions. We assessed maternal reports of daily positive and negative interactions with their children and spouses over the course of a week, to place caregiving stress in the context of daily family life.

CPCs may constitute a valuable early CVD risk marker, a potential mechanism, and a protective factor. A meta-analysis of over 1000 patients at high CVD risk found that $CD34^+KDR^+$ cells were prospectively associated with an increased risk of cardiovascular morbidity and mortality, independent of inflammatory and traditional CVD risk factors (Fadini, 2010). Thousands of studies have investigated CPCs (in fresh blood) and early EPCs (in culture) in relation to disease and regenerative cell-therapy. However, only a few have examined associations with psychological factors (Van Craenenbroeck, 2009; Chen et al., 2011; Dome, 2009; Fischer, 2009). None of these published studies used an *objectively defined exposure* to chronic stress (such as caregiving).

The immunologic definition of CPCs is still evolving and has suffered some confusion in the literature. We, and others, refer to the $CD45^+CD34^+KDR^+$ and $CD45^+CD133^+KDR^+$ cell populations derived from circulating blood as CPCs. We use the term CPCs to distinguish these rare cells found in fresh blood from their counterparts derived from cell culture models. Historically, $CD34^+KDR^+$ and $CD133^+KDR^+$ cells were termed endothelial progenitor cells (EPCs). EPCs were measured by a combination of surface markers (to specify phenotypes) and cell culture models (to investigate function). Subsequently, it was established that blood-derived "EPCs" that emerge early in cell culture ($\leqslant$7 days) are not true endothelial cells and do not form new blood vessels (Hirschi et al., 2008). Hence, these cultures of "early EPCs" are increasingly renamed circulating angiogenic cells (CACs) Aschbacher, 2016; Chen, 2016; Di Santo, 2009. We intentionally use the term CPCs, because cell culture models like CACs contain several different types of immune cells, and their phenotypes are influenced by cell culture media and conditions. Moreover, less than 1% of CACs in culture models express the stem cell markers, $CD34^+$ and $CD133^+$, while the majority express hematopoietic and monocytic markers ($CD45^+$ and $CD14^+$) Heiss, 2010. In sum, we use the term CPCs to refer to $CD45^+CD34^+KDR^+$ and $CD45^+CD133^+KDR^+$ cells.

In animal models, chronic social stress accelerates the development of hematopoietic stem cell pools in the bone marrow. In turn, this leads to an increase in pro-inflammatory monocytes and promotes their infiltration into atherosclerotic lesions (Heidt, 2014). Hematopoietic progenitor cells have the capacity develop into the major types of immune cells (including $CD14^+$ monocytes) dependent on their microenvironment and cytokine milieu (Lachmann, 2015). As an exploratory hypothesis, we investigated whether chronic stress would be associated with alterations in $CD14^+$ monocytes or with CPCs co-expressing $CD14^+$ Secondarily, we also investigated the associations of CPCs with traditional cardiovascular risk factors.

We hypothesized that mothers of children with autism spectrum disorders would have significantly greater levels of psychological distress and fewer $CD45^+CD34^+KDR^+$ and $CD45^+CD133^+KDR^+$ CPCs than mothers of healthy, neurotypical children. Furthermore, we tested whether differences in CPCs could be explained by pinpointing the most central characteristic of maternal caregiver stress – daily negative mother-child interactions. We contrasted these interactions with other sources of psychological distress, such as marital interactions, perceived stress, and depressive symptoms.

2. Methods

2.1. Participants

The current study was conducted as part of a larger study on chronic caregiving stress and cellular aging. Participants were 68 mothers living in the San Francisco Bay area, recruited through local schools, parenting publications, social media, mailings, child development centers, and through the University of California, San Francisco Sensory Neurodevelopment and Autism Program. Eligible mothers were non-smokers between 20 and 50 years of age, with at least one child between 2 and 16 years of age. Thirty-eight percent (n = 26) had one child, 47% (n = 32) had two, 10% (n = 7) had three, and 4% (n = 3) had four children. Inclusion criteria for mothers in the higher stress, caregiver group were caring for a child diagnosed with an autism spectrum disorder (including labels such as autism, Asperger syndrome, or pervasive developmental disorder not otherwise specified) and having a minimum perceived stress score (PSS) of 13 upon the initial phone screen. Mothers were eligible for the lower-stress, control group if they were caring for a neurotypical child without other chronic disease and reported PSS $\leqslant$19 during the phone screen. Overlap in PSS scores was permitted so that perceived stress could be better disentangled from the objective characteristic of caring for a child with an autism spectrum disorder. We then reassessed the PSS at the baseline visit to align our psychological and biological measures in time. Because depression is common in states of chronic stress, depression was allowed in the caregiving group. Thus, at recruitment, mothers were excluded from the control group, but not the chronic stress group, if they met criteria for current major depressive disorder or were taking antidepressants. Two controls who later started taking antidepressants were not excluded from the study as a whole, and subanalyses were included to test that their exclusion did not change the significance of the results. Exclusion criteria included major chronic diseases (e.g., diabetes, cardiovascular, autoimmune, history of stroke, brain injury, cancer, endocrine disorders), and regular use of steroid prescription medications. Participants meeting criteria for current posttraumatic stress, bipolar, or eating disorders were also excluded. This study was approved by the Committee for Human Research at the University of California, San Francisco, and all participants gave written consent.

2.2. Perceived stress scale (PSS)

The perceived stress scale-10 (Cohen et al., 1983) is a standard 10-item questionnaire that assesses subjective perceptions of