Published in final edited form as:

Thromb Haemost. 2017 April 03; 117(4): 816–818. doi:10.1160/TH16-10-0817.

Susceptibility to chronic social stress increases plaque progression, vulnerability and platelet activation

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Dear Sirs

Atherosclerotic cardiovascular disease (CVD) causes nearly 17.5 million deaths per year, a number that is projected to increase significantly within the next two decades (1).

Depression – the most common mental illness in the United States affecting up to 20 % of Americans during their lifetime (2, 3) – has been associated with 2.7-fold increased risk of acute myocardial infarction (AMI) (4) and two- to four-fold higher risk of mortality after a cardiac event (5–7). However, whether depression *per se* directly influences atherosclerotic CVD or is simply a co-morbidity remains unknown. In fact, most research effort in the field has concentrated on the effects of chronic stress on atherosclerosis (8–11), with no focus on depression-like symptoms—mostly because of the paucity of animal models that accurately reflect symptoms of affective disorders like depression. These studies have shown that chronic stress—a known risk factor for depression (6, 12–14) – affects atherosclerotic lesion progression, composition (8–10) and even myocardial infarction and sudden death in ApoE^{−/−} mice (11). However, no direct proof that depression affects atherosclerosis either *in vivo* or in humans exists. In the present study – using a validated and standardised protocol of chronic social defeat stress that results in a measurable depression-like phenotype in mice (15) – we tested the effect of depression on atherosclerotic plaque progression and

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Supplementary Material to this article is available online at www.thrombosis-online.com.

Conflicts of interest

Dr. Eric Nestler is Consultant for Merck Research Laboratories and had a research grant from J&J in the past five years. Dr. Scott Russo has received funding from Janssen Pharmaceuticals to study inflammation and depression. The other authors have no conflict to disclose.

composition and on platelet reactivity, all factors that contribute to the onset of myocardial infarction and stroke in humans.

Male ApoE^{-/-} mice on C57Bl/6 background were fed a high-fat diet and exposed to chronic social defeat stress (15). Details of Methods can be found in the Supplementary Material available online at www.thrombosis-online.com.

To test the hypothesis that depression directly affects atherosclerosis, ApoE^{-/-} mice on high-fat diet were subjected to chronic social defeat stress (15, 16). This model leads to the development of marked social avoidance associated with a constellation of overlapping behavioral and physiological changes reminiscent of depressive-like phenotype (16) (see Methods in the Supplementary Material available online at www.thrombosis-online.com). Using this protocol, social avoidance – measured as social interaction (SI) score, defined as the ratio of total time spent by the mouse in the interaction zone during each social interaction test session when the target is absent or present – is automatically captured using a robust method that ensures reproducibility and quantitative analysis of the depression-like phenotype in mice (15). ► Figure 1A shows the spectrum of avoidance behavior in our cohort of mice subjected to chronic social defeat and divided between depression-like (susceptible: S) and resilient (R) phenotypes as a function of their social interaction (SI) score (see Methods in the Supplementary Material available online at www.thrombosis-online.com). The scores of control (C) animals, on high fat diet but unexposed to chronic social defeat stress, are also shown. Plasma cholesterol (S: 2042 ± 404 mg/dl; C: 2070 ± 262 mg/dl), LDL (S: 1428 ± 345 mg/dl; C: 1375 ± 175 mg/dl), HDL (S: 522 ± 64 mg/dl; C: 592 ± 99 mg/dl) or triglyceride (S: 458 ± 29 mg/dl; C: 521 ± 27 mg/dl) did not differ between groups. S mice showed increased platelet aggregation versus control mice when stimulated with serotonin:epinephrine (5HT:EPI) at 24 hours after social defeat. In contrast, platelet reactivity to 5HT:EPI was not significantly different between S and C mice at six months (►Figure 1B), suggesting that the effect seen acutely after chronic social defeat exposure does not persist in the long term. Both control and susceptible ApoE^{-/-} mice showed the development of atherosclerotic lesions after six months of atherogenic diet. Analysis of aortic root sections by Oil Red-O staining revealed increased lipid accumulation in lesions of S versus C mice (►Figure 1C), suggesting a direct effect of depression on plaque progression and composition. We also found a negative correlation between Oil Red-O staining (% of stained area) and SI score (r: -0.79; p-value: 0.0005), indicating a direct correlation between the severity of depression-like symptoms and degree of lipid accumulation in the arterial wall. To investigate whether depression promotes the development of lesions with features of plaque vulnerability, we examined their cellular composition. We found increased plaque macrophage (MOMA-2+ cells) density in the lesions of S versus C mice (►Figure 1D) and, similarly to lipid accumulation, an inverse correlation between MOMA-2+ density and SI score (r: -0.41; p-value: 0.03). These observations further suggest that the severity of depression-like symptoms seems to directly affect the degree of plaque macrophage infiltration. On the other hand, no differences in vascular smooth muscle cell density (S: 26.8 ± 7.6 %; C: 26.7 ± 8.7 % stained area; P=NS) or plaque fibrosis (S: 42.6 ± 13.4 %; C: 38.3 ± 3.1 % stained area; P=NS) were observed between groups. To explore the effects of depression on plaque inflammation, we profiled the expression of 96 inflammation-related genes in the atherosclerotic aortas of S and C

mice (see Methods in the Supplementary Material available online at www.thrombosis-online.com). Among all differentially expressed genes (►Figure 1E), CCR6 (4.7 fold-increase), CCL19 (2.1 fold-increase), CCL5 (2.5 fold-increase) and CXCL13 (2.3 fold-increase) – selected for their known effects on atherosclerosis (17–20) – were additionally validated by qualitative PCR in S and C mice. Results confirmed the up-regulation of CCR6 (2.6 fold-increase), CCL19 (2.0 fold-increase), CCL5 (3.1 fold-increase) and CXCL13 (1.7 fold-increase) in the aorta of atherosclerotic S mice versus C (►Figure 1E).

Using a validated and reproducible model of chronic social defeat we have shown that the development of depression-like symptoms increases platelet aggregation and atherosclerosis in ApoE^{-/-} mice on high-fat diet. The results of this study provide strong evidence of a causal effect of depression on blood thrombogenicity and plaque vulnerability, two of the main components of Virchow's triad. As we previously reported in CAD patients with depression symptoms (21), we observed platelet hyperactivity to serotonin and epinephrine combination as agonist. These observations confirm a critical role of serotonergic and adrenergic pathways in mood disorders and cardiovascular disease (21–23). Aortic root histological analysis revealed a significant increase in lipid deposition and macrophages infiltration—well-established markers of plaque instability (24) – in susceptible mice versus controls. A pro-atherosclerotic inflammatory profile was identified when gene expression of 96 cytokines and receptors was tested in the atherosclerotic aortas of S versus C mice. We found that four cytokines, implicated in the progression of atherosclerosis (CCR6, CCL19, CCL5 and CXCL13) (17, 19, 20) were up-regulated in the atherosclerotic lesions of susceptible mice versus controls. These results are notable given that heightened systemic inflammation has been shown to directly regulate depression-like behaviors in susceptible mice (25) and may represent a shared risk factor in the comorbidity of depression and CVD risk. Together, these results indicate that depression-like phenotype not only promotes a prothrombotic state acutely after chronic stress in atherosclerotic mice, but also the development of features of plaque instability and a pro-atherogenic inflammatory phenotype in the long term. In conclusion, the results of this proof-of-concept study suggest a causal link between depression and atherosclerosis in patients with overt, or at high risk of, atherosclerotic cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge support from K23HL111339, R03HL135289, R21TR001739 to CG and from P50MH096890 to EJM and from the Fondation Leducq Transatlantic Network of Excellence Award (CAD Genomics) to CG.

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Figure 1.

A) Spectrum of avoidance behaviour. Susceptible (S, blue dots; n=33), resilient (R, black squares; n=7) and Control mice (C, red triangles; n=15) are shown. **B) Platelet reactivity to serotonin and epinephrine (5HT:EPI) at 24 hours and 6 months after social defeat.** 24h = 24 hours, 6m = 6 months. **C) Oil Red O staining: quantification (left) and representative aortic root sections of C and S mice (right).** Magnification 25 $\times$. Scale Bar: 1 mm. **D) Quantification of MOMA-2 immunostaining (left).** Representative sections from C and S mice (right). Magnification 50 $\times$. Scale Bar 500 μ m. **E) Volcano Plot of qPCR**

array data and gene expression analysis by Taqman PCR of selected candidate genes in the atherosclerotic aortas of S (n=10) normalised on C mice (n=3). FC: fold-change.

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