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Relation between resting amygdalar activity and cardiovascular events: a longitudinal and cohort study

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Summary

Background—Emotional stress is associated with increased risk of cardiovascular disease. We imaged the amygdala, a brain region involved in stress, to determine whether its resting metabolic activity predicts risk of subsequent cardiovascular events.

Methods—Individuals aged 30 years or older without known cardiovascular disease or active cancer disorders, who underwent ^{18}F-fluorodexoyglucose PET/CT at Massachusetts General Hospital (Boston, MA, USA) between Jan 1, 2005, and Dec 31, 2008, were studied longitudinally. Amygdalar activity, bone-marrow activity, and arterial inflammation were assessed with validated methods. In a separate cross-sectional study we analysed the relation between perceived stress, amygdalar activity, arterial inflammation, and C-reactive protein. Image analyses and cardiovascular disease event adjudication were done by mutually blinded researchers. Relations

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Contributors

AT, AI, RAPT, ALF, LMS, ZAF, and RKP drafted the study protocol and analysis plan. RAPT, AT, and RKP did the combined statistical analysis, and AT, AI, and RKP drafted the Article. All authors contributed to data collection, and the design, analysis, interpretation, and re-drafting of this Article.

See **Online** for appendix

Declaration of interests

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between amygdalar activity and cardiovascular disease events were assessed with Cox models, log-rank tests, and mediation (path) analyses.

Findings—293 patients (median age 55 years [IQR 45.0–65.5]) were included in the longitudinal study, 22 of whom had a cardiovascular disease event during median follow-up of 3.7 years (IQR 2.7–4.8). Amygdalar activity was associated with increased bone-marrow activity (r=0.47; p<0.0001), arterial inflammation (r=0.49; p<0.0001), and risk of cardiovascular disease events (standardised hazard ratio 1.59, 95% CI 1.27–1.98; p<0.0001), a finding that remained significant after multivariate adjustments. The association between amygdalar activity and cardiovascular disease events seemed to be mediated by increased bone-marrow activity and arterial inflammation in series. In the separate cross-sectional study of patients who underwent psychometric analysis (n=13), amygdalar activity was significantly associated with arterial inflammation (r=0.70; p=0.0083). Perceived stress was associated with amygdalar activity (r=0.56; p=0.0485), arterial inflammation (r=0.59; p=0.0345), and C-reactive protein (r=0.83; p=0.0210).

Interpretation—In this first study to link regional brain activity to subsequent cardiovascular disease, amygdalar activity independently and robustly predicted cardiovascular disease events. Amygdalar activity is involved partly via a path that includes increased bone-marrow activity and arterial inflammation. These findings provide novel insights into the mechanism through which emotional stressors can lead to cardiovascular disease in human beings.

Introduction

Psychosocial stress is both a byproduct of adversity and an important precipitant of morbidity. Chronic stress is associated with an increased risk of cardiovascular disease,[1,2] with an attributable risk that is on par with that of other major cardiovascular risk factors.[3–5] However, little is known about the mechanisms that translate stress into cardiovascular events.

Although several factors could account for the risk of cardiovascular disease attributable to stress, the brain's salience network, an ensemble of interconnected structures involved in complex functions such as cognition and emotion, is thought to have an important role. Activation of this network, which includes the amygdala as a key component,[6] leads to hormonal, autonomic, and behavioural changes typically associated with fear and stress.[7] The amygdala's efferent projections to the brainstem participate in the sympathetic responses to stress.[8] In murine models, stress increases proliferation of haemopoietic stem cells and progenitor cells in the bone marrow, accelerates innate immune cell output and cytokine production, and potentiates atherosclerosis.[9–13] However, whether a homologous pathway exists in human beings is unknown. Furthermore, although amygdalar reactivity is known to be heightened in individuals with pre-existing atherosclerosis,[14] neither human nor animal studies have yet shown whether amygdalar activation precedes and predisposes to the subsequent development of cardiovascular events.

Activation of the neural circuitry underlying the perception of fear-related stimuli can be reproducibly imaged using functional MRI (fMRI) and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT.[15,16] Amygdalar activity is upregulated in conditions marked by stress, such as post-traumatic stress disorder, anxiety, and depression.[16–20] By showing cellular glycolysis,

^{18}F-FDG PET/CT can be used to simultaneously quantify not only regional brain metabolism (activity),[16–19,21] but also haemopoietic tissue activity and large vessel arterial inflammation,[12,22] thereby making it uniquely suitable for investigation of linked activity among these systems and the pathogenesis of atherosclerosis. Accordingly, we used ^{18}F-FDG-PET/CT to test the hypotheses that amygdalar activity is associated with haemopoietic activity and arterial inflammation, and predicts the development of future cardiovascular disease events.

Methods

Study design and participants

We did two complementary imaging studies: a longitudinal outcomes study to assess the relation between resting amygdalar metabolic activity, atherosclerotic inflammation, and subsequent cardiovascular events, and a smaller, cross-sectional perceived stress study to assess the relation between psychometric measures of perceived stress, resting amygdalar metabolic activity, and atherosclerotic inflammation.

Participants were identified from a pool of 6088 patients who underwent ^{18}F-FDG PET/CT for clinical assessment (mainly for cancer screening) at the Massachusetts General Hospital (Boston, MA, USA) between Jan 1, 2005, and Dec 31, 2008. Predefined inclusion criteria included either absence of previous history of cancer or remission from cancer for at least 1 year before imaging (and throughout the follow-up period), absence of cardiovascular disease or acute or chronic inflammatory or autoimmune disease at time of imaging, and age older than 30 years. To ensure adequate information for events adjudication, an additional inclusion criterion was the availability of at least three clinical encounter notes in the medical records a minimum of 1 year apart. A full list of inclusion criteria is included in the appendix. All individuals who were included in a previous study[23] of the relation between arterial inflammation and cardiovascular disease events were included in our study if their scans showed the amygdala. The study protocol was approved by the Partners Human Research Committee. No specific informed consent was needed for this study.

Procedures

Two cardiologists (AT and QAT) blinded to PET/CT data used clinical records to adjudicate cardiovascular disease events, which were defined, according to the Framingham Heart Study,[24] as coronary death, myocardial infarction, coronary insufficiency, angina, cerebrovascular accidents, revascularisation, peripheral artery disease, and heart failure. Two additional and increasingly stringent subcategories of events were assessed as exploratory endpoints: major adverse cardiovascular events (which excluded angina without evidence of occlusive coronary disease), and atherosclerotic major adverse cardiovascular events (which required identification of potential culprit atherosclerotic plaque in association with the events; appendix).

^{18}F-FDG was given intravenously at a dose of ~370 MBq after an overnight fast. After tracer injection, individuals sat in a quiet waiting room and PET/CT was done around 1 h later with an integrated scanner (Biograph 64, Siemens Healthcare, Erlangen, Germany [or

similar]). Non-gated, non-contrast-enhanced CT (120 keV, ~50 mAs) was done for attenuation correction. Image analyses (appendix) were done by a radiologist (AI) who was blinded to the clinical data and used a dedicated workstation. Analysis of amygdalar activity was based on a validated approach that has shown associations with anxious temperament[19,25] and clinical manifestations of stress-related disorders.[16–19,21] ^{18}F-FDG uptake in the amygdala was determined by placing circular regions of interest in the right and left amygdalae and measuring the mean and maximum tracer accumulation (ie, standardised uptake value [SUV]) in each region of interest. Amygdalar activity was corrected for background cerebral (amygdalac, as mean temporal lobe SUV) and cerebellar ($amygdala_{cbl}$, as mean cerebellar SUV) activity.[26]

Bone-marrow activity, splenic activity, and arterial inflammation were measured according to previously validated methods[12,20] by deriving SUVs from the target tissue (bone marrow, spleen, or aortic and carotid walls) and correcting them for venous blood background activity to calculate target-to-background ratios. Arterial ^{18}F-FDG uptake is a well validated measure of arterial inflammation that relates to atherosclerotic macrophages[22] and predicts subsequent cardiovascular disease events.[23] ^{18}F-FDG uptake in subcutaneous adipose tissue was derived as a control measure of glycolytic activity. Coronary artery calcium score and visceral adipose tissue volume were derived from CT images (appendix).

Perceived stress study

To assess further the role of stress in the associations noted in the longitudinal study, in a separate cross-sectional study we tested the hypotheses that perceived stress is associated with resting amygdalar metabolic activity, arterial inflammation, and inflammatory biomarkers. 13 individuals with an increased burden of chronic stress (ie, history of post-traumatic stress disorder) were recruited from the community, completed the well validated, ten-item Perceived Stress Scale (PSS-10)[27] and underwent ^{18}F-FDG PET (Siemens mMR, Erlangen, Germany). Amygdalar activity, C-reactive protein (CRP), and arterial inflammation were measured (appendix). Further details of the perceived stress study are in the appendix.

Statistical analysis

Continuous variables are listed as mean (SD) or, when not normally distributed, median (IQR). We used Pearson product-moment correlation to assess univariate associations for normally distributed variables, and Spearman correlation coefficients for non-normally distributed variables. Cox proportional hazards models were used, with or without the addition of potential confounders as covariates, to calculate hazard ratios (HRs) and 95% CIs. Additionally, we did log-rank tests to generate Kaplan-Meier estimates (and associated curves) of cardiovascular event-free survival, comparing clinical events in patients with higher-versus-lower amygdalar activity. Mediation analysis—which tests a putative causal relation among variables (ie, a path)—was also done to test whether amygdalar activity exerts its effect on cardiovascular disease via postulated mediators (bone-marrow activity and arterial inflammation), either singularly or in series. Statistical significance was determined as p values less than 0.05. We used SPSS (version 23.0) for all statistical analyses (appendix).

Role of the funding source

There was no funding source for this study. AT, AI, RAPT, and RKP had full access to all study data, and AT had final responsibility for the decision to submit for publication.

Results

Imaging and cardiovascular disease events data were available for 293 people in the longitudinal outcomes study (figure 1). Baseline characteristics are listed in table 1. Individuals who developed subsequent cardiovascular events had an increased prevalence of several atherosclerotic risk factors compared with those who did not develop cardiovascular events. Of the atherosclerotic risk factors that were significant on univariate analyses, two remained significantly associated with cardiovascular disease events in multivariate analyses: age (standardised β 0.27; p=0.0003) and smoking (0.22; 0.0028). Furthermore, age and family history of coronary disease were associated with amygdalar activity (appendix).

During median follow-up of 3.7 years (IQR 2.7–4.8), 22 individuals experienced 39 cardiovascular disease events. The 22 index events were eight myocardial infarctions, three unstable angina, two peripheral arterial disease, six strokes, one heart failure, and two new onset angina (appendix). Amygdalar activity (ie, amygdalac and amygdala$_{cbl}$) robustly predicted the risk of developing a subsequent cardiovascular event (figure 2, table 2), yielding adjusted standardised HRs of approximately 1.6 (ie, a 1.6-times increased risk of a cardiovascular event for each increase of one SD in amygdalar signal). However, activity in the background brain structures (cerebral or cerebellar) or control extra-cranial tissue (subcutaneous fat) was not significantly associated with cardiovascular disease (appendix).

Furthermore, the relation between amygdalar activity and cardiovascular disease events remained significant after multivariate adjustments for cardiovascular risk factors, Framingham risk scores, and pre-existing atherosclerotic disease burden (table 2; appendix), and after correction for coronary artery calcium score or visceral adipose tissue volume (appendix). Notably, amygdalar activity generally remained associated with cardiovascular disease in subgroups with or without pre-clinical evidence of atherosclerosis at baseline (as coronary atherosclerotic calcification), subgroups with or without a high burden of coronary atherosclerotic risk factors, and subgroups with or without a previous history of cancer (table 3).

Amygdalar activity generally remained associated with cardiovascular events when more stringent definitions of events were used (ie, major adverse cardiovascular events and atherosclerotic major adverse cardiovascular events). The associated HRs increased relative to the stringency of the event definition (table 4). Additionally, amygdalar activity seemed to be associated with the timing of the cardiovascular disease event: individuals with higher resting amygdalar activity experienced subsequent cardiovascular disease events sooner than those with lower resting amygdalar activity (appendix).

To further explore the relation between amygdalar activity and cardiovascular disease, we compared outcomes for individuals with high amygdalar activity outcomes for those with low amygdalar activity. We determined the threshold values defining high activity by three

distinct approaches: receiver operating characteristic analysis, which yielded values with the highest accuracy to identify subsequent cardiovascular disease events; one or more SD above the mean; and as 90th percentile or greater. All remaining individuals were characterised as having low activity.

When the primary amygdalar imaging endpoint was dichotomised into high-activity and low-activity groups, Cox regression analyses yielded significantly increased HRs (table 5) and Kaplan-Meier analyses yielded significant group differences (figure 3). We noted similar findings when the secondary and post-hoc measures of amygdalar activity were similarly dichotomised (table 5). Additionally, amygdalar activity remained robustly predictive of cardiovascular disease when several other means of measuring it were used in sensitivity analyses (appendix).

Amygdalar activity correlated with haemopoietic tissue activity, expressed as ^{18}F-FDG-uptake in the bone marrow and spleen (table 6), and with arterial ^{18}F-FDG uptake, a well validated measure of arterial inflammation.[22,28] Moreover, measures of haemopoietic activity, especially in bone marrow, were associated with several measures of circulating blood cells, including total white blood cell count and neutrophil and lymphocyte counts (appendix). However, amygdalar activity did not correlate with ^{18}F-FDG-uptake in control tissue (ie, subcutaneous fat; table 6).

Bone-marrow activity was a significant mediator of the relation between amygdalar activity and arterial inflammation, accounting for a substantial 46% of the total effect. Arterial inflammation was a significant mediator of the relation between amygdalar activity and cardiovascular disease events, accounting for 39% of the total effect. Serial two-mediator analysis supported the hypothesised indirect path of increased amygdalar activity leading to increased bone-marrow activity leading to increased arterial inflammation leading to cardiovascular disease events (figure 4). These results suggest that bone-marrow activity and arterial inflammation, in series, have an important role in mediation of the association between amygdalar activity and cardiovascular disease events. Finally, when the path implicating bone-marrow activity was excluded, the residual path of increased amygdalar activity leading to increased arterial inflammation leading to cardiovascular disease events (figure 4) was also significant, suggesting that amygdalar activity also influences arterial inflammation and hence cardiovascular events through means other than haemopoiesis. That the mediators account for less than 100% of the total effects also suggests that amygdalar activity also affects cardiovascular disease events through means other than arterial inflammation.

In the cross-sectional study, in congruence with the longitudinal study's findings, amygdalar activity strongly correlated with arterial inflammation (r=0.70, p=0.0083). Perceived stress was associated with amygdalar activity (0.56; 0.0485; figure 5A), arterial inflammation (0.59; 0.0345, figure 5B) and CRP (0.83; 0.0210; figure 5C). Furthermore, amygdalar activity mediated most of the relation between perceived stress and arterial inflammation (p<0.05; appendix).

Discussion

Our results show, for the first time in human beings, that resting metabolic activity within the amygdala, a key component of the brain's salience network involved in stress, significantly predicts the development of cardiovascular disease independently of established cardiovascular risk factors. Furthermore, we showed that amygdalar activity is associated with increased haemopoietic activity and increased arterial inflammation. In the companion cross-sectional study, amygdalar activity was associated with perceived stress. In the longitudinal outcomes study, moreover, the link between amygdalar activity and cardiovascular disease events was substantially mediated by arterial inflammation (which in turn was substantially mediated by upregulated bone-marrow activity). These findings provide new and important insights, specifically that the amygdala could be a key structure in the mechanism linking stress to cardiovascular events, and that upregulation of haemopoietic tissue activity and increased atherosclerotic inflammation are additionally implicated in a neural–haemopoietic–arterial axis.

Psychological stress has long been thought of as an important human malady. Over the past several decades, increasing attention has been paid to the physical manifestations of stress. Despite evidence linking psychological stress and cardiovascular disease,[29] cardiovascular risk management has remained focused on other risk factors, possibly partly as a result of poor understanding of the mechanisms underlying stress-associated cardiovascular disease. Our study is clinically important because it advances this understanding and suggests targets for novel therapeutic approaches to reduce cardiovascular disease risk.

Stress prompts activation of both the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis, leading to increases in circulating catecholamines, glucocorticoids, and (eventually) inflammatory cytokines.[30–32] Additionally, stress can increase heart rate and blood pressure via the autonomic nervous system, all of which can contribute to endothelial dysfunction.[33] However, these mechanisms do not entirely explain the link between stress and cardiovascular disease. Work in animals has yielded important new insights into another (and potentially more important) mechanism by which stress might induce cardiovascular disease. Murine studies show that stress leads to mobilisation and release of neutrophils and monocytes, and increases activity of haemopoietic progenitor cells in the bone marrow.[13] Haemopoietic progenitors might accumulate in the circulation and the spleen and are thus poised to potentiate leucocyte supply by establishing extra-medullary haemopoiesis.[34–36] In mice, bone-marrow-derived monocytes released in response to variable stress or to stressful events (such as myocardial infarction) migrate to the arterial wall, where they instigate atherosclerotic inflammation.[11,13,37] Results from animal studies[38] point to increased haemopoiesis and arterial inflammation as important mechanisms in stress. They also prompt two crucial questions that were addressed by our study: is this mechanism relevant to human beings, and how does the brain participate?.

Our results clearly identify the amygdala as a key neural structure associated with future cardiovascular disease events. Previous human imaging studies demonstrated that amygdalar activity is correlated with the inflammatory response to stress[39] and the presence of pre-existing preclinical atherosclerosis.[14] However, a longitudinal pathogenetic link such as that

which we noted, to the best of our knowledge has never been identified. Furthermore, by demonstrating a significant pathway of increased amygdalar activity leading to increased bone-marrow activity leading to increased arterial inflammation leading to cardiovascular disease event (figure 6), our findings are a key addition to the literature. Increased amygdalar activity and its downstream consequences of upregulated haemopoietic and inflammatory activity could also be implicated in other medical conditions in which inflammation has an important role.

Our study has several limitations. First, the participants in the outcomes study were identified from a clinical database of patients who had undergone ^{18}F-FDG PET/CT for clinical indications (mainly cancer screening), thus possibly limiting the generalisability of our findings. However, the association between amygdalar activity and cardiovascular disease events remained robust in the subgroup of individuals who did not have history of cancer, suggesting that this potential confounder is not responsible for the main observations of this study. Second, 271 (93%) of 293 individuals in the outcomes study were white. However, in the separate cross-sectional perceived stress substudy, most participants were not white and none had a previous history of cancer (data not shown). In that substudy, the relation between amygdalar activity and arterial inflammation remained robust, lending support to the findings of the main study. Third, standard questionnaires were not used in the outcomes study, and thus the relation between perceived stress or mental disorder and cardiovascular disease events was not directly assessed. However, the perceived stress substudy showed a relation between perceived stress and both amygdalar activity and arterial inflammation, thus providing independent validation of the findings. Fourth, a positive mediation analysis, as we report here, is consistent with, but not demonstrative of, causation —particularly when a portion of the data are cross-sectional, as was the case with amygdalar activity, bone-marrow activity, and arterial inflammation. To infer causation, further longitudinal or interventional studies are needed. Additionally, the pathway from amygdalar activity to cardiovascular disease presented herein does not purport to encompass all possible pathological influences on any of its nodes. Candidate influences include, among others, sympathetic activity, hormones (eg, neuropeptide Y, GABAergic neurosteroids), and cytokines. Finally, the sample size of the main study was modest and only 22 participants had a cardiovascular disease event. These limitations are substantially counterbalanced by several important innovations, including the unique, simultaneous quantification of arterial, amygdalar, and bone-marrow activity, and their associations with cardiovascular disease events in human beings.

Acitvation of the brain stress network and its downstream consequences, including haemopoietic tissue activation and increased arterial inflammation, could be targets for therapies designed to interrupt a vicious cycle between stress and cardiovascular events. Our findings should prompt not only further investigation of the mechanisms that regulate this axis, but also studies of how to interrupt pathogenetic transmission along it. One possible future research avenue would be to experimentally induce amygdalar activation (eg, by stressful mental imagery[40] or presentation of fear-related stimuli[16]), and then examine the acute effect on bone-marrow activity and arterial inflammation. In a more ambitious vein, meditation has been shown to reduce amygdalar activity.[41] In a study of 226 individuals,[42] those randomly assigned to a 12-week stress-reduction course experienced an approximately

50% reduction in cardiovascular disease events compared with individuals who underwent cardiac rehabilitation but not stress management training.

Our findings raise the hypothesis that the benefits noted in that stress-reduction study[42] could partly have been due to a therapeutic action on the neural–haemopoietic–arterial axis described herein. In the future, larger studies will be needed to assess modulation of this axis, which could produce a substantial beneficial clinical effect. Such studies should examine the impact of various stress-management strategies (targeting upstream portions of this axis), in addition to pharmacotherapies that target other aspects of this axis. In the meantime, when encountering a patient with a stress syndrome, clinicians could reasonably consider the possibility that alleviation of stress might result in benefits to the cardiovascular system. Eventually, chronic stress could be treated as an important risk factor for cardiovascular disease, one that is routinely screened for and effectively managed, like other major cardiovascular disease risk factors.

Our study shows, for the first time, a relation between neural tissue activity and subsequent cardiovascular events and suggests that the brain's salience network, bone marrow, and arterial inflammation together form an axis that could accelerate the development of cardiovascular disease. Furthermore, our findings raise the possibility that efforts to attenuate psychosocial stress could produce benefits that extend beyond an improved sense of psychological wellbeing, and could beneficially impact the atherosclerotic milieu. Future studies of this neural–haemopoietic–arterial axis might lead to insights into how to further reduce the burden of cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before the study

Chronic stress carries an attributable risk for cardiovascular disease that is on par with other recognised risk factors, such as smoking, increased lipid concentrations, hypertension, and diabetes. Despite the prevalence and potency of this risk factor, little is known about the mechanisms that translate stress into cardiovascular disease events. To assess existing work, we searched PubMed with the terms “psychosocial stress”, “arterial inflammation”, “bone marrow”, “hematopoietic”, “human”, and “cardiovascular disease” for articles published in English before June 18, 2016. Although a link between stress, bone-marrow activity, and arterial inflammation had been identified in animal studies, this link had not previously been assessed in human beings. Furthermore, we found no studies in which the relation between neural tissue activity and cardiovascular disease events was assessed in either animal models or people.

Added value of this study

Our study provides several novel observations that together define a mechanism linking stress to cardiovascular events. We show for the first time in human beings that resting metabolic activity within the amygdala is significantly associated with the risk of developing cardiovascular disease independently of established cardiovascular risk factors. Furthermore, the link between amygdalar activity and cardiovascular disease events was substantially mediated by arterial inflammation (which in turn was substantially mediated by upregulated bone-marrow activity). These observations provide new and important insights, specifically that the amygdala could be a key structure in the mechanism linking stress to cardiovascular disease events, and that upregulation of haemopoietic tissue activity and increased atherosclerotic inflammation are additionally implicated in a neural–haemopoietic–arterial axis.

Implications of all the available evidence

Our results provide unique insights into mechanisms translating stress to cardiovascular disease and raise the possibility that alleviation of psychosocial stress could produce benefits that extend beyond an improved sense of psychological wellbeing, by improving the atherosclerotic milieu. Eventually, chronic stress could be treated as an important risk factor for cardiovascular disease, one that is routinely screened for and effectively managed, similar to other major cardiovascular disease risk factors.

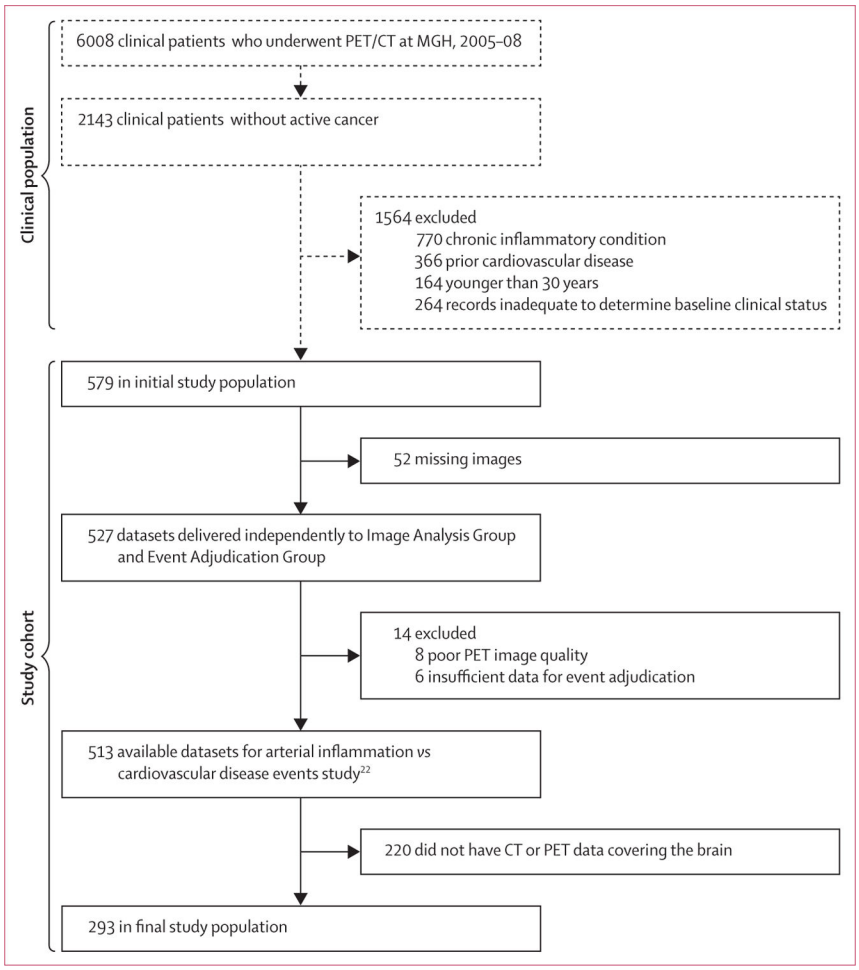

Figure 1: Study cohort

Eligible patients were selected on the basis of pre-defined criteria. All patients meeting these criteria were included. Image analyses and event adjudication were performed by mutually blinded investigators. ^{18}F-FDG=^{18}F-fluorodeoxyglucose. MGH=Massachusetts General Hospital.

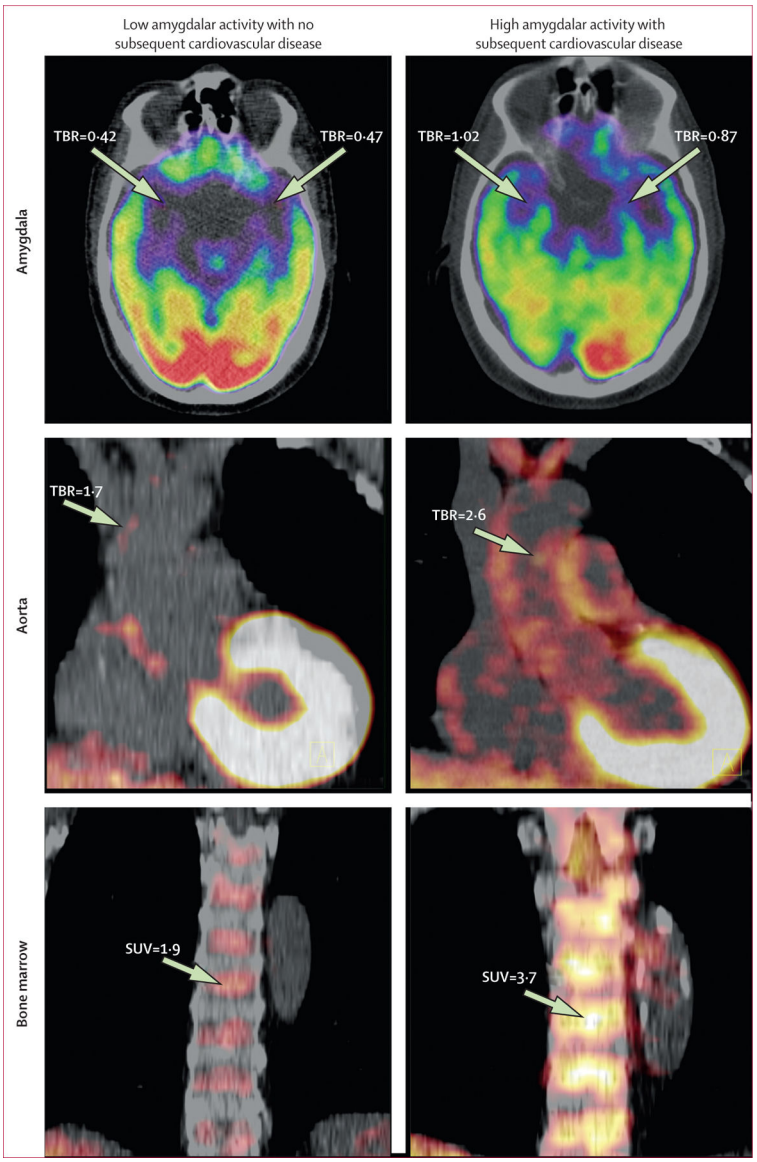

Figure 2: Amygdalar, arterial, and bone-marrow uptake of ^{18}F-FDG in individuals with and without subsequent cardiovascular disease events

Axial views of amygdala (upper left and right), coronal views of aorta (middle left and right), and coronal views of bone marrow (lower left and right) are shown. ^{18}F-FDG uptake was increased in the amygdala, bone marrow, and arterial wall (aorta), in a patient who experienced an ischaemic stroke during the follow-up period (right) compared with a patient who did not (left). ^{18}F-FDG=^{18}F fluorodeoxyglucose. SUV=standardised uptake value. TBR=target-to-background ratio.

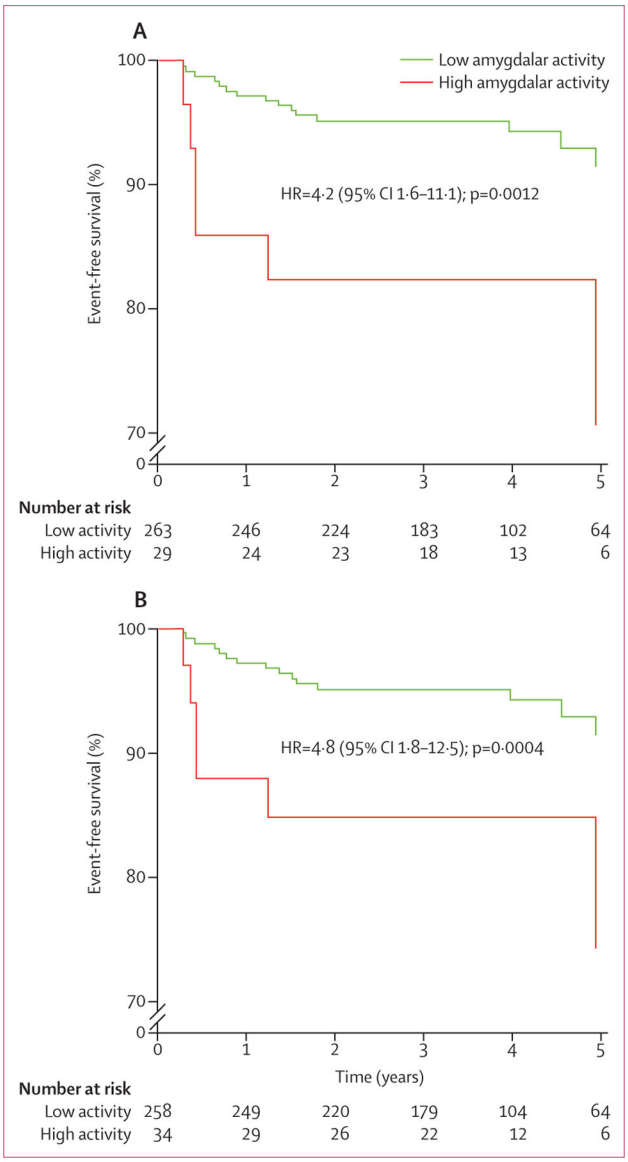

Figure 3: Kaplan-Meier survival curves of low *vs* high amygdalar activity based on the 90th percentile cutoff (A) or the mean (SD) cutoff (B)

Event-free survival for the primary amygdalar endpoint (max max amygdalac—ie, the maximum standardised uptake value for the right and left amygdalae, corrected for background cerebral tissue activity) are shown. p values were calculated with the log-rank test, and cox regression analyses were done to calculate HRs. HR=hazard ratio.

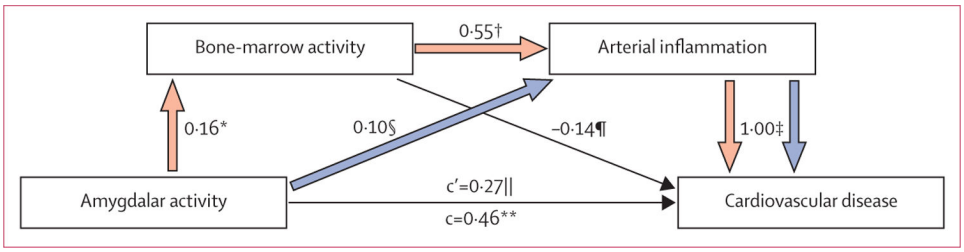

Figure 4: Serial mediation model for hypothesised pathway to a cardiovascular disease event

n=266. A single-mediator analysis showed that bone-marrow activity was a significant mediator of the relation between amygdalar activity and arterial inflammation. Another single-mediator analysis showed that arterial inflammation was a significant mediator of the relation between amygdalar activity and cardiovascular disease events. A serial two-mediator analysis testing the hypothesised indirect path of increased resting amygdalar activity leading to increased bone-marrow activity leading to increased arterial inflammation leading to cardiovascular disease events (red arrows) was significant. Additionally, excluding the path through bone-marrow activity, the residual path of increased resting amygdalar activity leading to increased arterial inflammation leading to cardiovascular disease event (blue arrows) was also significant. Amygdalar activity was assessed as the primary measure, max max amygdalac (ie, the maximum standardised uptake value for the right and left amygdalae, corrected for background cerebral tissue activity). Bone-marrow activity was measured by ^{18}F-FDG uptake in vertebral bone marrow corrected for background uptake in the superior vena cava. Arterial inflammation was measured by ^{18}F-FDG uptake in the aortic wall corrected for background uptake in the superior vena cava. In the figure, c represents the total effect of amygdalar activity on cardiovascular disease events, whereas c’ is the residual direct effect of amygdalar activity on cardiovascular disease events (independent of mediated effects). Standardised regression coefficients or log odds ratios are shown; all analyses incorporated age, sex, and baseline coronary artery calcification (as a control for pre-existing atherosclerotic disease burden) as covariates. The appendix contains additional explanation. ^{18}F-FDG=^{18}F fluorodeoxyglucose. *p=0.0073. †p<0.0001. ‡p=0.0044. §p=0.0432. ¶p=0.6409. ||p=0.2196. **p=0.013.

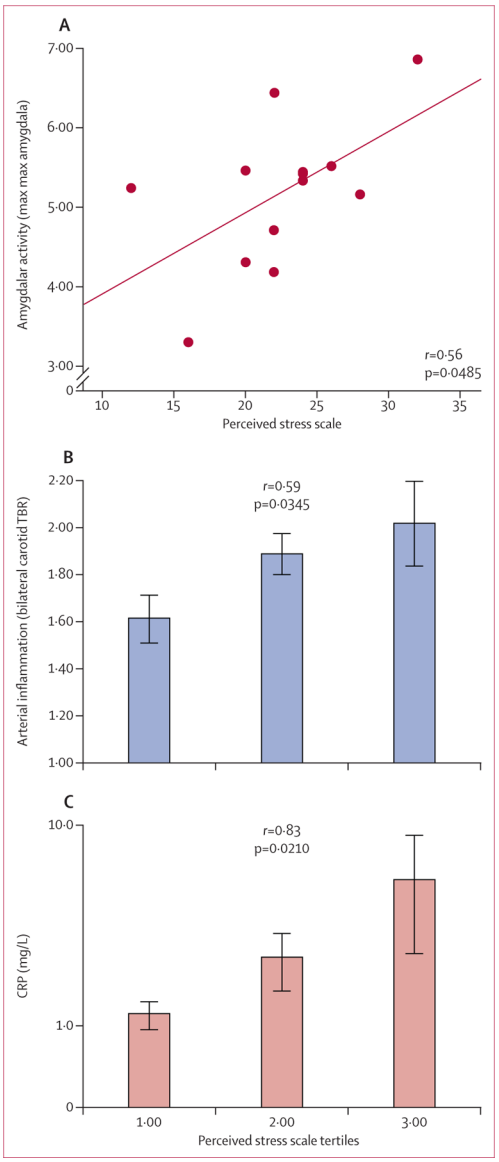

Figure 5: Perceived stress associated with amygdalar activity (A), arterial inflammation (B), and CRP (C) in cross-sectional validation sub-study

Perceived stress was assessed with a validated questionnaire. Error bars in (B) and (C) represent the standard error of the mean. TBR=target-to-background ratio. CRP=C-reactive protein.

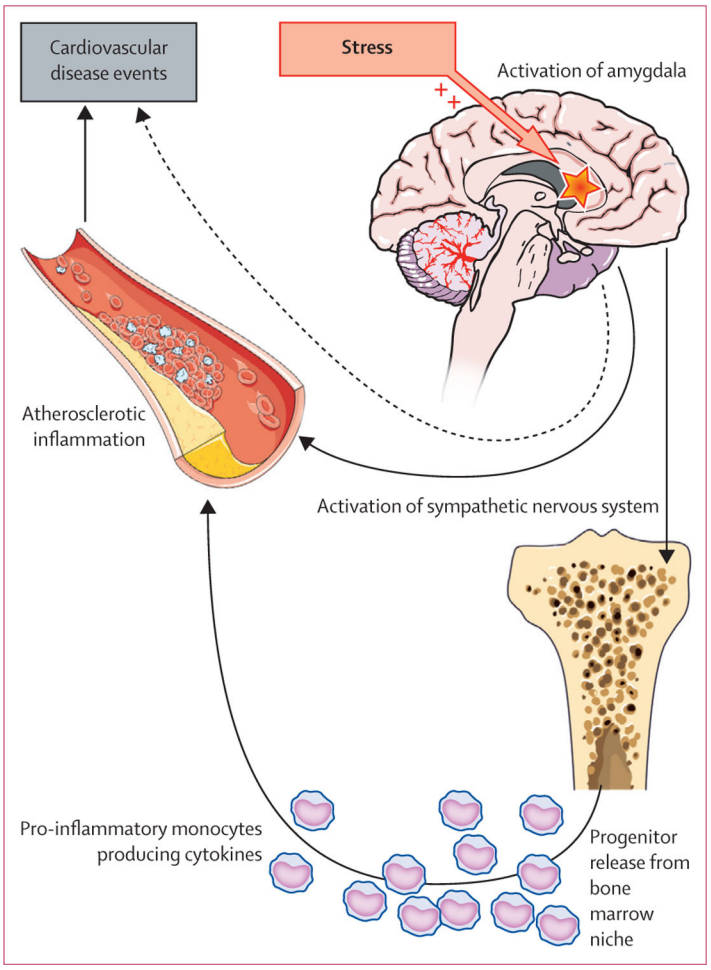

Figure 6: A model of stress leading to atherosclerotic inflammation

Data suggest that at least two biologically significant pathways link amygdalar activity to cardiovascular disease events in human beings. One of these pathways is sympathetic. The other, which is the object of this study, includes activation of the bone marrow (and release of inflammatory cells), which in turn lead to atherosclerotic inflammation and its atherothrombotic manifestations.

Table 1:

Baseline characteristics of participants in outcomes study

	Full cohort (n=293)	No cardiovascular event (n=271)	Cardiovascular event (n=22)	p value (event *vs* no event)
Median age, years (IQR)	55.0 (45.0–65.5)	55.0 (44.0–64.0)	64.5 (60.0–78.3)	<0.0001
Male	124 (42%)	111 (41%)	13 (59%)	0.10
White race	271 (93%)	243 (89%)	20 (91%)	0.85
Current smoker	27 (9%)	20 (7%)	7 (32%)	0.0001
Hypertension	103 (35%)	89 (32%)	14 (64%)	0.0035
Diabetes mellitus	25 (8%)	19 (7%)	6 (27%)	0.0010
Hyperlipidaemia	85 (29%)	74 (27%)	11 (50 %)	0.0241
Mean total cholesterol (SD)	190.7 (47.4)	193.4 (47.9)	174.6 (42.1)	0.10
Mean LDL cholesterol (SD)	109.6 (38.9)	111.4 (39.2)	98.6 (36.2)	0.17
Statin therapy	61 (21%)	51 (19%)	10 (46%)	0.0030
Mean Framingham risk score (SD)	5.9 (6.2)	5.1 (5.9)	10.6 (5.9)	0.0002
Median body-mass index (IQR)	26.8 (23.4–31.0)	26.6 (23.2–30.9)	27.0 (24.8–32.6)	0.36
Coronary artery calcium score		..	..	0.0012
0–10	204 (70%)	192 (71%)	12 (55%)	..
11–99	39 (13%)	33 (12%)	6 (27%)	..
≥100	33 (11%)	29 (11%)	4 (18 %)	..
History of cancer	256 (87%)	241 (89%)	15 (68%)	0.0114
Previous chemotherapy	234 (80%)	221 (82%)	13 (59%)	0.0047
History of depression or anxiety *	29 (10%)	25 (9%)	4 (20%)	0.13
Antidepressant drug use *	27 (9%)	24 (9%)	3 (15%)	0.42

Data are n (%), unless otherwise specified. The coronary artery calcium score is a measure derived from coronary CT. The presence of calcium in the coronary artery suggests the presence of coronary atherosclerotic disease.

* Data for depression and anxiety and for antidepressant drug use were available for 288 participants—268 in the no events group and 20 in the cardiovascular disease events group.

Table 2:

Univariate and multivariate analysis of neural tissue activity *vs* cardiovascular disease events

	Max max amygdala$_c$ (primary measure)		Mean mean amygdala$_c$ (secondary measure)		Mean max amygdala$_c$ (secondary measure)		Mean L amygdala$_{cbl}$ (post-hoc measure)	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**
Univariate								
Per unit change	14.1 (4.0–50.0)	<0.0001	41.1 (8.7–194.5)	<0.0001	19.4 (4.7–79.9)	<0.0001	101.4 (12.5–822.4)	<0.0001
Per SD change	1.59 (1.27–1.98)	<0.0001	1.58 (1.30–1.91)	<0.0001	1.57 (1.27–1.95)	<0.0001	1.71 (1.33–2.20)	<0.0001
Covariates: age and sex								
Per unit change	5.0 (1.3–19.1)	0.0193	11.5 (2.0–66.5)	0.0064	5.7 (1.2–25.8)	0.0246	48.0 (6.7–342.0)	0.0001
Per SD change	1.32 (1.05–1.68)	0.0193	1.35 (1.09–1.67)	0.0064	1.35 (1.30–1.64)	0.0246	1.58 (1.25–2.00)	0.0001
Covariate: Framingham risk score								
Per unit change	4.5 (1.3–15.7)	0.0192	8.8 (1.8–43.7)	0.0078	5.1 (1.2–20.8)	0.0237	29.0 (4.6–183.7)	0.0003
Per SD change	1.30 (1.04–1.62)	0.0192	1.31 (1.08–1.67)	0.0078	1.28 (1.03–1.59)	0.0237	1.49 (1.20–1.85)	0.0003
Covariates: combined cardiac risk factors *								
Per unit change	7.6 (2.0–28.4)	0.0027	11.0 (1.8–65.5)	0.0087	8.4 (1.8–39.3)	0.0066	26.1 (2.6–260.5)	0.0054
Per SD change	1.42 (1.13–1.79)	0.0027	1.34 (1.13–1.79)	0.0087	1.38 (1.09–1.75)	0.0066	1.47 (1.12–1.93)	0.0054
Covariate: pre-existing atherosclerotic disease (CAC score)								
Per unit change	10.7 (2.7–42.9)	0.0008	31.6 (2.7–42.9)	<0.0001	14.3 (3.0–68.0)	0.0008	55.2 (5.0–614.3)	0.0011
Per SD change	1.51 (1.19–1.93)	0.0008	1.53 (1.24–1.88)	<0.0001	1.50 (1.08–1.90)	0.0008	1.61 (1.21–2.14)	0.0011
Covariate: history of depression or anxiety								
Per unit change	18.1 (5.0–65.5)	<0.0001	51.0 (10.3–251.7)	<0.0001	25.1 (6.0–105.8)	<0.0001	125.0 (14.4–1081.3)	<0.0001
Per SD change	1.66 (1.32–2.08)	<0.0001	1.62 (1.33–1.97)	<0.0001	1.63 (1.31–2.03)	<0.0001	1.77 (1.37–2.29)	<0.0001
Covariate: antidepressant use								
Per unit change	17.3 (4.8–62.2)	<0.0001	48.5 (10.0–235.5)	<0.0001	24.1 (5.8–100.1)	<0.0001	118.3 (14.0–997.5)	<0.0001
Per SD change	1.65 (1.32–2.06)	<0.0001	1.61 (1.33–1.95)	<0.0001	1.62 (1.31–2.01)	<0.0001	1.76 (1.37–2.26)	<0.0001

We measured amygdalar activity with several different approaches, providing a primary, two secondary, and one post-hoc measure. Amygdalar activity was corrected for background cerebral (c) or cerebellar (cbl) neural tissue activity. Max max is the maximum SUV for the right and left amygdalae. Mean mean is the mean of the mean SUVs in the right and left amygdalae. Mean max is the mean of the maximum SUVs in the right and left amygdalae. Mean L is the mean SUV for the left amygdala. CAC=coronary artery calcium. HR=hazard ratio. SUV=standardised uptake value.

*The cardiac risk factors that were entered into this model were each of the standard cardiovascular risk factors that were significantly associated with the development of events on the basis of univariate analysis (table 1)—age, smoking, hypertension, diabetes, dyslipidaemia, and family history—which were entered as cofactors in a stepwise (backward) conditional manner.

Table 3:

Subgroup analyses of amygdalar activity *vs* cardiovascular disease

	Max max amygdala$_c$ (primary measure)		Mean mean amygdala$_c$ (secondary measure)		Mean max amygdala$_c$ (secondary measure)		Mean L amygdala$_{cbl}$ (post-hoc measure)	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**
Individuals with previous subclinical coronary artery disease (CAC>0)								
Per unit change	7.1 (1.5–34.2)	0.0151	19.6 (2.1–180.3)	0.0086	7.8 (1.3–45.7)	0.0236	186.0 (3.9–8956.3)	0.0082
Per SD change	1.41 (1.07–1.85)	0.0151	1.44 (1.10–1.89)	0.0086	1.37 (1.04–1.79)	0.0236	1.86 (1.17–2.94)	0.0082
Individuals without previous subclinical coronary artery disease (CAC=0)								
Per unit change	19.9 (2.7–144.3)	0.0031	49.1 (5.1–473.9)	0.0008	34.5 (3.7–324.4)	0.0020	122.9 (7.9–1903.6)	0.0006
Per SD change	1.69 (1.19–144.34)	0.0031	1.61 (1.22–2.13)	0.0008	1.71 (1.22–2.41)	0.0020	1.77 (1.28–2.44)	0.0006
Individuals with ≥3 coronary risk factors								
Per unit change	13.3 (3.1–57.4)	0.0050	23.4 (3.7–147.3)	0.0008	19.4 (3.6–105.7)	0.0006	56.7 (7.7–416.2)	<0.0001
Per SD change	1.57 (1.22–2.03)	0.0050	1.47 (1.18–1.85)	0.0008	1.57 (1.21–2.03)	0.0006	1.61 (1.27–2.04)	<0.0001
Individuals with <3 coronary risk factors								
Per unit change	12.0 (1.1–128.8)	0.0401	57.8 (2.7–1227.7)	0.0093	15.8 (1.2–212.5)	0.0378	4.2 (0.0–9658.5)	0.72
Per SD change	1.54 (1.02–2.34)	0.0401	1.62 (1.13–2.39)	0.0093	1.52 (1.02–2.26)	0.0378	1.18 (0.47–2.96)	0.72
Individuals with previous cancer								
Per unit change	9.9 (1.5–64.3)	0.0164	46.5 (4.0–544.9)	0.0022	12.7 (1.6–103.1)	0.0171	330.2 (5.2–21 007.7)	0.0062
Per SD change	1.49 (1.08–2.07)	0.0164	1.60 (1.18–2.17)	0.0022	1.47 (1.07–2.02)	0.0171	2.00 (1.22–3.25)	0.0062
Individuals without previous cancer								
Per unit change	12.9 (2.2–76.5)	0.0050	18.8 (2.0–174.7)	0.0098	18.2 (2.4–139.8)	0.0054	24.5 (1.5–397.9)	0.0244
Per SD change	1.56 (1.15–2.13)	0.0050	1.43 (1.09–1.88)	0.0098	1.55 (1.14–2.12)	0.0054	1.46 (1.05–2.03)	0.0244

We measured amygdalar activity with several different approaches, providing a primary, two secondary, and one post-hoc measure. Amygdalar activity was corrected for background cerebral (c) or cerebellar (cbl) neural tissue activity. No additional adjustments were included in the subgroup analyses. Coronary risk factors include age older than 55 years, male sex, current smoker, hypertension, diabetes, and dyslipidaemia. Max max is the maximum SUV for the right and left amygdalae. Mean mean is the mean of the mean SUVs in the right and left amygdalae. Mean max is the mean of the maximum SUVs in the right and left amygdalae. Mean L is the mean SUV for the left amygdala. CAC=coronary artery calcium. HR=hazard ratio. SUV=standardised uptake value.

Table 4:

Analysis of neural tissue activity *vs* events—additional event definitions

	Max max amygdala$_c$ (primary measure)		Mean mean amygdala$_c$ (secondary measure)		Mean max amygdala$_c$ (secondary measure)		Mean L amygdala$_{cbl}$ (post-hoc measure)	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**	**HR (95% CI)**	**p value**
Cardiovascular disease								
Per unit change	14.1 (4.0–50.0)	<0.0001	41.1 (8.7–194.5)	<0.0001	19.4 (4.7–79.9)	<0.0001	101.4 (12.5–822.4)	<0.0001
Per SD change	1.59 (1.27–1.98)	<0.0001	1.58 (1.30–1.91)	<0.0001	1.57 (1.27–1.95)	<0.0001	1.71 (1.33–2.20)	<0.0001
MACE								
Per unit change	15.9 (4.4–58.1)	<0.0001	45.7 (9.1–228.9)	<0.0001	21.7 (5.1–92.8)	<0.0001	114.2 (12.9–1014)	<0.0001
Per SD change	1.62 (1.29–2.03)	<0.0001	1.60 (1.31–1.95)	<0.0001	1.6 (1.28–1.99)	<0.0001	1.75 (1.35–2.27)	<0.0001
AMACE								
Per unit change	23.7 (1.6–350.0)	0.0212	432.9 (4.2–44499.4)	0.0102	41.0 (1.3–1278.2)	0.0343	315 (3.8–26196.9)	0.0108
Per SD change	1.74 (1.09–2.78)	0.0212	2.11 (1.19–3.72)	0.0102	1.76 (1.04–3.00)	0.0343	1.98 (1.17–3.33)	0.0108

We measured amygdalar activity with several different approaches, providing a primary, two secondary, and one post-hoc measure. Amygdalar activity was corrected for background cerebral (c) or cerebellar (cbl) neural tissue activity. Max max is the maximum SUV for the right and left amygdalae. Mean mean is the mean of the mean SUVs in the right and left amygdalae. Mean max is the mean of the maximum SUVs in the right and left amygdalae. Mean L is the mean SUV for the left amygdala. HR=hazard ratio. MACE=major adverse cardiovascular event. AMACE=atherosclerotic major adverse cardiovascular event (requires confirmed presence of an atherosclerotic culprit lesion). SUV=standardised uptake value.

Table 5:

Adjusted HRs for high *vs* low amygdalar activity

	Determination of high *vs* low cutoff	Adjusted HR (95% CI)	p
Primary amygdalar measure			
Max max $amygdala_c$	Above *vs* below ROC-optimised threshold	5.8 (2.1–16.0)	<0.0001
Max max $amygdala_c$	Above *vs* below mean+1 SD	4.8 (1.8–12.5)	0.0013
Max max $amygdala_c$	Above *vs* below 90th percentile	4.2 (1.6–11.1)	0.0030
Secondary amygdalar measure			
Mean mean $amygdala_c$	Above *vs* below ROC-optimised threshold	25.3 (9.1–70.5)	<0.0001
Mean max $amygdala_c$	Above *vs* below ROC-optimised threshold	9.2 (3.5–23.9)	<0.0001
Post-hoc amygdalar measure			
Mean L $amygdala_{cbl}$	Above *vs* below ROC-optimised threshold	40.5 (15.3–107.1)	<0.0001

Amygdalar activity is corrected for background cerebral (c) or cerebellar (cbl) neural tissue activity. Max max is the maximum SUV for the right and left amygdalae. Mean mean is the mean of the mean SUVs in the right and left amygdalae. Mean max is the mean of the maximum SUVs in the right and left amygdalae. HR=hazard ratio. ROC=receiver operating characteristic. SUV=standardised uptake value.

Table 6:

Correlation of amygdalar activity with haemopoietic activity and arterial inflammation

	Mean mean		Mean max		Max max	
	Correlation coefficient	**p value**	**Correlation coefficient**	**p value**	**Correlation coefficient**	**p value**
Aortic inflammation	0.49	<0.0001	0.45	<0.0001	0.41	<0.0001
Carotid inflammation *	0.47	<0.0001	0.43	<0.0001	0.40	<0.0001
Splenic activity	0.50	<0.0001	0.47	<0.0001	0.46	<0.0001
Bone-marrow activity	0.44	<0.0001	0.40	<0.0001	0.40	<0.0001
Control tissue uptake of ^{18}F-FDG uptake (subcutaneous fat)	0.02	0.73	0.02	0.80	0.02	0.79

Pearson product-moment correlations. All measures are uncorrected SUVs. Mean mean is the mean of the mean SUVs in the right and left amygdalae. Mean max is the mean of the maximum SUVs in the right and left amygdalae. Max max is the maximum SUV for the right and left amygdalae. ^{18}F-FDG=^{18}F fluorodeoxyglucose. SUV=standardised uptake value.

* Mean of the right and left carotid mean maximum SUVs.