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BASIC SCIENCE

BDNFVal66met polymorphism: a potential bridge between depression and thrombosis

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Aims Epidemiological studies strongly suggest a link between stress, depression, and cardiovascular diseases (CVDs); the mechanistic correlation, however, is poorly understood. A single-nucleotide polymorphism in the BDNF gene (BDNFVal66Met), associated with depression and anxiety, has been proposed as a genetic risk factor for CVD. Using a knock-in mouse carrying the BDNFVal66Met human polymorphism, which phenocopies psychiatric-related symptoms found in humans, we investigated the impact of this SNP on thrombosis.

Methods and results $BDNF^{Met/Met}$ mice displayed a depressive-like phenotype concomitantly with hypercoagulable state and platelet hyperreactivity. Proteomic analysis of aorta secretome from $BDNF^{Met/Met}$ and wild-type (WT) mice showed differential expression of proteins involved in the coagulation and inflammatory cascades. The BDNF Met allele predisposed to carotid artery thrombosis $FeCl_3$-induced and to death after collagen/epinephrine injection. Interestingly, transfection with $BDNF^{Met}$ construct induced a prothrombotic/proinflammatory phenotype in WT cells. SIRT1 activation, using resveratrol and/or CAY10591, prevented thrombus formation and restored the physiological levels of coagulation and of platelet markers in $BDNF^{Met/Met}$ mice and/or cells transfected with the Met allele. Conversely, inhibition of SIRT1 by sirtinol and/or by specific siRNA induced the prothrombotic/proinflammatory phenotype in WT mice and cells. Finally, we found that BDNF Met homozygosity is associated with increased risk of acute myocardial infarction (AMI) in humans.

Conclusion Activation of platelets, alteration in coagulation pathways, and changes in vessel wall protein expression in $BDNF^{Met/Met}$ mice recapitulate well the features occurring in the anxiety/depression condition. Furthermore, our data suggest that the BDNFVal66Met polymorphism contribute to the individual propensity for arterial thrombosis related to AMI.

Keywords BDNFVal66Met polymorphism • Depression • Thrombosis • Platelet • Vascular inflammation

Translational perspective

Epidemiological studies suggest a strong link between depression and incidence of acute coronary syndrome (ACS). Using a knock-in mouse carrying the BDNFVal66Met human polymorphism that phenocopies many of the human psychiatric-related symptoms, we show that modification of the BDNF gene suffices to enhance both a depressive and a prothrombotic/proinflammatory phenotype, and that SIRT1 activation by resveratrol prevents the prothrombotic/proinflammatory status. We also show that in humans the Met homozygosity associates with acute myocardial infarction, independently of age, sex, and major cardiovascular risk factors. Future studies will be directed to unveil the mechanistic link between BDNFVal66Met polymorphism, depression and ACS, thus opening the way to novel therapeutic approaches.

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Introduction

Epidemiological studies have shown a robust association between stress, depression, and increased morbidity and mortality in acute coronary syndrome (ACS). A recent review suggests that depression should be included among the risk factors (RFs) for adverse medical outcomes in patients with ACS.[1] The depressive status, resulting in an increased prevalence of cardiovascular RFs and in potential non-adherence to secondary prevention measures, is associated with an unhealthy behaviour, which in turn may cause an increased occurrence of adverse outcomes.[2] Neuroendocrine dysfunction and disturbances in cardiac autonomic control, endothelial dysfunction, inflammation, and enhanced platelet reactivity are among the mechanisms potentially implicated in the connection between depression and adverse cardiac events.[3] Indeed, the rupture of an atherosclerotic plaque coupled with activation of circulating platelets, leading to the occurrence of acute thrombosis, is the major cause of ACS.

Several polymorphisms in genes (e.g. serotonin, cannabinoid receptor 1, glucocorticoid receptors) associated with mood disorders have also been linked to the development of coronary artery disease (CAD).[4] Recently, single-nucleotide polymorphisms (SNPs) in the brain-derived neurotrophic factor (*BDNF*) gene, which encodes for a neurotrophin that plays critical roles in neuronal strength and morphology and vascular development,[5,6] have been included in this list.[7,8] In particular, the rs6265 SNP leading to a valine to methionine substitution at position 66 in the BDNF protein (BDNFVal66Met) has been repeatedly associated with an increased susceptibility to depression and anxiety,[9] whereas association studies of this SNP with CAD led to conflicting results.[7,8,10–12]

In view of the role of acute vascular occlusion in ACS, we hypothesized an influence of the BDNFVal66Met polymorphism on thrombosis susceptibility. Taking advantage of a genetic knock-in mouse carrying the human BDNFVal66Met polymorphism, which phenocopies many of the psychiatric-related symptoms found in human carriers,[13] we have investigated the potential role of this SNP on thrombus formation.

Materials and methods

See Supplementary material online, Methods.

Results

BDNFVal66Met polymorphism affects basal depressive-like behaviour in mice

Chronic restraint stress has been shown to increase the immobilization time only in the heterozygous $BDNF^{+/Met}$, but not in the WT mice.[14] Here, we showed that even in the absence of stress the immobilization time was increased in the homozygous $BDNF^{Met/Met}$, compared with WT mice (see Supplementary material online, *Figure S1*), confirming that homozygous BDNF knock-in mice reproduce the phenotype observed in subjects carrying this SNP.[9]

Platelet function is increased in BDNF Val66Met mice

$BDNF^{Met/Met}$ mice had a significant higher number of circulating (WT: $1046 \pm 38.50 \times 103/\mu L$ vs. $BDNF^{Met/Met}$: $1296 \pm 53.64 \times 10^3/\mu L$, $P < 0.001$) and reticulated platelets (WT: $8.20 \pm 0.56\%$ vs. $BDNF^{Met/Met}$: $10.44 \pm 0.78\%$, $P < 0.01$), and an increased mean platelet volume (MPV; WT: 6.37 ± 0.04 fl vs. $BDNF^{Met/Met}$: 6.65 ± 0.14 fl, $P < 0.01$) compared with WT.

Washed platelets (WPs) from $BDNF^{Met/Met}$ mice showed higher aggregation in response to collagen and thrombin (*Figure 1A* and *B* and Supplementary material online, *Figure S2A* and *B*). Likewise, lower concentrations of ADP were required to elicit platelet-rich plasma aggregation of $BDNF^{Met/Met}$ mice compared with WT (*Figure 1C* and Supplementary material online, *Figure S2C*). Concomitantly, an increased binding of JON/A-PE antibody and of fibrinogen-FITC, markers of functional GPIIB/IIIa receptor, was measured in $BDNF^{Met/Met}$ WP exposed to different concentrations of thrombin or ADP (*Figure 1D* and *E*). The increased binding of fibrinogen was accompanied by an increased adhesion of $BDNF^{Met/Met}$ platelets to fibrinogen-coated surfaces (*Figure 1F* and Supplementary material online, *Figure S3A*). In addition, clot retraction experiments showed increased amounts of serum extrusion in clots of $BDNF^{Met/Met}$ compared with WT mice (*Figure 1G*), which reflects alterations in cytoskeleton dynamics. P-selectin expression and platelet–leucocyte aggregates (*Figure 1H* and *I* and Supplementary material online, *Figure S3B*), as well as the levels of plasma thrombospondin-1 (TSP-1) (WT: 37.56 ± 5.32 vs. $BDNF^{Met/Met}$: 69.58 ± 7.64 ng/mL, $P < 0.05$) and of serum thromboxane B_2 (WT: 455.4 ± 40.75 vs. $BDNF^{Met/Met}$: 661.5 ± 47.16 ng/mL, $P < 0.05$) were also significantly higher in $BDNF^{Met/Met}$ mice, consistent with increased platelets activation.

Influence of BDNFVal66Met polymorphism on coagulation

The interaction between blood cells and plasma factors in blood clotting was then analysed. Whole blood recalcification test showed similar coagulation times in the two groups of mice (*Figure 2A*), as confirmed by FII, FV, FVII, FVIII, FIX, FX, FXI, and FXII activities (see Supplementary material online, *Figure S4*). Clotting formation time was shorter in $BDNF^{Met/Met}$ than in WT mice (*Figure 2B*), consistent with higher levels of functional fibrinogen (*Figure 2C*) and platelet hyperreactivity (*Figure 1*). Accordingly, greater maximum clot firmness and maximum clot elasticity were observed in $BDNF^{Met/Met}$ mice (*Figure 2D* and *E*). In addition, clot formation was associated with an increased TF activity of circulating microparticles (MP-TF) and leucocytes in $BDNF^{Met/Met}$ mice (*Figure 2F* and *G*).

Tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) in plasma did not differ in the two groups, but the ratio tPA/PAI-1, which reflects the endogenous fibrinolytic activity,[15] was significantly lower in $BDNF^{Met/Met}$ mice (*Figure 2H–L*).

Aortic tissue characterization

To investigate the increased procoagulant potential of blood from $BDNF^{Met/Met}$ mice, we explored the components of aortic secretome, e.g. the supernatants of cultured aortic fragments. Proteomic analysis identified a total of 144 proteins (see Supplementary

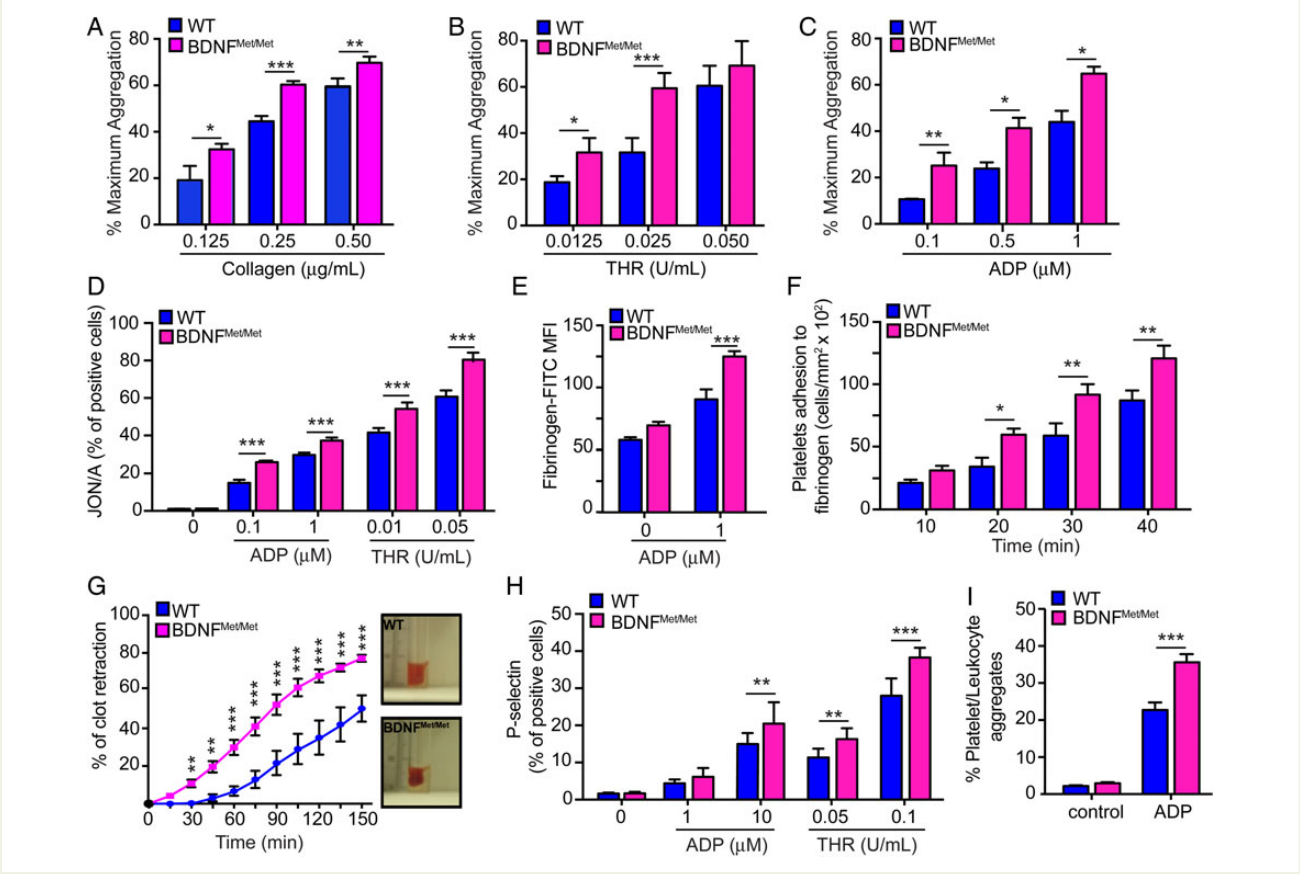

Figure 1 Platelet function is increased in BDNF$^{Met/Met}$ mice. Percentage of maximum platelet aggregation in response to (*A*) collagen, (*B*) thrombin (THR), and (*C*) ADP in WT vs. BDNF$^{Met/Met}$ mice ($n = 5–7$/group). Activation of GPIIbIIIa detected by flow cytometry with (*D*) JON/A-PE antibody and (*E*) fibrinogen-FITC in washed platelets (WP) from WT vs. BDNF$^{Met/Met}$ mice. (*F*) Quantitation of WP adherence on fibrinogen-coated surfaces. (*G*) Quantitation of clot retraction over time, and representative images at 60 min after addition of THR to platelet-rich plasma. (*H*) P-selectin expression detected by flow cytometry in WP, and (*I*) percentage of platelet/leucocyte aggregates in whole blood. Mean ± SEM ($n = 6–8$/group); $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, two-way repeated-measures ANOVA followed by Bonferroni post-hoc test.

material online, *Table S1*). Eight proteins were uniquely detectable in WT and 18 in BDNF$^{Met/Met}$ mice; among proteins expressed in both groups, 16 were down-regulated and 16 up-regulated in BDNF$^{Met/Met}$ mice (see Supplementary material online, *Figure S5* and *Table S2*).

Gene Ontology (GO) term overrepresentation analysis of up-regulated proteins revealed a significant enrichment in endopeptidase activity associated with response to cytokine stimulus, together with fatty acid oxidation, TCA cycle, and myosin complex (see Supplementary material online, *Figure S6A*). Moreover, KEGG pathway analysis revealed that five proteins up-regulated in BDNF$^{Met/Met}$ mice (see Supplementary material online, *Table S3*) are involved in the complement and coagulation cascades. Conversely, GO analysis revealed that down-regulated proteins were mainly associated with the actin cytoskeleton and microtubules (see Supplementary material online, *Figure S6B* and *Table S4*).

We focused our attention on two proteins: Gelsolin, given its involvement in actin remodelling and regulation of clot firmness,[16–18] and alpha1-antitrypsin (A1AT), a proinflammatory protein.[19] Consistent with the aorta secretome, lower amounts of Gelsolin and higher concentrations of A1AT were measured in plasma of BDNF$^{Met/Met}$ mice (*Figure 3A* and *B* and Supplementary material online, *Table S2*). This was accompanied by greater interleukin-6 (IL-6) levels, higher erythrocyte sedimentation rate (ESR), and elevated numbers of circulating leucocytes, in particular monocytes and neutrophils (*Figure 3C–H*). These data support the hypothesis that the BDNFVal66Met polymorphism modulates the fibrinolytic process affecting the clot structure and promotes a proinflammatory state.

We assessed whether the changes in Gelsolin abundance in secretome and plasma were paralleled by changes in tissue and cells. Unlike secretome and plasma, Gelsolin expression was higher in aortic tissue and in circulating platelets and leucocytes of BDNF$^{Met/Met}$ compared with WT mice (*Figure 4A–D*).

Intriguingly, expression of SorCS2, a sortilin family member engaged by BDNFMet prodomain in the regulation of morphological neuron changes,[20] and TF expression and activity were greater in BDNF$^{Met/Met}$ aortas (*Figure 4E–G*). Interestingly, decreased SIRT1 expression and increased acetylation of its target RelA/p65 lysine310 (p65K310ac)[21] were observed in aortas and/or circulating leucocytes of BDNF$^{Met/Met}$ mice (*Figure 4H–M*).

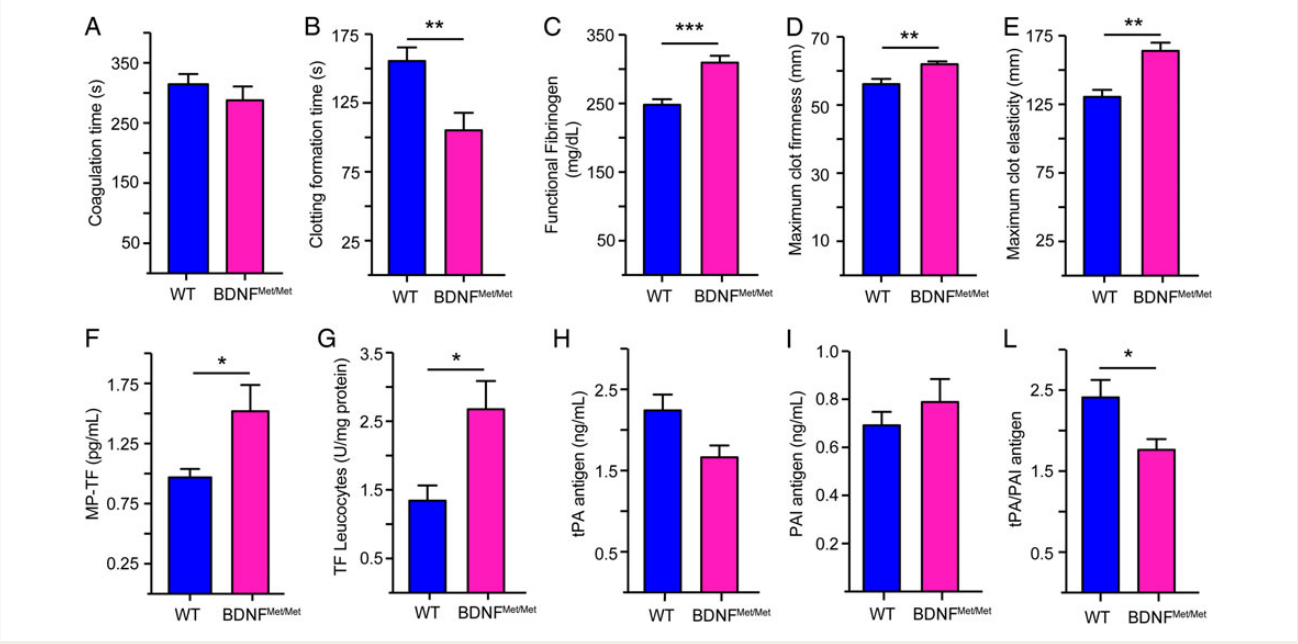

Figure 2 BDNFVal66Met polymorphism influences coagulation. Thromboelastographic analyses were carried out in whole blood of WT and BDNF$^{Met/Met}$ mice: (*A*) clotting time, (*B*) clot formation time, (*D*) maximum clot firmness, and (*E*) maximum clot elasticity ($n = 8–11$/group). (*C*) Functional fibrinogen, (*F*) circulating TF microparticles (MP-TF), (*H*) tPA, and (*I*) PAI-1 (*L*) tPA/PAI-1 ratio were measured in plasma from WT and BDNF$^{Met/Met}$ mice ($n = 9$/group). (*G*) TF procoagulant activity in circulating leucocytes from WT and BDNF$^{Met/Met}$ mice ($n = 6–8$/group). Mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ at unpaired Student's *t*-test.

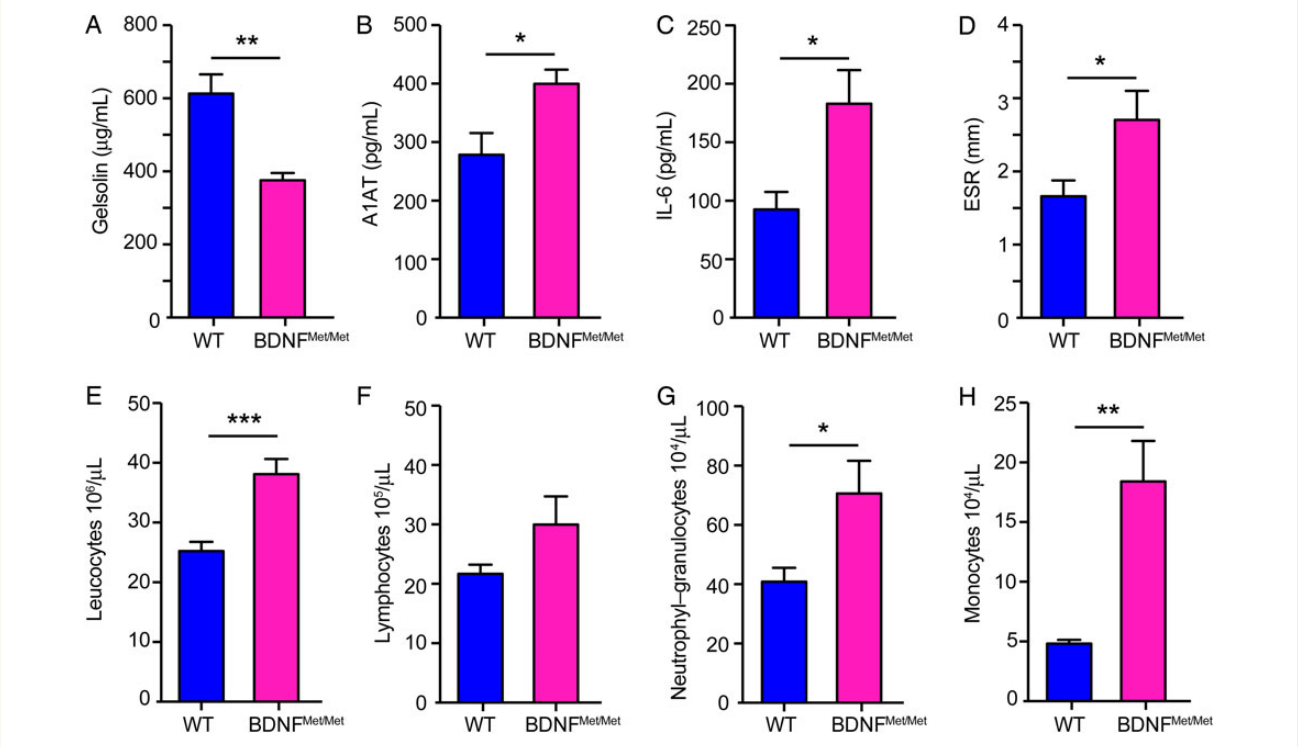

Figure 3 Inflammatory markers are increased in BNDF Val66Met mutant mice. Plasma levels of (*A*) Gelsolin, (*B*) alpha-1-antitrypsin (A1AT) ($n = 6$/group), and (*C*) IL-6 ($n = 10$/group), and (*D*) whole blood erythrocyte sedimentation rate (ESR, $n = 8$/group) of WT vs. BDNF$^{Met/Met}$ mice. Number of (*E*) leucocytes, (*F*) lymphocytes, (*G*) neutrophils-granulocytes, and (*H*) monocytes in whole blood from WT vs. BDNF$^{Met/Met}$ mice ($n = 6$/group). Mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ at unpaired Student's *t*-test.

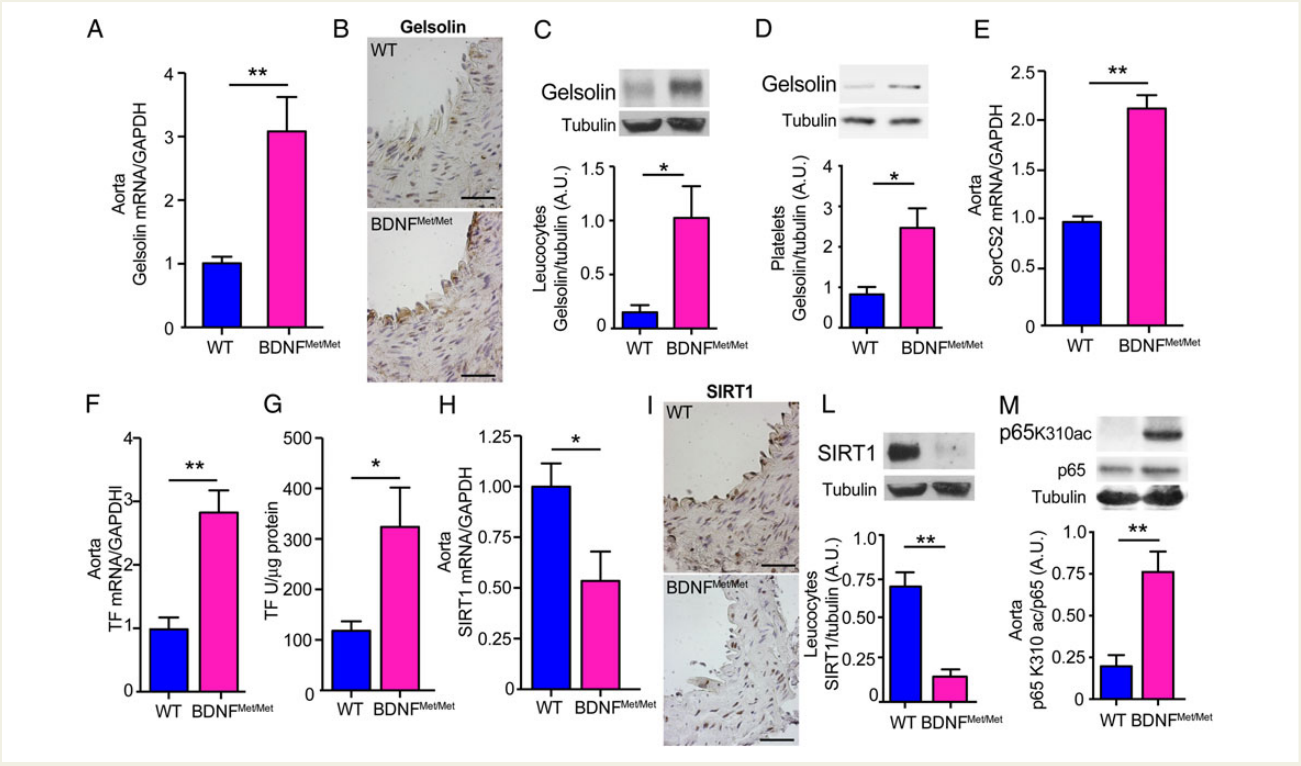

Figure 4 BDNFVal66Met polymorphism affects the expression and/or activity of proteins associated with inflammation, coagulation and fibrinolytic processes. Gelsolin (*A*) mRNA levels ($n = 12$/group) and (*B*) representative immunoperoxidase staining of aorta tissue, and protein expression in (*C*) circulating leucocytes and (*D*) platelets ($n = 5–6$/group). SorCS2 (*E*) and TF (*F*) mRNA levels ($n = 8$/group) and (*G*) TF activity ($n = 13$/group) in aorta tissue. SIRT1 (*H*) mRNA levels and (*I*) representative immunoperoxidase staining of aorta tissue, and protein expression in (*L*) circulating leucocytes ($n = 6$/group). (*M*) Acetylation of RelA/p65 lysine 310 (p65K310 ac) in aorta tissue. mRNA values are referred to baseline. Mean ± SEM; $^*P < 0.05$, $^{**}P < 0.01$ at unpaired Student's *t*-test.

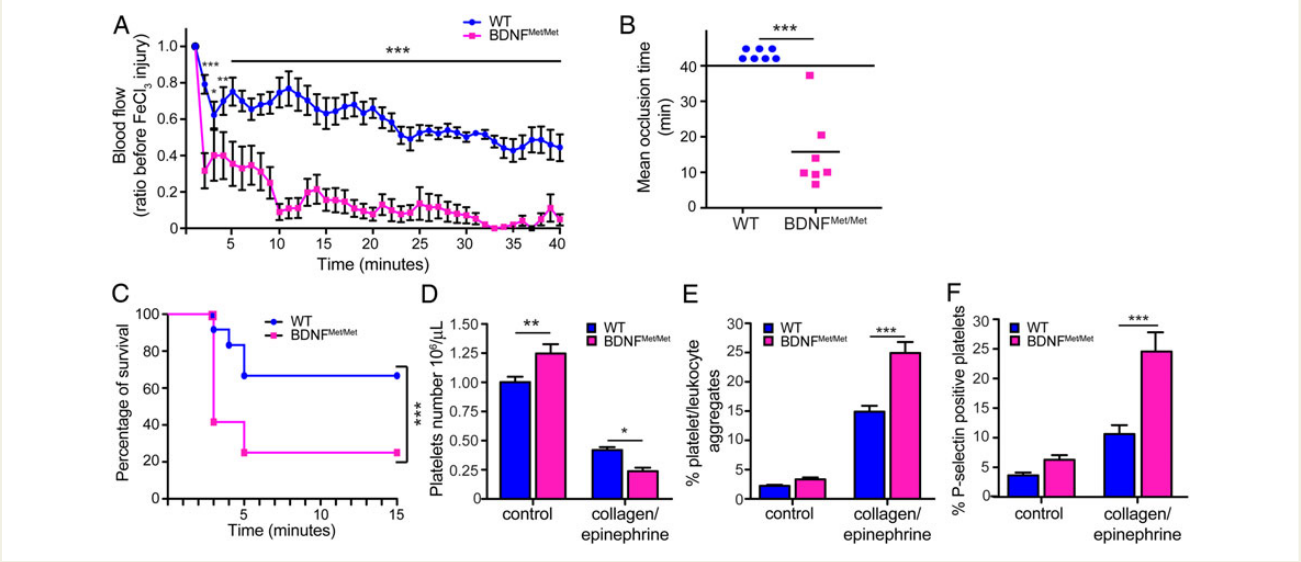

Figure 5 BDNFVal66Met polymorphism predisposes to thrombosis. (*A*) Blood flow in carotid arteries of mice expressed relative to the value before injury ($n = 7$/group). (*B*) Time to occlusion in WT and BDNF$^{Met/Met}$ mice: horizontal bars indicate the mean value for each group. (*C*) Kaplan–Meier graph showing the percentage of animals that survived after intravenous injection of collagen and epinephrine ($n = 12$/group). (*D*) Platelet count, and percentage (*E*) of platelet/leucocyte aggregates, and (*F*) of P-selectin positive platelets in mice 2 min after the intravenous injection of collagen and epinephrine ($n = 6$/group). Statistical analysis was performed by unpaired Student's *t*-test or by two-way repeated-measures ANOVA followed by Bonferroni post-hoc test. Mean ± SEM; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

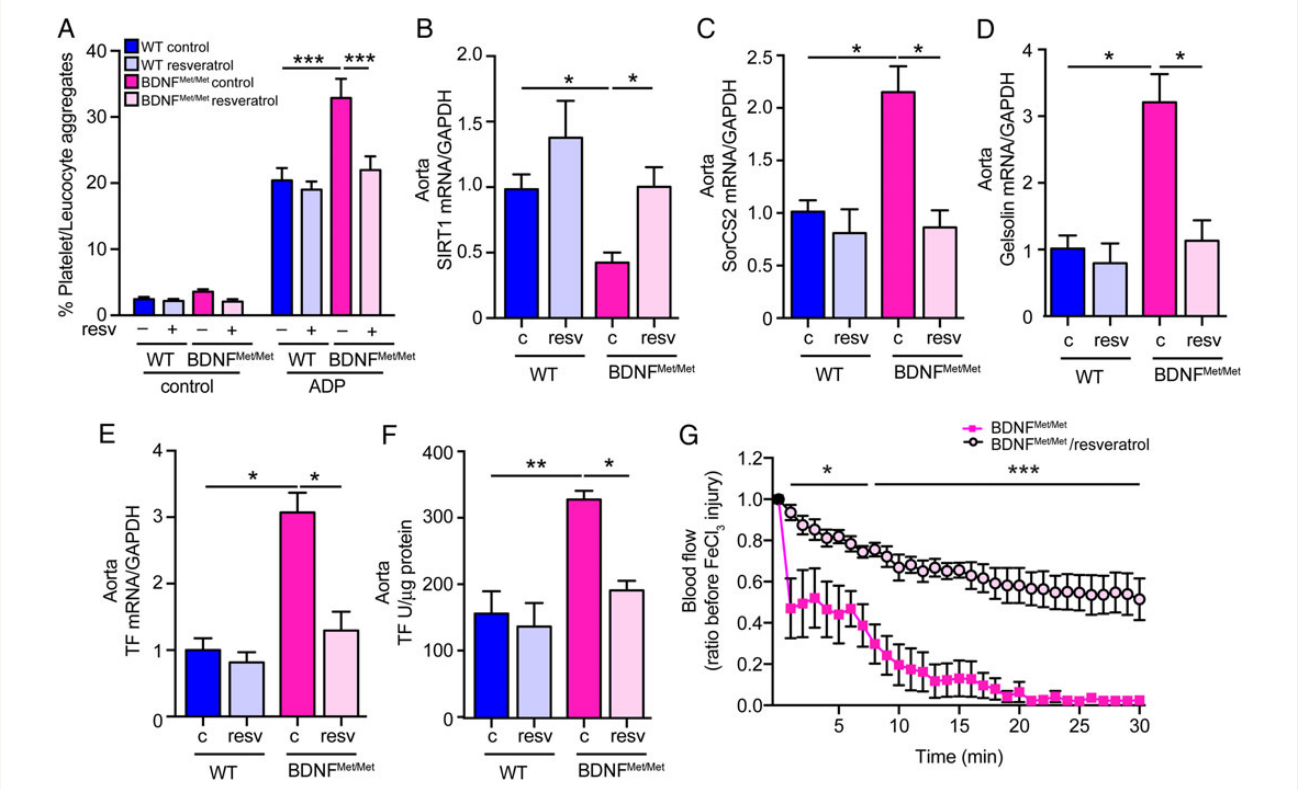

Figure 6 Resveratrol prevents prothrombotic phenotype in BDNF$^{Met/Met}$ mice. Mice were treated with DMSO (control) or resveratrol (2.3 mg/kg per day intraperitoneally for 7 days); (*A*) percentage of platelet/leucocyte aggregates was analysed in whole blood, mRNA levels of (*B*) SIRT1, (*C*) SorCS2, (*D*) Gelsolin, (*E*) TF in aorta tissue and (*F*) activity of TF in carotid artery. (*G*) Blood flow in the carotid arteries of mice expressed relative to the value before injury. Statistical analysis was performed by unpaired Student's *t*-test or by two-way repeated-measures ANOVA followed by Bonferroni post-hoc test. Mean ± SEM ($n = 6$/group); *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

BDNFVal66Met polymorphism predisposes to thrombosis

Having established that in BDNF$^{Met/Met}$ mice platelet hyperreactivity, abnormal blood clotting, and increased expression of proteins linked to thrombosis/inflammation occur, we investigated the impact of this SNP using two experimental models of thrombosis.

Carotid artery thrombus formation was induced by topical application of $FeCl_3$ 5% in BDNF$^{Met/Met}$ and WT mice. Blood flow was reduced by 83% and by 48% in BDNF$^{Met/Met}$ and WT mice, respectively, within the 40-min observation time. In particular, the mean time to total occlusion (defined as a flow reduction >90%) was shorter in BDNF$^{Met/Met}$ mice (*Figure 5A* and *B*).

The thrombotic phenotype detected in BDNF$^{Met/Met}$ mice was confirmed in the platelet-dependent pulmonary thromboembolism model. Thromboembolic death after intravenous collagen plus epinephrine injection occurred in 75% BDNF$^{Met/Met}$ (9 of 12) and 35% WT (4 of 12) mice (*Figure 5C*). After injection of collagen/epinephrine, circulating platelet counts were lower in BDNF$^{Met/Met}$ mice, indicating that *in vivo* platelet activation and sequestration were more sustained (*Figure 5D*). In addition, a significantly greater expression of platelet-associated P-selectin and of platelet/leucocyte aggregates after injection of collagen/epinephrine was found in the whole blood of BDNF$^{Met/Met}$ mice (*Figure 5E–F*).

Resveratrol prevents the prothrombotic phenotype in BDNF$^{Met/Met}$ mice

Subsequent experiments were performed to elucidate the mechanism(s) by which the Met allele affects thrombosis, focusing on a potential role of SIRT1.[22–24]

Activation of SIRT1 by the natural dietary compound resveratrol reduced the number of circulating platelets and leucocytes by 27 and 40%, respectively, in BDNF$^{Met/Met}$, without influencing cell count in WT mice (see Supplementary material online, *Figure S7*). In addition, resveratrol decreased platelet activation and rescued the procoagulant phenotype only in BDNF$^{Met/Met}$ mice, e.g. reducing platelet–leucocyte aggregate percentage (*Figure 6A*), plasma concentrations of TSP-1, MP-TF, and functional fibrinogen and increasing plasma Gelsolin (see Supplementary material online, *Table S5*). Treatment of mutant mice with resveratrol increased SIRT1 expression and down-regulated SorCS2, Gelsolin and TF back to the levels of WT aortae (*Figure 6B–F*). In addition, this treatment completely prevented thrombus formation in BDNF$^{Met/Met}$ mice (*Figure 6G*), without modifying arterial thrombosis in WT mice (see Supplementary material online, *Figure S7*).[24]

Inhibition of SIRT1 with sirtinol, which is known to increase arterial thrombogenicity and TF activity,[22] increased the expression of SorCS2, TF, and Gelsolin in WT mice, inducing a phenotype

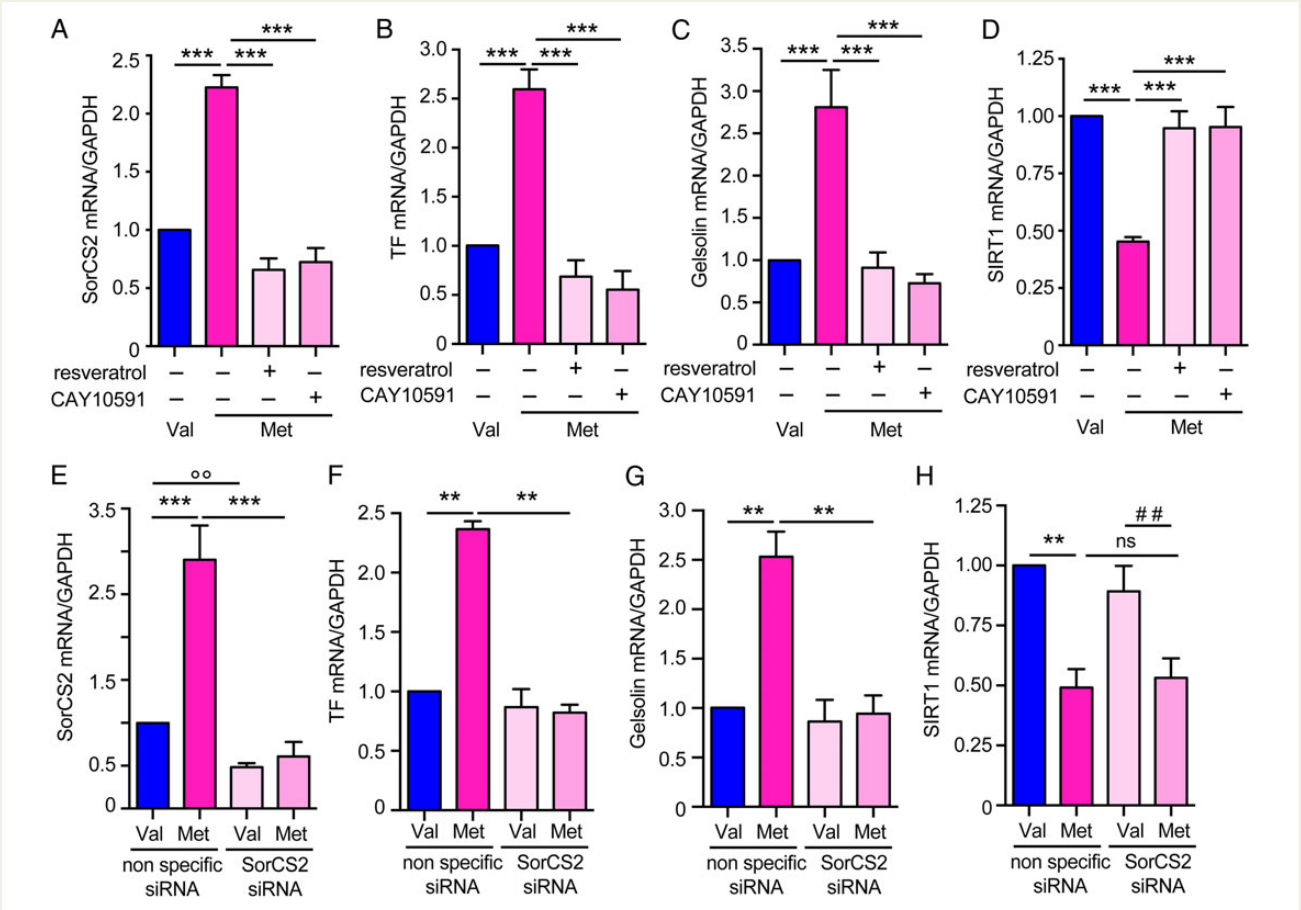

Figure 7 BDNFMet sequence modulates *in vitro* prothrombotic genes *via* SIRT1/SorCS2 pathway. HeLa cells transfected with Val or Met sequence were (*A*–*D*) incubated with resveratrol (10 μM) or CAY10591 (10 μM) for 6 h, or (*E*–*H*) transfected with SorCS2-directed siRNA or non-specific siRNA, as indicated: mRNAs for (*A* and *E*) SorCS2, (*B* and *F*) TF, (*C* and *G*) Gelsolin, and (*D* and *H*) SIRT1 were determinated. Statistical analysis was performed by one-way ANOVA, followed by Dunn's post-hoc test. ($n = 4$/group). Mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.005$ vs. $HeLa^{Met}$ cells, $^{\circ\circ}P < 0.01$ vs. $HeLa^{Val}$ cells transfected with SorCS2 siRNA.

similar to that of $BDNF^{Met/Met}$ mice (see Supplementary material online, *Figure S8*).

BDNFMet sequence modulates *in vitro* prothrombotic genes via SIRT1/SorCS2 pathway

To understand whether BDNFVal66Met polymorphism *per se* suffices to modulate prothrombotic genes, *in vitro* experiments on HeLA cells, transfected with plasmid expressing the BDNFVal or Met sequence, were carried out. Consistent with *in vivo* data, HeLA cells expressing Met mutation ($HeLA^{Met}$) showed higher levels of SorCS2, TF, and Gelsolin and lower levels of SIRT1 (*Figure 7A–D*). Treatment of $HeLA^{Met}$ cells with resveratrol or with CAY10591, a selective SIRT1 activator, down-regulated SorCS2, TF, and Gelsolin and concomitantly up-regulated SIRT1 expression (*Figure 7A–D*). The same treatments failed to influence the expression of these proteins in control $HeLA^{Val}$ cells (data not shown). Silencing with specific SorCS2 siRNA completely suppressed TF and Gelsolin overexpression in $HeLA^{Met}$ cells, with no effects on $HeLA^{Val}$ cells (*Figure 7E–G*). Remarkably, SIRT1 expression was not affected by SorCS2 inhibition both in $HeLA^{Val}$ and in $HeLA^{Met}$ (*Figure 7H*). Finally, inhibition of SIRT1, by sirtinol or by specific SIRT1 siRNA, up-regulated SorCS2, Gelsolin, and TF only in $HeLa^{Val}$ cells (see Supplementary material online, *Figure S9*).

Association of rs6265 with acute myocardial infarction in humans

To assess whether the BDNFVal66Met polymorphism is associated with a thrombotic state in a clinical setting, we took advantage of a retrospective study at our Centre (see Supplementary material online), which recruited patients with at least one coronary stenosis ≥70% undergoing coronary artery bypass graft. Nine hundred and seventy-nine subjects (see Supplementary material online, *Table S6*) were stratified according to the onset of the CAD: patients with (acute myocardial infarction, AMI; $n = 286$) or without (stable or unstable angina; $n = 693$) overt signs of an acute coronary thrombotic event. Genotyping of BDNF rs6265 identified 59 A/A homozygotes, 326 G/A heterozygotes, and 594 G/G homozygotes,

resulting in a minor allele (A) frequency of 23%, consistent with data reported in Caucasians.[25]

Association analysis revealed that BDNF rs6265 was significantly associated with AMI in the genotypic ($\chi^2 = 7.783$, df = 2, $P = 0.0204$) and recessive ($\chi^2 = 5.258$, df = 1, $P = 0.0219$) models, e.g. the homozygosity AA was significantly more frequent in patients with an overt thrombotic event at CAD onset than in other CAD patients (see Supplementary material online, *Table S7*). Notably, the sample size of $n = 979$ achieved 71% power to deem as significant the observed genotypic effect size (see Supplementary material online, *Table S7*). Consistently, logistic regression analysis showed a significant association of the minor allele A with an AMI onset of CAD in our patients, with an odds ratio [95% confidence interval] of 1.857 [1.086–3.173] ($P = 0.0236$), assuming a recessive genetic effect. This was true also when the analysis was corrected both for age and sex ($P = 0.0257$), and for age, sex, and major vascular RFs ($P = 0.0334$; *Table 1*).

Discussion

The relationship between depression and ACS has been amply debated, but the physiological and behavioural underpinnings of this association remain poorly understood. Our study indicates that the substitution at codon 66 of valine with methionine in the BDNF prodomain promotes a concomitant depressive and prothrombotic/proinflammatory phenotype in mice. Platelet activation, alterations in coagulation pathways, and changes in vessel wall protein expression are all events that occur in both $BDNF^{Met/Met}$ mice and patients with anxiety/depression. Further, we showed that Met homozygosity is associated with AMI in a cohort of patients with severe CAD.Increased GPIb, P-selectin, and β-thromboglobulin expression, greater platelet–leucocyte interactions, and higher levels of A1AT, TSP-1, and functional fibrinogen, all contributing to ACS risk,[26] have been previously reported in patients with depression.[19,27–29] Likewise, lower levels of SIRT1 in different tissues/cells, including circulating leucocytes, have been linked to inflammatory processes,[30] and thrombosis through modulation of TF expression and activity,[22,23] and have been reported in patients with depression or ACS.[19,29–31] Of note, the associations between SIRT1 SNPs and CAD, mood disorders or inflammation have been recently reported.[32–34]

The increased amount of TF associated with MPs, circulating leucocytes and carotid arteries, besides platelet activation, well explains the increased propensity to thrombosis observed in $BDNF^{Met/Met}$ mice exposed to $FeCl_3$.[35] A critical role for $BDNF^{Met/Met}$ platelets in the worsening of thrombosis occurrence is also suggested by the pulmonary thromboembolism model. Available data, however, do not allow us to discriminate the role of vessel wall procoagulant activity vs. the prothrombotic phenotype of circulating platelets in the occurrence of thrombus formation, with either experimental model used. The role of intrinsic vascular changes is further supported by the observation that tPA/PAI-1 ratio is decreased in $BDNF^{Met/Met}$ mice, which may result in reduced fibrinolysis.[15] The tPA-PAI-1 imbalance is known to play an important role in the pathophysiology of mental and thromboembolic disorders.[36,37] tPA facilitates clot dissolution and participates in several brain functions, including the response to stress, learning, and memory.[38]

An alteration in the fibrinolytic process is also supported by the evidence of modification in the Gelsolin levels detected in $BDNF^{Met/Met}$ mice. Gelsolin in plasma is known to clear F-actin from the circulation, supporting the lytic action of plasmin;[16,17] its cytoplasmic isoform controls several cellular processes, including platelet formation and activation.[18,39] Lower amounts of Gelsolin were previously observed in plasma of rats with myocardial ischaemia and in patients with myocardial infarction.[40,41] In particular, the lack of F-actin removal, consequent to reductions in circulating levels of Gelsolin, may favour platelet aggregation and lead to the formation of bigger thrombi less prone to lysis, which could lead to fatal thrombosis.[42] Interestingly, opposite to circulating levels, we found greater amounts of Gelsolin in platelets of $BDNF^{Met/Met}$ mice, which is in accordance with reported data in ischemic rats and in ACS patients,[40,41] suggesting an alteration in protein trafficking.

In addition, activation of inflammation has been proposed as a link between depression and heart disease: specifically, an increase in proinflammatory cytokines, e.g. IL-6, results in a prothrombotic phenotype with endothelial perturbation and activation of circulating platelets.[4] Consistent with this hypothesis, the $BDNF^{Met/Met}$ mouse well recapitulates the association between depression/anxiety, inflammatory state (e.g. increased plasma IL-6, monocytes, and ESR) and thrombosis. Nevertheless, the molecular mechanisms underlying these phenomena are not yet understood.

Recently, it has been reported that the $proBNDF^{Met}$ interacts with SorCS2 and promotes neurons alteration.[20] Here, we show that the $BDNF^{Met}$ sequence transfected in HeLa cells modulates proinflammatory/thrombotic genes via SIRT1/SorCS2 pathway, suggesting a direct effect of the BDNFVal66Met polymorphism on cell activation. In addition, treatment with resveratrol reverted the prothrombotic phenotype in $BDNF^{Met/Met}$ mice (see Supplementary

Table 1 Association of the minor allele of rs6265 with AMI onset in CAD patients.

Model	Minor allele	TEST	NMISS	OR	STAT	*P*-value
1	A	REC	979	1.857	2.263	0.0236
2	A	REC	979	1.844	2.230	0.0257
3	A	REC	969	1.816	2.127	0.0334

Logistic Regression Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: adjusted for age, sex, body mass index, diabetes, dyslipidemia, hypertension, and smoking habit; TEST, code for the test assuming a recessive effect; NMISS, number of non-missing patients included in analysis; for 10 patients one covariate (BMI) was unknown; OR, odds ratio; STAT, coefficient of *t*-statistics; *P*, asymptotic *P*-value for *t*-statistics.

material online, *Figure S10*) supporting the potential protective effects of SIRT1 in atherothrombosis.[31] However, the use of non-selective SIRT1 activator may represent a limitation of *in vivo* study.

These findings are consistent with the reported involvement of BDNFMet genotype in the predisposition to CAD associated with depression.[7,8] In contrast, data concerning the impact of BDNFVal66Met polymorphism in CAD patients without depressive disorders are conflicting: indeed, protection against unstable angina and/or no effect of this genotype were reported.[10–12] Based on the evidence that the BDNFVal66Met polymorphism is linked to the occurrence of acute thrombosis in mice, we genotyped patients with severe CAD previously enrolled at our Centre, to look for the prevalence of the Met allele in patients presenting with an overt, acute coronary thrombotic event. Remarkably, Met homozygosity was significantly associated with AMI, independently of age, sex and major cardiovascular RFs. The main limitations of this study include its retrospective design, single-centre site, and inadequate power: thus, it needs to be confirmed in appropriately designed prospective studies. Nevertheless, these data provide support for a contribution of this BDNF polymorphism in the individual propensity for arterial thrombosis related to the occurrence of AMI.

Deciphering the role of BDNFVal66Met-related pathway in thrombosis might point out new tailored approaches for the prevention of the cardiovascular risk. Studies elucidating the mechanistic link between depression and ACS will possibly pave the way for designing single-targeted drugs with beneficial effects on both mood and cardiovascular disorders.

Supplementary material

Supplementary Material is available at *European Heart Journal* online.

Authors' contributions

P.A., C.B., S.S.B., M.B., G.I.C., and A.I., E.T. performed statistical analysis. S.S.B., and E.T. handled funding and supervision. P.A., S.S.B., M.B., G.I.C., S.G., A.I., A.P., E.T., and J.P.W. acquired the data. S.S.B. conceived and designed the research. S.S.B., E.T., and G.I.C. drafted the manuscript. F.S.L. made critical revision of the manuscript for key intellectual content.

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