

# BDNF polymorphism predicts the rate of decline in skilled task performance and hippocampal volume in healthy individuals

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Numerous studies have indicated a link between the presence of polymorphism in brain-derived neurotrophic factor (BDNF) and cognitive and affective disorders. However, only a few have studied these effects longitudinally along with structural changes in the brain. This study was carried out to investigate whether valine-to-methionine substitution at position 66 (val66met) of pro-BDNF could be linked to alterations in the rate of decline in skilled task performance and structural changes in hippocampal volume. Participants consisted of 144 healthy Caucasian pilots (aged 40–69 years) who completed a minimum of 3 consecutive annual visits. Standardized flight simulator score (SFSS) was measured as a reliable and quantifiable indicator for skilled task performance. In addition, a subset of these individuals was assessed for hippocampal volume alterations using magnetic resonance imaging. We found that val66met substitution in BDNF correlated longitudinally with the rate of decline in SFSS. Structurally, age-dependent hippocampal volume changes were also significantly altered by this substitution. Our study suggests that val66met polymorphism in BDNF can be linked to the rate of decline in skilled task performance. Furthermore, this polymorphism could be used as a predictor of the effects of age on the structure of the hippocampus in healthy individuals. Such results have implications for understanding possible disabilities in older adults performing skilled tasks who are at a higher risk for cognitive and affective disorders.

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## Introduction

The neurotrophin family is considered recent in the evolutionary scale and has not been identified in *Caenorhabditis elegans*.<sup>1</sup> It has been estimated that nerve growth factor and brain-derived neurotrophic factor (BDNF) evolved ~600 million years ago from a common ancestral gene.<sup>2</sup> However, unlike nerve growth factor, BDNF has been remarkably conserved in lower vertebrates and in mammals with ~90% homology between fish and mammalian BDNF. The extremely high conservation rate in the BDNF gene suggests that alterations in this gene have not been evolutionarily tolerated.<sup>2</sup> For this reason, the occurrence of single-nucleotide polymorphism(s) (SNPs) in BDNF may lead to significant structural and functional consequences. BDNF binding and internalization have been shown to have important roles in neuronal survival, differentiation,<sup>3</sup> axonal path finding,<sup>4</sup> regulation of dendritic trafficking to post-synaptic densities,<sup>5</sup> protection against neuronal death in the hippocampus<sup>6</sup> and induction and maintenance of late-phase potentiation.<sup>7</sup> Furthermore, reduced levels of cortical BDNF have been shown to correlate with the severity of pathology in mouse models of Alzheimer's disease (AD).<sup>8</sup> These studies emphasize the fact that any abnormalities in BDNF would exert significant functional implications on the brain.

The most widely studied polymorphism in BDNF is val66met substitution (rs6265), which has consistently been linked to the occurrence of depression in elderly and adolescents,<sup>9,10</sup> depressive symptoms associated with AD<sup>11</sup> and stroke.<sup>12</sup> Other psychiatric conditions linked to this substitution include anorexia nervosa,<sup>13</sup> anxiety-related disorders,<sup>14</sup> depressive episodes in bipolar disorder,<sup>15</sup> suicidal behavior,<sup>16</sup> schizophrenia<sup>17</sup> and introversion.<sup>18</sup>

The G-to-A substitution in the coding exon VIII of the BDNF gene (G196A) leads to substitution of amino-acid valine to methionine at position 66 of the pro-BDNF protein (val66met). The prevalence of this substitution has been found to be the highest in Asia (~43%) and lowest in Sub-Saharan Africa (~0.5%). In the United States, the prevalence of met carriers has been reported to be around 18–32%. Interestingly, small Native-American communities in Arizona show higher prevalence of met alleles (40%).<sup>19,20</sup>

The val66met substitution occurs in the pro-domain of the BDNF protein and is thus unlikely to affect the intrinsic activity of mature BDNF. However, it seems that val66met substitution alters BDNF processing and thus the outcome of BDNF-TrkB signaling. It has been suggested that val66met substitution can alter the rate of activity-dependent BDNF release by (1) affecting its proper folding and sorting into

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**Figure 1** Schematic representation of possible mechanism by which val66met substitution in BDNF leads to failed activity-dependent release of BDNF or reduced intracellular transport. Val66met substitution changes the dynamics of interaction between BDNF and two important proteins: (1) Sortilin, is involved in intracellular sorting and pro-neurotrophin signaling. Val66met substitution leads to reduction of BDNF interaction with this protein, which results in mis-sorting into the constitutive secretory pathway instead of activity-dependent release. (2) Translin is a highly conserved protein involved in mRNA transport. An exon found in all BDNF mRNA splice variants contains a specific translin-binding region, which is essential for appropriate BDNF mRNA dendritic targeting. It has been shown that Val66met substitution diminishes BDNF mRNA interaction with translin, which leads to reduced translocation of *BDNF* mRNA to dendrites.

secretory vesicles and/or (2) reducing its intracellular distribution: Within the Golgi apparatus, BDNF can be sorted into regulated (activity dependent) or constitutive secretory pathways. Sortilin, a member of the vacuolar protein sorting 10 family, is highly enriched in the Golgi apparatus and has an important role in BDNF processing.<sup>21</sup> Through binding to the pro-domain region of BDNF, sortilin has an important role in sorting BDNF into the regulated secretory pathway. The occurrence of val66met substitution in this region leads to a significant decrease in BDNF interaction with sortilin<sup>21</sup> thereby mis-sorting of BDNF into the constitutive secretory instead of the regulated secretory pathway (Figure 1). In neurons, BDNF is not exclusively synthesized in the somata. It has been shown that fragments of *BDNF* mRNA are transported from cell body to dendrites for local synthesis.<sup>22</sup> BDNF gene expression is achieved by formations of two types of BDNF mRNA, those with short 3' untranslated region and others with long 3' untranslated region. The long 3' untranslated region of the *BDNF* mRNA is transported to the dendrites, whereas the short 3' untranslated region remains within the cell body.<sup>23</sup> It has been suggested that by reducing interaction with translin, that is, an mRNA-binding protein, G-to-A substitution in the *BDNF* gene leads to failed translocation of *BDNF* mRNA to dendrites,<sup>24</sup> thus reducing its intracellular distribution.

Owing to the critical role of BDNF in synaptic plasticity particularly in the hippocampus and the significant consequences of val66met substitution on BDNF processing and intracellular distribution, we asked whether this polymorphism can alter the rate of decline in skilled task performance along with changes in the hippocampal volume in the absence of any neurological or cognitive disorders.

Multiple studies have suggested that abnormalities in production, transport and signaling of BDNF have a significant role in affective disorders.<sup>25</sup> Volumetric brain alterations particularly in the hippocampus have also been linked to BDNF polymorphism.<sup>26</sup> Using a meta-analysis of 399 healthy individuals, Hajek *et al.*<sup>26</sup> reported a significant reduction in bilateral hippocampal volume in met carriers. Furthermore, Cathomas *et al.*<sup>27</sup> studied the relationship between multiple SNPs on the *BDNF* gene and verbal episodic memory. From 55 SNPs studied, only val66met polymorphism correlated, showing poorer performance in met carriers.<sup>28</sup> However, no study has investigated the correlation between skilled task performance in healthy individuals and BDNF polymorphisms. Such information could help us in understanding the complex physiology of cognitive and affective alterations associated with normal aging in older individuals attempting to perform skilled tasks such as driving, aviation or operating machinery.

This study was carried out to investigate whether val66met substitution in BDNF can (1) predict the rate of decline in performance of skilled tasks, (2) lead to structural alterations in the hippocampus and (3) alter the pattern of relationship between aging and hippocampal volume. To address these questions, we investigated the rate of decline in the skilled task of piloting an aircraft as determined by a standardized flight simulator score (SFSS). Standard flight simulation is a reliable and highly quantifiable method for measuring executive functions that are dependent on several aspects of cognition, in particular memory and attention, shown by a significant correlation between SFSS and cognition measured by CogScreen-AE.<sup>29,30</sup>

**Table 1** Demographic information

Parameter	Met carriers (n = 55)	Non-met carriers (n = 89)
Age range (years)	42–69	40–69
Age (mean ± s.d.)	56.8 ± 0.9	57.1 ± 0.9*
Education range (years)	13–20	12–20
Education (mean ± s.d.)	17 ± 0.2	17 ± 0.2**
Apoε4 (%)	21 (38.0%)	55 (62.0%)
Females (%)	7 (7.9%)	15 (6.0%)
% Aviation expertise rating VFR/IFR/CFII-ATP <sup>a</sup>	25.4/58.2/16.3%	22.4/49.4/28.0%
Log hours range	310–13000	310–13990
Log hours (mean ± s.d.)	1913.8 ± 2454	2717.2 ± 2845

Abbreviations: BDNF, brain-derived neurotrophic factor; CFII/ATP, certified flight instructor/air transport planes; IFR, instrument flight rules; VFR, visual flight rules.

\* $P = 0.7936$ .

\*\* $P = 0.6319$ .

The homozygous (met/met) and heterozygous (val/met) haplotypes of the BDNF rs6265 polymorphism were both included as met carriers. <sup>a</sup>IFR, allowed to fly using instruments for accurate navigation after at least 40 h of training. CFII/ATP, certified to fly air-transport planes. VFR, flying under visual conditions for pilots when first get a license and are restricted to flying only in good visibility conditions.

## Materials and methods

**Participants.** Participants included 144 Caucasian pilots who completed a minimum of 3 annual visits (Table 1) as part of the ongoing longitudinal Stanford/VA Aviation Study, approved by the Institutional Review Board of Stanford University. After complete description of the study to the subjects, written informed consent was obtained. Enrollment criteria included age older than 40 years, current Federal Aviation Administration medical certificate and currently flying. The participants were either recreational pilots, certified flight instructors or civilian air-transport pilots. Upon enrollment, participants were assigned to one of three levels of expertise based on their Federal Aviation Administration proficiency rating: (1) least expertise: VFR (qualified to fly under visual flight rules only); (2) moderate expertise: IFR (also qualified to fly under instrument flight rules); and (3) most expertise: CFII or ATP (certified flight instructor for IFR students or rated for flying air-transport planes). Each rating requires progressively more advanced training and more hours of training.<sup>31</sup> Participants were healthy as determined by their regular physical and mental examinations performed by trained physicians. The absence of cognitive impairment was confirmed by CogScreen-AE, that is, a computerized battery for screening and monitoring cognitive abilities relevant to flying.<sup>32</sup>

**Flight simulation.** Standard flight simulation enabled us to investigate the ability to navigate an aircraft in a quantifiable and reproducible manner.<sup>33</sup> We used a Frasca 141 flight simulator (Urbana, IL, USA), which simulates flying a small single-engine aircraft. Each individual went through six practice sessions and a 3-week break before the first baseline visit. The baseline visit and each visit thereafter consisted of morning and afternoon flights, 75-min in duration each, during which pilots had to face flight scenarios with emergency situations such as engine malfunctions and/or incoming air traffic. For each subsequent flight, several

components were randomly varied and multiple versions of the flight scenario were presented to reduce learning of specific maneuvers and air traffic control messages. The scoring system produced 23 variables that measured a combination of the reaction time (in seconds) and deviations from ideal positions (altitude in feet, heading in degrees and airspeed in knots).<sup>33</sup> Standardized Z-scores were computed to match different measurements. In addition, an overall flight performance summary score, that is, SFSS was computed. The scores collected represent the accuracy of executing the air traffic control commands, traffic avoidance, time to detect engine emergencies and executing a visual approach to landing. The primary measure of the outcome was the average rate of change per year in overall SFSS as determined by the slope of SFSS. Each flight simulation was followed by 40–60 min battery of cognitive tests including CogScreen AE.<sup>32</sup> These tests were repeated annually.

**Genotyping.** Blood and saliva samples were collected during the first visit. We used an Illumina Bead Array platform (Illumina, San Diego, CA, USA) for high-throughput multiplexed SNP genotyping using Human 610 Quad gene chip. The system uses a high-density BeadArray technology in combination with an allele-specific extension, adapter ligation and amplification assay protocol.<sup>34</sup> For quality control, Illumina's BeadStudio Software (version 3.1) was used to validate the genotypes. Golden Helix SNP and Variation Suite (SVS Version 7.2.2, Bozeman, MT, USA) software was used for the analysis. As ApoE polymorphism markers were not present on HumanHap 650Y chip, a sample of the DNA was separately used for ApoE alleles determination,<sup>35</sup> which led to successful genotyping in 98.6% of participants.

**Imaging.** Around one-third of participants (43 individuals) underwent structural magnetic resonance imaging (MRI) neuroimaging. From these, 65% (28 individuals) returned 1–2 times for follow-up imaging. The average time lag between the baseline simulator visit and the first MRI scan was 3.85 years (s.d. = 3.0 years).

The MRI data were acquired at the Veterans Affairs Palo Alto Health Care System on a 1.5-T (General Electric Medical Systems, Milwaukee, WI, USA) MRI scanner. The following structural MRI sequences were performed on all participants using a standard head coil: (1) a spin-echo, sagittal localizer two-dimensional sequence of 5-mm thick slices; (2) a proton density and T2-weighted spin-echo MRI, repetition time/echo time/echo time2 = 5000/30/80 ms, 51 oblique axial 3 mm slices covering the entire brain and angulated parallel to the long axis of the hippocampal formation (1.00 × 1.00 mm<sup>2</sup> in plane resolution); and (3) three-dimensional spoiled gradient recalled acquisition in the steady state (GRASS) MRI of the entire brain, repetition time/echo time = 9/2 ms, perpendicular to the long axis of the hippocampus.

The volume of the hippocampus was determined using high-dimensional brain warping software (Surgical Navigation Technologies, Louisville, CO, USA). The Surgical Navigation Technologies method has been validated and compared with manual tracing of the hippocampus before (left:  $r = 0.92$ ; right:  $r = 0.91$ ;  $n = 60$ ).<sup>36</sup> This method, originally developed by

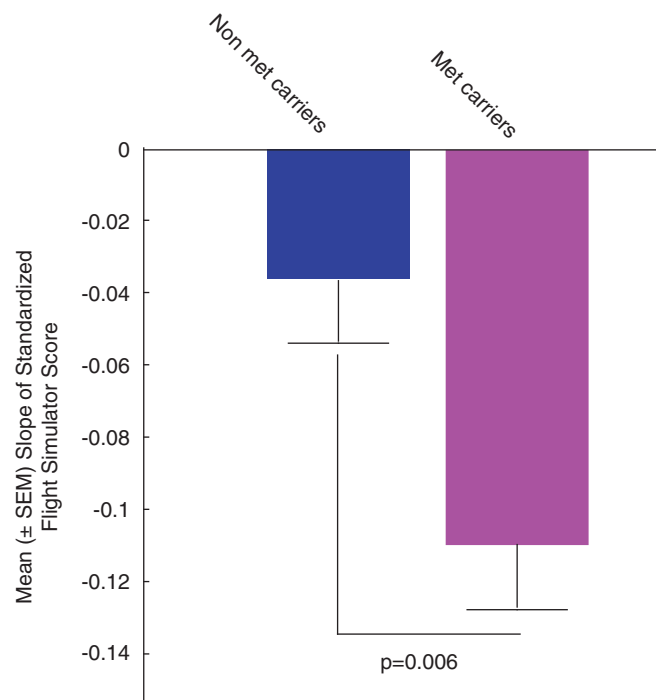
Christensen *et al.*,<sup>37</sup> has been used in numerous studies on AD,<sup>38,39</sup> aging<sup>36,40,41</sup> and schizophrenia.<sup>42</sup> The Surgical Navigation Technologies semi-automated technique involves manual placement of global and hippocampal landmarks, followed by automated atlas mapping. In this study, global landmarks were placed at external boundaries of the brain image (by manually adjusting the angle and dimension of a three-dimensional proportional box to fully enclose the brain). Next, 22 local landmarks were placed at the boundaries of the hippocampus: 1 at the head, 1 at the tail and 4 per image (that is, at the superior, inferior, medial and lateral boundaries) on 5 equally spaced images perpendicular to the long axis of the ipsilateral hippocampus. The automated hippocampal mapping algorithm applied a coarse transformation followed by a fine and fluid transformation to match the individual image to a template brain. The final output included numerical volumes of the right and left hippocampi. In this study, we report total hippocampal volumes, normalized to each subject's total intracranial volume.

**Statistical analyses.** The primary outcome for skilled task performance was SFSS.<sup>31</sup> The average rate of change in SFSS was computed by calculating the slope of linear regression between SFSS and the number of three consecutive annual visits yielding a fitted slope. The analysis of variance and Student's *t*-tests were used to test the difference among met positive (met/val and met/met) and met negative (val/val) groups. A *P*-value <0.05 was considered significant.

## Results

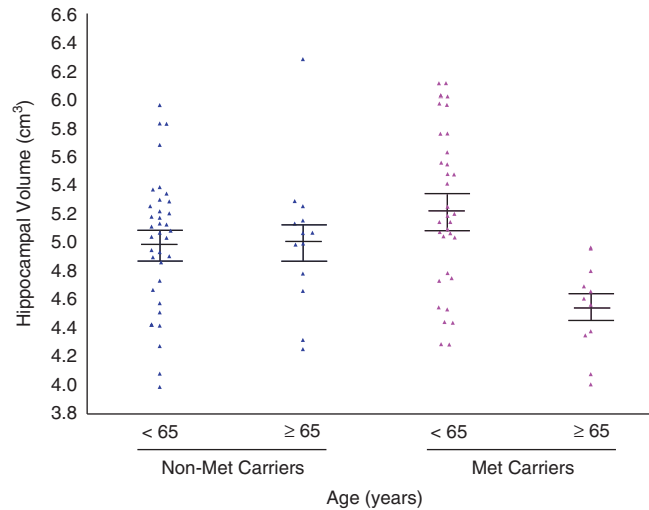
**Demographic characteristics.** A total of 144 individuals underwent 3 consecutive annual flight simulations. Of these, 55 (38.2%) were met carriers (met/val or met/met) and 89 (61.8%) were non-met carriers (val/val). The prevalence of met carriers was close to demographics reported in healthy individuals.<sup>43</sup> As shown in Table 1, met carriers and non-met carriers were demographically similar. No significant differences were found in age and education levels between the two groups (Table 1). Both groups had average of 17 years of education. ApoE genotyping showed that 52% of the participants were ApoE33, 25% ApoE34, 16.6% ApoE23, 2.77% ApoE44, 1.38% ApoE22 and 0.69% were ApoE24. We found no significant effects of ApoE4 alleles on the rate of decline of SFSS ( $t = -0.673$ ;  $P = 0.5020$ , d.f. = 140) and hippocampal volume ( $t = 1.534$ ,  $P = 0.1286$ , d.f. = 84).

**Standard flight simulator score.** Only the data derived from the first three consecutive annual visits were used for this part of study. The effect of polymorphisms in *BDNF* was investigated by quantifying the rate of decline in SFSS during three consecutive annual visits using a linear regression. The average rate of decline in SFSS over the next 2 years was significantly higher in met carriers than in non-met carriers (met carriers =  $-0.109 \pm 0.134$  ( $n = 55$ ), non-met carriers =  $-0.036 \pm 0.165$  ( $n = 89$ ),  $F = 7.696$ ,  $P = 0.0060$ ) (Figure 2).



**Figure 2** The slope of the SFSS in individuals with and without val66met substitution. As shown, we found a significant (ANOVA,  $F = 7.696$ ,  $P = 0.0060$ ) reduction in the slope of flight simulator score in met carriers (mean  $\pm$  s.d., slope =  $-0.110 \pm 0.135$ ,  $n = 55$ ) compared with non-met carriers (slope =  $-0.036 \pm 0.165$ ,  $n = 89$ ) during the first 2 years of follow-up.





**Figure 3** The total volume of the hippocampus (both left and right) in individuals with and without val66met substitution. No significant differences were found in the size of the hippocampus, before and after 65 years in non-met carriers (before 65 =  $100.0 \pm 9.63$  and after 65 =  $100.556 \pm 10.265$ ;  $F = 0.031$ ,  $P$ -value = 0.8610). However, there was a significant reduction in the size of the hippocampus in met carriers after the age of 65 years (before 65 =  $104.654 \pm 9.938$  and after 65 =  $90.918 \pm 6.522$ ,  $F = 17.53$ ,  $P$ -value = 0.0001). As the result, we found a significant difference in the size of the hippocampus after the age of 65 years between met carriers and non-met carriers ( $F = 7.207$ ,  $P$ -value = 0.014). The number of individuals in their sixth, seventh and eighth decades of life were 15, 15 and 6 among met carriers and 19, 26 and 5 among non-met carriers, respectively.

**Effects of flight experience.** No significant differences were found in mean log hours of flight between met and non-met carriers (met carriers =  $1913.7 \pm 2454$  h; non-met carriers =  $2717.2 \pm 2845$  h,  $t$ -value = 1.733,  $P = 0.0852$ , d.f. = 142). To further test the possible effects of experience on the results, we compared a subgroup of individuals with very similar log hours (met carriers =  $559.9 \pm 184.81$  h, non-met carriers =  $589.77 \pm 197.77$  h). Our investigation showed a significant difference ( $P = 0.0043$ ,  $t$ -value = 2.974, d.f. = 56) in the average rate of decline in SFSS for met carriers compared with non-met carriers, indicating that the difference in the slope of the SFSS was not significantly influenced by flight experience.

**Hippocampal volume.** The total volume of the entire hippocampus (right plus left) normalized to the total intracranial volume was quantified in one-third of individuals during the course of the study.

**Cross-sectional analysis.** The total volume of the hippocampus was compared between met and non-met carriers. We found no significant effects of val66met substitution on the average hippocampal size for all visits (met-carriers =  $5.042 \pm 0.552$  cm<sup>3</sup>,  $n = 19$ , non-met carriers =  $5.026 \pm 0.487$  cm<sup>3</sup>,  $n = 24$ ,  $F = 0.047$ ,  $P = 0.8290$ ). We also investigated the link between hippocampal volume and SFSS and found no significant correlation between the two parameters ( $r = -0.101$ ,  $P = 0.346$ ), which was not affected by BDNF polymorphism (met carriers,  $r = -0.058$ ,  $P = 0.7290$ , non-met carriers  $r = -0.117$ ,  $P = 0.4140$ ).

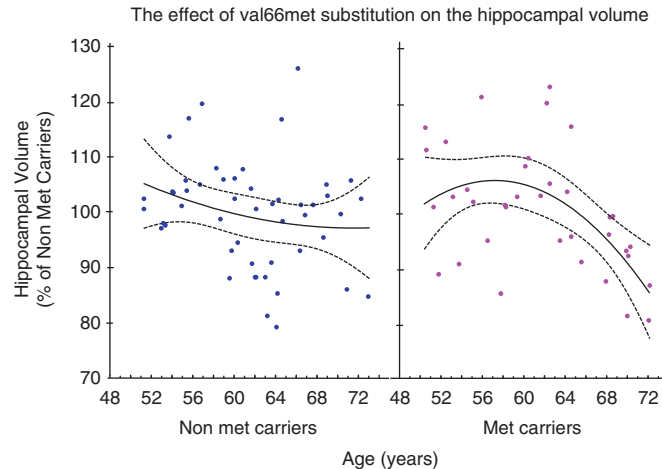
It has been shown that there is a negative correlation between hippocampal volume and age in healthy individuals.<sup>44</sup> The question was raised whether val66met substitution can alter the pattern of relationship between hippocampal volume and age. Although we found no significant correlation

between the age at MRI and the volume of hippocampus in non-met carriers ( $r = -0.178$ ,  $P = 0.2639$ ), a significant negative correlation was detected between the two among met carriers ( $r = -0.447$ ,  $P = 0.0150$ ). Furthermore, the slope of the age-related decline in hippocampal volume in met carriers (slope =  $-0.038$ ) was two folds higher than that in non-met carriers (slope =  $-0.016$ ) (Figure 3). Our results also showed that atrophy of the hippocampus became significant after the age of 65 years. Unlike non-met carriers who showed no difference in hippocampal volume before and after the age of 65 years, we found a significant shrinkage in hippocampal volume among met carriers after the age of 65 years (Figure 4).

**Longitudinal analysis.** Around 65% ( $n = 28$ ) of participants who underwent MRI, returned an average of  $4.36 \pm 2.78$  years later for follow-up imaging. The slope of hippocampal volume alterations for each individual was computed. The average slope of hippocampal volume alterations was quantified longitudinally among met carriers ( $n = 12$ ) and non-met carriers ( $n = 16$ ), even though the follow-up interval was not consistent. We found no significant effects of val66met substitution on the rate of decline in hippocampal volume ( $r = -0.089$ ,  $P = 0.6500$ , met carriers =  $-0.105 \pm 0.124$  and non-met carriers =  $-0.084 \pm 0.118$ ).

## Discussion

In this study, we found that val66met substitution in BDNF could predict the rate of decline in skilled task performance in middle-aged and older healthy individuals. Furthermore, the rate of decline in hippocampal volume could be significantly affected by this substitution. These results are consistent with a significant reduction in hippocampal volume after the age of 65 years in met carriers as compared with non-met carriers, reported here.



**Figure 4** The relationship between the total volume of the hippocampus and the age and the effects of val66met substitution. Using a polynomial fitting curve, we found a ( $r = -0.447$ ,  $P = 0.0150$ ) correlation between age and the volume of the hippocampus in met carriers. No such correlation was found in non-met carriers ( $r = -0.178$ ,  $P = 0.2639$ ). Furthermore, the slope of the regression in met carriers (slope =  $-0.038$ ) was twice the value of the slope of regression in non-met carriers (slope =  $-0.016$ ).

Our finding of a two-fold increase in the rate of decline in SFSS among met carriers during the first 2 years of follow-up is consistent with the poorer performance and functioning of met carriers described by Egan *et al.*<sup>45</sup> The negative effect of val66met substitution on the hippocampus and synaptic activity has been supported by decreased levels of *N*-acetyl-aspartate, an intracellular marker for neuronal and synaptic structural integrity.<sup>46</sup> Furthermore, it has been shown that met carrier individuals show lower hippocampal activity than do non-met carriers during encoding and retrieval activities.<sup>47</sup> This is supported by a number of studies reporting 11–15% reduction in the volume of hippocampus in healthy individuals with val66met substitution.<sup>48–52</sup> The effect of age on hippocampal volume in met carriers has also been described in association with changes in the amygdala.<sup>53</sup> Our results are also in concordance with studies that showed no effects of val66met substitution on hippocampal volume in younger adults.<sup>47</sup> Interestingly, val66met substitution leads to lower scores in episodic memory in schizophrenic patients when compared with their healthy relatives.<sup>54</sup> Finally, as already noted, BDNF abnormalities have been associated with affective abnormalities, which in turn might affect motivation and performance on cognitive tests and skilled tasks.

We did not find a significant correlation between SFSS and hippocampal volume in our healthy participants. This could be due to two reasons: (1) executive functioning is not solely dependent on the hippocampus. Multiple other brain structures and circuits have vital roles in this function and (2) measurements of hippocampal volume and SFSS, for each individual, were determined at different intervals, which would make it difficult to detect a significant correlation.

Although the direct consequences of val66met substitution on BDNF levels remain to be clarified, there are a number of studies that suggest molecular mechanisms by which it could lead to a more severe decline in executive function and reduced hippocampal volume. Postmortem examination of the parietal cortex of AD patients has shown > 70% decrease in BDNF mRNA<sup>55</sup> and 40% decrease in pro-BDNF levels.<sup>56</sup>

The role of met alleles on cognition in AD has been controversial. It has been reported that the frequency of A alleles (met) in a Japanese AD population was lower than that of controls by 5%.<sup>57</sup> In the Mainland Chinese Han population, although a similar frequency of A alleles was found among AD and controls, they reported a higher frequency of A alleles in ApoE4 female carriers with AD.<sup>58</sup> However, multiple studies have failed to find higher frequency of A alleles in AD.<sup>59–62</sup> These controversial results might be explained by additional polymorphism(s) that may have a role in the occurrence of AD. One example is the C270T polymorphism in the non-coding area of the *BDNF* gene, which has been linked to a higher risk of AD in a Japanese population.<sup>63</sup> In addition, the presence of several polymorphisms might have synergic effects on the occurrence of cognitive dysfunction. As mentioned above, Bian *et al.*<sup>58</sup> found a higher frequency of val66met substitution in AD patients only in female ApoE4 carriers subgroup.

Our finding of the relationship between decline in skilled task function and val66met polymorphism may have important implications for intervention. BDNF has a major role in activity-dependent plasticity in the hippocampus.<sup>64,65</sup> Indeed, BDNF null mice show pronounced synaptic fatigue in CA1 synapses during high-frequency stimulation, reduced number of docked vesicles and decline in synaptic markers in this area.<sup>66</sup> Accordingly, *BDNF* gene delivery has been shown to reverse neuronal atrophy and cognitive deficits in aged primates and rodent models of AD.<sup>67</sup> Furthermore, *BDNF* knockout mice show decreased serotonergic axonal terminals, reduced levels of 5-HT and its metabolite (5-HIAA) in the hippocampus<sup>68</sup> and increased depression-like behaviors.<sup>69</sup> As val66met polymorphism leads to decreased levels of activity-dependent BDNF release,<sup>45</sup> strategies to therapeutically increase BDNF levels including gene therapy and/or the use of small molecules binding to NTRK2 receptors<sup>70</sup> might have successful outcome in treating depression and its associated cognitive deficits than selective serotonin reuptake inhibitors (SSRIs) that would target one particular system. There is already evidence that different anti-

depressive therapies including antidepressants,<sup>71</sup> electroconvulsive therapy<sup>72</sup> and repetitive transcranial magnetic stimulation<sup>73</sup> have all shown to increase BDNF levels in animal models of depression. Thus, there is the possibility that further research in therapeutics might lead to better treatments for cognitive and affective disorders.

There are a number of limitations to this study. First, for quantification of skilled task performance and decline in SFSS, we followed the participants for three consecutive annual visits. There is no doubt that much longer follow-ups and bigger samples size are required to better define the trend and confirm the link with val66met polymorphism. Second, only about a third of individuals enrolled in this study had neuroimaging for hippocampal volume alterations. Similar to the first point, larger sample size and measurement in multiple consecutive annual visits are required to confirm our findings. Having a larger group would help us to better determine the role of other factors such as gender, ethnicity, presence of other polymorphisms and expertise on the effects of val66met substitution on both cognition and brain structural changes that may impact SFSS.

Testing executive function in this study was performed using a flight simulator. Although the result of this study might have some implication for performance in pilots, it may also reflect age-dependent alterations in executive functions in the general public performing any skilled tasks that involve working with complex machinery. Thus, we anticipate the need of a larger longitudinal study using additional means of simulations.

In conclusion, we found that the val66met substitution was able to predict the rate of regression in SFSS and may be considered as a tool to evaluate the longitudinal decline in cognitive functioning of healthy individuals. Furthermore, it seems to modify the nature of the relationship between age and hippocampal volume. Careful genetic and neurochemical studies of BDNF may eventually lead to therapeutic interventions of benefit to older adults with cognitive and affective disorders.

## Conflict of interest

The authors declare no conflict of interest.

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