Effect of Bcl-2 rs956572 SNP on regional gray matter volumes and cognitive function in elderly males without dementia

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Abstract The Bcl-2 gene is a major regulator of neural plasticity and cellular resilience. A single-nucleotide polymorphism (SNP) in the Bcl-2 gene, Bcl-2 rs956572, significantly modulates the expression of Bcl-2 protein and cellular vulnerability to apoptosis. This study investigated the association between the Bcl-2 rs956572 SNP and brain structural abnormalities

in non-demented elders, and to test the relationship between neuropsychological performance and regional gray matter (GM) volumes. Our sample comprised 97 non-demented elderly men with a mean age of 80.6 ± 5.6 years (range, 65 to 92 years). Cognitive test results, magnetic resonance imaging, and genotyping of Bcl-2 rs956572 were examined for each subject. The differences

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in regional GM volumes between G homozygotes and A-allele carriers were tested using optimized voxel-based morphometry. Subjects with G homozygotes exhibited significantly worse performance in the language domain of the Cognitive Abilities Screening Instrument (CASI; $p=0.009$). They also showed significantly smaller GM volumes in the right middle temporal gyrus (MTG) (BA 21), but larger GM volumes in the left precuneus (BA 31), right lingual gyrus (BA 18), and left superior occipital gyrus (BA 19) relative to A-allele carriers ($p<0.001$). A trend toward a positive correlation between right MTG GM volumes and the language domain of CASI was also evident ($r=0.181$; $p=0.081$). The findings suggest that Bcl-2 rs956572 SNP may modulate cognitive function and regional GM volume in non-demented elderly men, and that this polymorphism may affect language performance through its effect on the right MTG.

Keywords Bcl-2 · MRI · Volumetry · Cognition · Aged · Polymorphism

Introduction

The anti-apoptotic protein B cell CLL/lymphoma 2 (Bcl-2) is a major anti-apoptotic protein that inhibits apoptotic and necrotic cell death induced by a diverse set of adverse conditions (Chen and Manji 2006). Bcl-2 also plays critical roles in neuronal morphogenesis and synaptic plasticity (Chen et al. 1997; Jonas 2006), and reduced Bcl-2 function is hypothesized to contribute to the impairment of cellular plasticity and resilience in neuropsychiatric patients (Chen and Manji 2006). Accumulated evidence supports the hypothesis that Bcl-2 is important in the etiology of cognitive deficits, and that this accounts for the therapeutic reaction to anti-dementia agents (Caraci et al. 2005; Sultana et al. 2010).

Cognitive impairment is frequently observed in patients with status epilepticus and is closely associated with selective neuronal loss. A recent animal study provided evidence that higher levels of Bcl-2 play a key role in preventing cognitive impairment induced by status epilepticus, through suppressing apoptotic neuronal cell death (Jun et al. 2009). Nicergoline, a drug used for age-dependent cognitive impairment, was found to upregulate Bcl-2 expression and protein levels, thereby exerting neuroprotective effects against β -amyloid (A β)

toxicity (Caraci et al. 2005). Furthermore, the beneficial effect of Bcl-2 in supporting central neurons may occur through intracellular calcium signaling, which stimulates the regenerative response and neuritogenesis (Jiao et al. 2005). This mechanism may be responsible for cognitive performance and may be involved in the pathogenesis of cognitive impairment (Berridge 2011). Collectively, these various findings suggest that Bcl-2 might play a critical role in modulating cognitive performance.

Recently, reporting on their research with B lymphoblast cell lines, Uemura and colleagues (2011) stated that an intronic single-nucleotide polymorphism (SNP) in the Bcl-2 gene (rs956572) exerts functional effects on Bcl-2 expression in bipolar disorder patients. The Bcl-2 messenger RNA (mRNA) and protein levels were lowest in patients with the G/G genotype, compared with patients with other genotypes (G/A and A/A) and with healthy subjects. However, a separate *in vivo* study found the A/A genotype to be associated with significantly lower Bcl-2 mRNA expression and lower protein levels, compared with G/G genotype (Machado-Vieira et al. 2011). Both studies showed that the Bcl-2 polymorphism was associated with intracellular calcium homeostasis in lymphoblast cells taken from bipolar disorder patients. A growing body of evidence indicates a relationship between Bcl-2 level and cognition (Sultana et al. 2010), and further suggests that calcium signaling could modulate cognitive function in humans (Berridge 2011). Thus, further research to determine whether this genetic variant may be associated with cognitive performance is warranted.

Because of the important role of Bcl-2 in neural plasticity and cellular resilience, Salvatore et al. (2009) investigated the genetic effect of Bcl-2 rs956572 on regional brain gray matter (GM) volumes in healthy individuals ($n=47$) aged 19 to 60 years. They reported that, compared with G homozygotes, A-carriers showed less GM volume in left ventral striatum. A more recent study reported that structural alterations in cognitively normal elders were observable well before the onset of dementia, and that such alterations involving GM may be associated with subtle cognitive decline in groups of clinically normal persons (Smith 2011). Cognitive function is a complex and major issue in the aged, and increased vulnerability to Bcl-2-related apoptosis appears to be involved in the aging process (Toman and Fiskum 2011). Therefore, we tested the hypothesis that the Bcl-2 rs956572 polymorphism would affect some regional GM

volumes as well as specific cognitive function in elderly people without dementia.

Methods

Subjects and instruments

This study included 97 elderly Han Chinese male subjects recruited from the community and a public veterans home in northern Taiwan. Each subject was evaluated by a trained research assistant using a diagnostic structured Mini-International Neuropsychiatric Interview (Sheehan et al. 1998). Daily activities and cognitive functions were assessed using the Clinical Dementia Rating Scale (CDR). The exclusion criteria included the following: (1) presence of any diagnosis on Axis I of the DSM-IV, such as mood disorder or psychotic disorder; (2) neurobiological disorder, such as dementia, head injury, stroke, or Parkinson's disease; (3) severe medical illness such as malignancy, heart failure, and renal failure; (4) illiteracy; (5) subjects with CDR score of greater than 0.5, or a score of 50 or less on the Cognitive Abilities Screening Instrument, Chinese version (CASI C-2.0; Lin et al. 2002); and (6) having ferromagnetic foreign bodies or implants anywhere in the body that are electrically, magnetically, or mechanically activated. A study by Liu et al. (1994) found that, when using a cutoff CASI score of less than or equal to 50 for dementia, the sensitivity was 0.88 and the specificity was 0.94. This exclusion criterion screened out patients with possible dementia.

Our inclusion and exclusion criteria resulted in the recruitment of a group of non-demented elderly subjects. All participants had sufficient visual and auditory acuity to undergo cognitive testing. They were administered the CASI C-2.0 test and the Wechsler Digit Span Forward (DSF) and Backward (DSB) tests. The CASI test, which is a 100-point cognitive test and provides quantitative assessment in nine domains of cognitive function (long-term memory, short-term memory, attention, concentration/mental manipulation, orientation, abstraction and judgment, language, visual construction, and list-generating fluency), was designed for cross-cultural studies and for individuals with little or no formal education (Teng et al. 1994). It has been adapted into Chinese, and we used the Chinese version (Lin et al. 2002).

The research was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Taipei Veterans General Hospital. Written informed consent was obtained from all the subjects after they had been given an adequate understanding of the study.

Genotyping

Genomic DNA was extracted from peripheral blood with a commercial kit (Qiagen, Gentra Puregene Blood Kit). Genotyping procedures for identifying the rs956572 were performed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The following PCR primers which were synthesized by MISSION BIOTECH Co. (Taiwan) were used in the present study: forward, 5-AGAGGAAA GAGCACACAC-3 and reverse, 5-AGAACTCTAC TTCCAGGC-3. PCR reactions were performed in a 12.5- μ l final volume containing 1 $\times$ PCR buffer, 1.0 mM Mg²⁺, 0.2 mM dNTPs, 5 pmol of each primer and 0.3 U Taq polymerase. PCR cycles were the following: 95°C for 5 min followed by 35 cycles each of 95°C for 30 s, 53°C for 30 s, and 72°C for 30 s. A final extension step was undertaken at 72°C for 5 min. The 567-base pair (bp) sequences of the Bcl-2 gene were amplified by PCR, and their products were digested with restriction endonuclease DdeI (New England BioLabs Inc.). The ancestral allele G yielded three bands of 298, 108, and 161 bp, while the mutant allele A yielded two bands of 406 and 161 bp.

MRI acquisition

All MR scanning was performed at National Yang-Ming University, Taiwan, using a 3.0T Siemens MRI scanner with 12 channel head coil (Siemens Magnetom Tim Trio, Erlangen, Germany). High-resolution structural MR images were acquired with 3D magnetization-prepared rapid gradient echo sequence (TR=2,530 ms, TE=3.5 ms, TI=1,100 ms, FOV=256 mm, flip angle=7°, matrix size=256 $\times$ 256, 192 sagittal slices, voxel size=1.0 $\times$ 1.0 $\times$ 1.0 mm, no gap). All the images were acquired parallel to the anterior commissure–posterior commissure line. To minimize motion artifact generated during image acquisition, each subject's head was immobilized with cushions inside the coil.

DARTEL-based T1 VBM analysis

A Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL)-based T1 voxel-based morphometry (VBM) approach was used for preprocessing and subsequent analysis of whole-brain T1-weighted volumetric images (Ashburner 2007; Ashburner and Friston 2000). Individual T1-weighted volumetric images were analyzed using Gaser's VBM8 toolbox (<http://dbm.neuro.uni-jena.de>) within Statistical Parametric Mapping (SPM8, Wellcome Institute of Neurology, University College London, UK) executed in MATLAB 2010a (The MathWorks, Natick, MA, USA) under Linux 64-bit environment with default settings. VBM8 toolbox, which is an extension of the unified segmentation model (Ashburner and Friston 2005), included the following steps to improve the quality of brain image segmentation: denoising, intensity inhomogeneity correction, and tissue segmentation. All images from each participant were carefully checked by an experienced radiologist to ensure that they had no scanner artifacts, motion problems, or gross anatomical abnormalities. The anterior commissure was set as the origin of each T1-weighted image.

After the above procedures, segmentation approach in the VBM8 toolbox was applied in the initial native space combined the following techniques. Noise reduction was performed on each native space T1-weight structural image, using the spatial adaptive nonlocal means denoising filter (Manjon et al. 2010). Then, whole-brain native space T1-weighted images with a higher signal-to-noise ratio were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) components; for this step, we used an adaptive maximum a posteriori segmentation approach (Rajapakse et al. 1997) with partial volume estimation technique (Tohka et al. 2004). Images were further refined by applying an iterative hidden Markov random field model (Cuadra et al. 2005) to remove isolated voxels which were unlikely to belong to a determinate tissue type, and to improve the quality of tissue segmentation. To achieve higher accuracy of registration between subjects, the native space GM, WM, and CSF segments were imported into a rigidly aligned space and were iteratively registered to group-based templates, which were generated from all images included in the current study through nonlinear warping using DARTEL toolbox (Ashburner 2007) that implemented in SPM8.

The deformation parameters obtained in the previous step were used to modulate the GM, WM, and CSF tissue maps of participants' brains so as to compare volumetric differences across groups. Since DARTEL works with images according to the average brain size of all participants, additional affine transformation between average group space and Montreal Neurological Institute (MNI) standard space was needed. Because the MNI standard space was constructed by affine registration of a number of subjects to a common standard coordinate system, it was reasonable to use only affine transformation to achieve a suitable alignment between these two spaces. Finally, the modulated tissue segments were converted into an isotropic voxel resolution of $1 \times 1 \times 1 \text{ mm}^3$. All normalized, segmented, and modulated MNI standard space images were smoothed with an 8-mm Gaussian kernel ahead of tissue volume calculation and voxel-wised group comparisons. Segmented tissue volumes (i.e., GM, WM, and CSF) were estimated in cubic millimeters by counting the voxels representing GM, WM, and CSF in standard space. Total intracranial volume (TIV) was determined as the sum of GM, WM, and CSF volumes.

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 program (SPSS Inc., Chicago, IL). Student's *t* test and Chi-square test were applied to compare the continuous and categorical variables between the two groups (A-carriers and G/G), respectively. Smoothed modulated gray matter segments were analyzed with SPM8 utilizing the framework of General Linear Model. Analysis of covariance (ANCOVA) was employed by co-varying the age, education, and TIV to investigate the regional gray matter volume differences between two genotypic groups. To avoid possible partial volume effects around the margin between GM and WM, all voxels with a GM probability value lower than 0.2 (range from 0 to 1) were eliminated. The differences were deemed to be significant at the individual voxel level when the uncorrected *p* value was less than 0.001 and the extended cluster size was more than 338 voxels which was calculated from the expected number of voxels per cluster according to the theory of Gaussian random fields. We used the *icbm2tal* function from the GingerALE toolbox (The BrainMap Development Team; <http://brainmap.org/ale/index.html>) to transform MNI coordinates into Talairach coordinates and to minimize coordinate transformation discrepancy

between MNI and Talairach space. Anatomical structures of the coordinates representing significant clusters were identified on the basis of the Talairach and Tournoux atlas (Talairach and Tournoux 1988). To evaluate the neuroanatomical correlates of individual differences between SNP genotypes, partial correlation analysis using age, education level, and TIV as confounding covariates was performed to correlate the clinical scores (only the scores showing group differences) with the regional GM volume in whole participants. To our knowledge, using familywise error (FWE)-corrected p value surely reduces type I error (false positive) but also suffers from a lack of the power to detect a difference that actually exists. As a result, the findings could be false negative while using more conservative method. Therefore, the statistical criteria of uncorrected p value could make a balance that minimized type II errors as well as controlling type I errors as possible, and be applied in previous VBM studies (Bitter et al. 2011; Luders et al. 2009; Nenadic et al. 2010). In current study, we reported both uncorrected and FWE-corrected p value to provide comprehensive information of any possible relationship between Bcl-2 SNP and regional gray matter volumes. The regional gray matter volumes were extracted and summed up from the peak coordinates showing significant differences.

Results

From a total of 154 participants ≥ 65 years old without alleged medical or neurological disease, 55 subjects were excluded from MRI examination due to psychotic disorders ($n=4$), depressive disorders ($n=15$), illiteracy ($n=16$), and possible dementia ($n=20$). For the 99 subjects who completed MR scanning, images of two subjects were excluded from analysis due to the presence of brain mass. Finally, our study sample comprised 97 ethnic Chinese males with a mean age of 80.6 ± 5.6 years (range, 65 to 92 years). With regard to educational background, the sample had been schooled for an average of 5.3 ± 5.1 years (range, 0 to 18 years). Their mean DSF, DSB, and total CASI scores were as follows: DSF, 12.1 ± 2.9 (range, 3 to 18); DSB, 3.7 ± 2.8 (range, 0 to 15); and CASI, 86.9 ± 9.4 (range, 63 to 99). The demographic and neuropsychological characteristics of the Bcl-2 G homozygotes and A-allele carriers are reported in Table 1. We found no significant differences in DSF, DSB, and CASI total scores between G homozygotes

and A-allele carriers. Of the nine domains of CASI, the language domain scores were significantly associated with the Bcl-2 genotype. Carriers of the G homozygote showed decreased language ability compared with A-allele carriers ($p=0.009$).

By using SPSS software, ANOVA test did not yield any significant results for differences in total intracranial volume and total GM volume between the Bcl-2 genotype groups. DARTEL-based T1 VBM analyses revealed that G homozygote carriers had smaller GM volumes in the right middle temporal gyrus (MTG) (BA 21), but larger GM volumes of the left precuneus (BA 7), right lingual gyrus (BA 18), and left superior occipital gyrus (SOG) (BA 19) than did A-allele carriers (Table 2 and Fig. 1). Results were then checked under the criteria as FWE-corrected $p < 0.05$, but no significant difference was found between two groups in current study. Due to the main effect of Bcl-2 on cognitive function which was found in language domain of CASI, we only tested whether the affected GM volumes were correlated with language score of CASI, using age, education, and TIV as covariates. We found a trend toward a positive correlation between larger volumes of right MTG and the language score ($r=0.181$; $p=0.081$.) We reanalyzed the ANCOVA test without using educational level as a covariate (only controlling for age and TIV), and the results remained similar to the above findings (Supplementary Table 1, Supplementary Figure 1). On the other hand, we replaced one confounding factor TIV to total brain volume (TBV) and calculated the ANCOVA test using age, educational level, and TBV as a covariate. There was no significant difference between conditions under TIV and TBV as confounding factor (Supplementary Table 2, Supplementary Figure 2).

Discussion

To the best of our knowledge, this was the first study to examine the effect of the Bcl-2 gene on cognitive function and brain structural changes in elderly people. The major findings of this study showed that non-demented elders who bore Bcl-2 rs956572 G homozygotes exhibited worse language performance and had smaller GM volumes in the right MTG compared with A-allele carriers, and the reduced volume in this region was related to poor language performance. In contrast, larger volumes were found in the left precuneus, right lingual gyrus, and left SOG of G homozygote carriers compared

Table 1 Demographical characteristics and neuropsychiatric tests between the Bcl-2 G homozygotes and A-allele carriers in elderly men without dementia

Group	A-carriers (N=68)	GG (N=29)	<i>F</i> or χ^2	<i>p</i> value
Age (years)	80.8±5.7	80.0±4.5	0.424	0.517
Education (years)	5.6±5.1	4.7±5.4	0.663	0.418
TIV (liter)	1.5±1.0	1.5±1.0	0.459	0.500
Handedness (left/right)	3/65	2/27	2.801	0.246
Digit span task				
Forward	11.9±3.1	12.6±2.1	0.010	0.920
Backward	4.0±2.9	2.9±2.4	2.983	0.087
CASI total score	87.7±8.7	85.1±10.8	1.507	0.223
Long-term memory	9.2±1.3	8.8±1.6	1.595	0.210
Short-term memory	10.7±1.6	10.3±1.8	0.915	0.341
Attention	6.4±1.2	6.6±1.1	0.386	0.536
Concentration/mental manipulation	7.2±2.5	6.3±2.9	2.145	0.146
Orientation	17.5±1.6	17.8±0.9	0.539	0.465
Abstraction	9.3±2.2	9.0±2.2	0.403	0.527
Language	9.6±1.0	8.9±1.4	7.049	0.009*
Visual construction	8.9±1.7	8.2±2.3	0.097	0.097
List-generating fluency	8.6±2.0	8.9±1.8	0.498	0.498

Data are presented as mean±standard deviation

CASI Cognitive Abilities Screening Instrument

* $p<0.05$

with A-allele carriers. Our findings supported the hypothesis that this Bcl-2 functional polymorphism may affect regional GM volumes and specific cognitive functions in non-demented elderly males.

Bcl-2, an anti-apoptotic protein, is the prototype of the Bcl-2 family that has been shown to regulate neuronal cell death during normal development, and has also been implicated in many models of acute and

Table 2 Regional gray matter volume differences between the Bcl-2 G homozygotes and A-allele carriers

MNI atlas coordinates			Voxel size	Anatomical region			Nearest Brodmann area	Regional GMV mean (SD; mm ³)		Z-Score	FWE-corrected <i>p</i>
<i>X</i>	<i>Y</i>	<i>Z</i>									
A-Carriers larger than GG (<i>p</i> <0.001, <i>k</i> =338)								A-Carriers	GG		
57	−3	−31	673	Right	Temporal lobe	Middle temporal gyrus	Brodmann area 21	0.33 (0.05)	0.29 (0.03)	3.53	0.773
GG larger than A-Carriers (<i>p</i> <0.001, <i>k</i> =338)								A-Carriers	GG		
−8	−52	41	3906	Left	Parietal lobe	Precuneus	Brodmann area 31	2.02 (0.24)	2.19 (0.25)	3.86	0.080
3	−90	−2	501	Right	Occipital lobe	Lingual gyrus	Brodmann area 18	0.22 (0.02)	0.23 (0.03)	3.66	0.596
−38	−78	37	742	Left	Occipital lobe	Superior occipital gyrus	Brodmann area 19	0.31 (0.05)	0.35 (0.04)	3.61	0.548

Z-scores are for the peak voxel of each regional clusters with uncorrected $p\leq 0.001$ as well as a cluster threshold of 338 voxels from *F* test, controlling age, education, and total intracranial volume as covariates

MNI Montreal Neurological Institute

Fig. 1 Regions showing gray matter volume differences between groups. *Red* color map: G homozygotes exhibited smaller regional GM volumes in right middle temporal gyrus (a); *Blue* color map: G homozygotes exhibited larger

regional GM volumes in left precuneus (b), right lingual gyrus (c), and left superior occipital gyrus (d) than A-allele carriers. ($p_{\text{uncorrected}} < 0.001$, cluster size = 338 mm³)

chronic neurodegeneration (Shacka and Roth 2005). Neuronal overexpression of Bcl-2 in transgenic mice increases the number of neurons in many brain regions by inhibiting naturally occurring neuronal cell death (Martinou et al. 1994). Considering the anti-apoptotic property of Bcl-2, our findings that subjects carrying the Bcl-2 G/G genotype performed more poorly on the language domain of the CASI and had smaller right-MTG volumes were in line with the conclusion of Uemura et al. (2011), who stated that the Bcl-2 G/G genotype is associated with lower protein and mRNA expression. However, our results were not congruent with those of a separate recent study (Machado-Vieira et al. 2011).

We found that Bcl-2 rs956572 G homozygote carriers had reduced right MTG GM volumes relative to A-allele carriers, as well as decreased language ability. We also found language score to be positively correlated with right MTG GM volume, which is consistent with the hypothesis that the right MTG is implicated in language processing (Krug et al. 2011; Sugiura et al. 2011). Our findings further support the hypothesis that the causal pathways may be influenced by Bcl-2 rs956572 polymorphism. Furthermore, Bcl-2 has been implicated in the pathogenesis of mood disorders (Kim et al. 2010; Uemura et al. 2011). One study found smaller right MTG volumes in elders with remitted geriatric depression relative to healthy control subjects (Yuan et al. 2008). A recent positron emission tomography (PET) study found that adult subjects with bipolar depression exhibited significant cerebral glucose hypometabolism in the MTG bilaterally, compared with matched healthy subjects (Brooks et al. 2009). Another PET study observed increased metabolism in depressed geriatric patients relative to comparison subjects, in the right MTG (Smith et al. 2009). Because of the range of structural and functional findings reported in the literature, further research is warranted to investigate the possibility that Bcl-2 genetic variants may act through MTG to affect geriatric mood disorder.

We found that G homozygote carriers showed greater GM volumes in the left precuneus, right lingual gyrus, and left SOG, compared with A-allele carriers. Thus, the beneficial effect of G/G in these regions contrasts with its detrimental effect in the right MTG. The basis for this discrepancy is at present unknown, but suggests that these areas may be sensitive to Bcl-2-related apoptosis. The precuneus and the occipital lobe are supplied by the posterior cerebral artery and partly by the anterior cerebral

artery, and these arteries intermingle for anastomosis in the medial parietal lobe. For both arteries, the precuneus and the occipital lobe constitute the last border zone of the cerebral artery network (Bogousslavsky and Caplan 2001). Asllani et al. (2009) demonstrated that an age-related decline of cerebral blood flow was evident in the GM of some brain regions, including the precuneus. An earlier functional imaging study of Alzheimer's disease (AD) patients revealed that hypoperfusion in the left precuneus appeared to be a main substrate of cognitive decline (Nobili et al. 2005). The right precuneus was also reported to be the site of maximum perfusion decrease in a group of patients with mild to moderate AD (Nobili et al. 2002). Evidently, Bcl-2 may modulate cell death after cerebral ischemia (Ouyang and Giffard 2004). Thus, the GM volumes in these areas may be selectively affected by this functional Bcl-2 polymorphism during the aging and degenerating processes.

Our finding that Bcl-2 rs956572 SNP exerts differential effects on regional GM volumes further supports the concept of genetic pleiotropy, as recently advocated by neuroscience researchers (Savitz et al. 2006). Such genetic pleiotropic effects were also found in a functional study of COMT Val (158) Met (rs4680) polymorphism, which made use of neuroimaging. In that study, Honea et al. (2009) demonstrated significant decreases in volume in a cluster of voxels which included the left hippocampus and parahippocampal gyrus for Val158 allele carriers relative to Met158 homozygote carriers. In contrast, analysis of the dorsolateral prefrontal cortex (including BA 9, 10, 45, and 46) showed a trend towards volume reduction in the Met158 carriers. Future studies should explore the potential pleiotropic influence of the Bcl-2 rs956572 polymorphism on the cellular and molecular determinants of the volumetric differences between specific brain regions.

Research by Salvatore et al. (2009) found that the only region affected by the Bcl-2 rs956572 genetic variation is the left ventral striatum, which is known to play key roles in the neurobiology of emotional regulation and in the pathogenesis of mood disorders. We were unable to replicate this finding but found additional brain regions to be affected by this genetic variant. However, the discrepancies between the results of these two studies may be due to differences in the samples' gender, age, or ethnic characteristics. Our study included only males, whereas Salvatore et al. had enrolled subjects of both genders. In addition, our sample was older (range, 65 to 92 years) than that of Salvatore et al. (range, 19 to

60 years). Furthermore, in the report by Salvatore et al., a more stringent criterion for significance was used (i.e., whole-brain FWE correction at a voxel level) as compared to the one used herein, and that could also explain the apparent difference. The fact that we found more brain regions to be affected by Bcl-2 rs956572 polymorphism than did Salvatore et al. suggests that this polymorphism may exert a genetic effect on the brain's aging process.

The strength of the current study was the relatively large sample that we used, which was also—by international standards—fairly homogenous (Chinese elderly males without dementia). The need for sufficient sample sizes in genetic imaging studies is increasingly recognized, and our sample size met the requirements recommended by previous researchers (Meyer-Lindenberg and Weinberger 2006). However, our study was limited by several factors, the first being the nature of its cross-sectional design. It is difficult to determine whether the Bcl-2 rs956572 SNP influences age-related or alternatively aging-related brain GM changes and cognitive impairment in elders. Age-related changes would occur within a specific age range, while aging-related changes would be caused by the aging process itself. A prospective study would elucidate this issue. Second, the current study enrolled only aged male veterans, and therefore, its findings cannot be generalized to other population sections or females. Third, the study's results may simply indicate that linkage disequilibrium with the Bcl-2 rs956572 polymorphism and other functional polymorphisms in the Bcl-2 gene, or another nearby gene, affects language performance and brain structure in elderly people. Finally, the exclusion criterion (CDR score >0.5) would not necessarily have excluded all subjects with mild cognitive impairment (Tognoni et al. 2005).

In conclusion, we found that Bcl-2 rs956572 SNP could modulate regional GM volumes and language performance in aged individuals without dementia. The rs956572 genetic effect on language performance may partially act through its effect on right MTG GM volume. Our findings further support the hypothesis that Bcl-2 exerts a genetic effect on the brain aging process.

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