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# Synergistic associations of *COMT* and *BDNF* with executive function in aging are selective and modified by *APOE*

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## Abstract

Genetic polymorphisms of *Catechol-O-methyltransferase (COMT*) and *Brain-derived neurotrophic factor (BDNF*) have shown promising but inconsistent linkages with executive function (EF) in normal aging. We tested (a) independent contributions of *COMT* and *BDNF* risk, (b) potential magnification by risk-related interactions or additive effects with age, and (c) effect modification through stratification by *Apolipoprotein E* (*APOE*; risk (ε4+)). Multiple linear regression models were applied with non-demented older adults (*N* = 634; range: 53–95 years) for an EF latent variable. No independent effects of *BDNF* or *COMT* on EF were observed. Additive (but not interactive) effects of *COMT*, *BDNF*, and age showed that older adults with a high-risk allelic combination performed differentially worse. Of two tested models of synergistic effects, the additive approach selectively supported a magnification hypothesis, which was qualified by the presence or absence of *APOE* ε4.

### Keywords

Aging; Executive Function; *Apolipoprotein E*; *Catechol-O-methyl transferase*; *Brain-derived neurotrophic factor*; Victoria Longitudinal Study



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**Disclosure Statement**

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## 1. Introduction

Genetic associations in complex and multifaceted neurocognitive phenotypes are known to be detectable but relatively small in magnitude. Such associations are likely to be polygenic, interactive, or combinatorial in influence. They operate through relevant neurobiological mechanisms, and vary in influence according to the match between endogenous factors and neurocognitive domain or clinical outcome status (Deary et al., 2004; Goldberg and Weinberger, 2004; Gomar et al., 2011; Green et al., 2008; McClearn, 2006). Recent advances in understanding relevant molecular genetics, neurophysiological mechanisms, and key structural and functional aspects of specific cognitive phenotypes have led to increasing attention to potential associations among dopaminergic and neurotrophic related genes expressed in the prefrontal cortex and influencing aging changes in executive functions (Bäckman et al., 2006; Harris and Deary, 2011; Savitz et al., 2006). Two polymorphisms have received sustained attention for their potential contributions to aging-related individual differences in executive function (EF): *Catechol-O-methyl transferase* (*COMT*; rs4680) and *Brain-derived neurotrophic factor* (*BDNF*; rs6265) (Bilder et al., 2004; Miyajima et al., 2008; Payton, 2009; Starr et al., 2007). The third polymorphism we consider is *Apolipoprotein E* (*APOE*; rs7412, rs429358). *APOE* has received considerable attention for both predictive and modifying roles in normal cognitive aging, mild cognitive impairment (MCI), and Alzheimer's disease (AD) (Brainerd et al., 2011; Farlow et al., 2004; Harris and Deary, 2011; Kantarci et al., 2012; Saunders et al., 1993). In this study we examined both the independent, interactive, and additive effects of the first two polymorphisms on EF as well a subsequent potential vulnerability conveyed with effect modification by *APOE*-related cognitive risk.

EF is a complex neurocognitive phenotype that may vary with aging in terms of both latent structure and performance on manifest variables (Luszcz, 2011). Quantitative modeling and empirical results with younger adults, normal older adults, and clinical populations have confirmed that unidimensional (single-factor) solutions are typically observed in normal and clinical (impaired) aging (de Frias et al., 2009; McFall et al., 2013). We assembled two common markers (each) of EF inhibition (Hayling Sentence Completion, Stroop) and EF shifting (Brixton Spatial Anticipation, Color Trails). In order to avoid multiple significance tests and enhance the construct and measurement characteristics of these four manifest variables, we performed confirmatory factor analyses on the performance data, resulting in a replicated and validated latent variable representation of EF for non-demented older adults (de Frias et al., 2006).

Biological-to-neurocognitive rationales for exploring the *COMT* and *BDNF* SNPs in the context of EF are available (e.g., Erickson et al., 2008; Miyajima et al., 2008; Savitz et al., 2006; Starr et al., 2007). We summarize the most pertinent aspects of the proposed neural mechanisms as they are currently related to non-demented aging. The Val158Met *COMT* rs4680 polymorphism at codon 158 on chromosome 22q11 increases COMT enzymatic activity that in turn decreases dopamine levels particularly in the prefrontal cortex (Chen et al., 2004). This results in *COMT* homozygotes for the Met allele having greater dopamine levels than the Val allele homozygotes. Thus, non-demented older adults with any Val allele (Val-Val, Val-Met) may be at higher risk for EF impairment than those with the Met-Met

combination (Nagel et al., 2008; Wishart et al., 2011). However, a variety of phenotypic associations have been observed for this polymorphism, with such characteristics linked to the tonic-phasic dopamine hypothesis (Bilder et al., 2004; Egan et al., 2001). Regarding BDNF, this factor is mainly present in the hippocampus and prefrontal cortex, and it may play a role in such phenotypes as episodic memory, global cognitive functioning, and executive functions, perhaps interactively, additively, or differentially by age and gender (Komulainen et al., 2008; Raz et al., 2009; Savitz et al., 2006). Although not quite to the extent as *COMT*, this polymorphism has produced multiple phenotypic associations, likely due to variations in endogenous and environmental factors in the context of other relevant genes and measures of neurocognitive performance (Mandelman and Grigorenko, 2012).

Informed by overlapping neurobiological mechanisms and EF phenotypic expressions, but in the absence of a specific theory regarding the mechanisms of their potential synergistic associations, we recruited related theoretical perspectives linking them with non-demented aging. Specifically, an aging magnification or dynamic vulnerability perspective (e.g., Belsky et al., 2009; Fotuhi et al., 2009; Lindenberger et al., 2008) suggests that a combination of risk alleles from *BDNF* and *COMT* could effectively intensify the deleterious effects of brain aging on select neurocognitive phenotypes. We examined two ways of representing vulnerability effects in this study (Gomar et al., 2011; Harris et al., in press; McClearn, 2006). First, we examined interactive or multiplicative (e.g., gene x gene interactions, ending with gene x gene x age interactions) models to test moderating biological relationship between *COMT*, *BDNF*, and age. The genotype of each polymorphism was coded from 1–3 (3 = highest risk) and age was evaluated as a continuous variable. We reasoned that, if the interactive model would hold, adults with relatively non-risk (or even protective) alleles for either *COMT* or *BDNF* (or younger age) would be at a lower risk for cognitive decrements. Conceivably, removing even one risk factor could reduce some risk associated with either *COMT* or *BDNF* risk alleles, because at least one factor is moderating the others to produce the deleterious effect on EF. Second, as an alternative representation of genetic-plus-aging vulnerability, we performed parallel tests of additive effects. This additive model of genetic risk included subsets and the full following calculation, *COMT* + *BDNF* + age. The additive model represents the notion that panels or combinations of risk biomarkers will influence cognitive phenotypic performance in normal aging and in early cognitive impairment (e.g., Gomar et al., 2011), even in the absence of independent or multiplicative associations. An additive model (Purcell et al., 2009; Harris et al., in press; Verhaaren et al., 2013) could indicate that a non-risk (or protective) allele for *BDNF* or *COMT* or younger age would effectively only eliminate the risk for one of the risk factors, but the risk associated with the other factors could still be present and influential. For convenience, we refer to both interactive and additive effects as synergistic associations with EF throughout the paper. For both biological and cognitive reasons, BDNF and COMT have been studied independently (rarely in addition or interaction) in the prefrontal cortex in non-demented older adults. For example, BDNF may interact with COMT levels in the prefrontal cortex through basal ganglia-thalamocortical loops (e.g., Alexander et al., 1986). Conceivably, decreases in the secretion of BDNF may be associated with normal cognitive decline and additional *COMT* effects may further regulate the effects of cognitive deficits. In

the *BDNF* Val66Met polymorphism, *BDNF* Met homozygotes may be expected to produce selective cognitive deficits, as compared to *BDNF* Val homozygotes.

To our knowledge, the present additive effects model has not been reported for these two SNPs in neurocognitive aging (for other examples see Bertolino et al., 2006; Canli et al., 2008; McIntosh et al., 2013; Purcell et al., 2009; Verhaaren et al., 2013). However, independent and interaction effects of *COMT*, *BDNF*, and age have indicated suggestive results. For example, Wishart and colleagues (2011) examined a single EF test (Trail Making Test) and found COMT-EF effects in the expected direction and no *BDNF* x *COMT* interaction effect. However, in a follow-up analysis, adults with the combined risk alleles for *COMT* and *ANKK1* (*Ankyrin Repeat and Kinase domain containing 1*) performed worst on the EF task. Regarding genetic vulnerability and aging, Nagel and colleagues (2008) examined the performance of younger and older groups on the Wisconsin Card Sorting Test (EF measure) as magnified by gene x gene interactions. For a younger group and an older group the deleterious effects of *COMT* Val carriers were visible in the older group of adults, and this was modulated by whether individuals were carriers of the *BDNF* Met allele. Other examples are appearing in related literatures (e.g., Gomar et al., 2011; McFall et al., 2014; Deshmukh et al., 2009).

The presence or absence of *COMT* and *BDNF* interactive and additive associations may be due to the moderating role of other unmeasured genetic variants. Therefore, to investigate whether an additional neurogenetic indicator of cognitive health and vulnerability might modulate the synergistic effect for EF performance, we examined effect modification by stratifying the groups by allelic risk for *APOE*, the most widely studied neurocognitive vulnerability gene in aging (Harris and Deary, 2011; Verghese et al., 2011). There are many studies with *APOE* risk and cognitive impairment (e.g., Sachs-Ericsson et al., 2010; Small et al., 2004). The *APOE* genotype is involved in central nervous system repair and function, and is differentiated by three alleles: ε2, ε3, and ε4. The ε4 allele (both homozygosity and heterozygosity) is consistently linked to risk factors for cognitive aging decline, impairment, and dementia (e.g., Brainerd et al., 2011; Elias-Sonnenschein et al., 2011; Wisdom et al., 2011) in comparison to the ε2 allele, which has been found to be protective in numerous studies (Corder et al., 1994; de-Almada et al., 2011; Panza et al., 2000). The *APOE* gene has been reported to have an antagonistic pleiotropy effect, whereby the presence or absence of the ε4 allele may moderate the appearance of age differences (Jochemsen et al., 2011), as well as other grouping and modification effects (e.g., Edland et al., 2003; Niti et al., 2008; Risacher et al., 2013; Woodard et al., 2012). We investigated genetic and aging effects as stratified by *APOE* allelic risk (i.e., the commonly implemented dichotomous comparison between risk (ε4+) group and no risk (ε4−) group) for a large sample of older adults.

We extend previous research by including a larger heterogeneous sample of well-characterized older adults, a wide band (40 years) of age within the sample, and an informative battery of four EF measures, including two tests each of shifting and inhibition, as represented by a quantitatively derived EF latent variable. Specifically, we tested independent, interactive, and additive effects pertaining to whether those with *COMT* risk alleles, *BDNF* risk alleles, and older age vulnerability performed worse on EF. Subsequently, we tested the effect modification by *APOE* allelic risk. Therefore, four

research questions were examined. First, do carriers of the risk allele for *COMT* (Val+) and *BDNF* (Met+) perform worse on EF? Second, do either interactive (gene x gene) or additive (gene + gene) effects demonstrate synergistic associations, such that adults with combined risk alleles perform worse? Third, does age have an interactive or additive effect with *COMT*, *BDNF*, or both *COMT* and *BDNF*, such that older age magnifies the deleterious effect for genetic risk carriers? Fourth, do adults in *APOE* risk (ε4+) group perform more poorly than adults in the reduced *APOE* (ε4−) risk group for additive or interactive associations of *COMT*, *BDNF*, and age?

## 2. Method

### 2.1. Participants

This study uses recent data from the Victoria Longitudinal Study (VLS), a long-term project examining biomedical, health, and neurocognitive aspects of aging. General information on recruitment, methodological, and VLS characteristics are available elsewhere (e.g., Dixon and de Frias, 2004; Dolcos et al., 2012). All volunteers in the VLS were initially healthy, enrolled through advertisements, and received a small honorarium for their participation. The VLS and all present data collection procedures are in full and certified compliance with prevailing human/institutional research ethics guidelines. Written informed consent was obtained from all participants. All participants were Caucasian with complete access to Canadian national health care. The present sample reflects the implementation of exclusionary criteria affecting individuals with (a) diagnosis or history of dementia, (b) anti-psychotic medication, (c) Mini Mental State Exam scores less than 24, (d) uncontrolled hypertension, (e) insulin-controlled diabetes, and (f) history of serious head injury (e.g., hospitalized). Accordingly, $n = 634$ participants (age range = 53–95, mean age = 70.58 (SD = 8.65)) including 423 females and 211 males with genetic data were included.

### 2.2. DNA Extraction and Genotyping

Saliva was collected according to standard procedures from Oragene DNA Genotek and stored at room temperature in Oragene® disks until DNA extraction. DNA was manually extracted from 0.8 ml of saliva sample mix using the manufacturer's protocol with adjusted reagent volumes. Genotyping was carried out by using a PCR-RFLP strategy to analyze the allele status for *BDNF* (rs6265), *COMT* (rs4680), and (*APOE*; rs7412, rs429358). Genotyping was successful for the targeted SNPs for all present participants. Table 1 presents participant characteristics and allele frequency by genotype for *BDNF*, *COMT*, and *APOE*. The genotype frequencies for the three examined genotypes did not differ significantly from Hardy-Weinberg equilibrium: *BDNF* rs6265 ($\chi^2 = 0.837$, $p = 0.36$), *COMT* rs4680 ($\chi^2 = 2.786$, $p = 0.10$), and *APOE* rs7412, rs429358 ($\chi^2 = 0.545$, $p = 0.909$) or among any baseline characteristics. For purposes of analyses we included all three allelic combinations for *COMT* and *BDNF* (Met/Met, Met/Val, and Val/Val). For evaluating modification by *APOE*, we deleted all ε2/ε4 carriers and then compared patterns between ε4+ carriers and ε4− carriers.

### 2.3. Executive Function Measures

Two dimensions of EF (inhibition, shifting) were each measured by two standard and frequently used tests for both behavioral and clinical studies in older adults (for details see: de Frias et al., 2006, 2009; McFall et al., 2013, 2014).

**2.3.1. Hayling Sentence Completion (Hayling; Inhibition)—**The test consists of two sections, each comprising fifteen sentences. In the first section, participants must state the last word that correctly completes the sentence. In the second section, the participants must say a word that is not at all related to the sentence. The standardized scores are based on an error score from the first section and the speed of each response from both sections, which are then combined to obtain the final score (1 = impaired to 10 = superior).

**2.3.2. Stroop (Inhibition)—**The test consists of three parts. In part A, participants are asked to name four different colors that appear as 24 dots in six different rows. In part B, the same colors appear but are printed as common words. In part C, each different color is represented as a textual representation, with the text being the name of its corresponding color. The participants are measured based on latencies. The final score is the standardized Stroop interference index ([Part C- Part A]/Part A), with a lower index reflecting better performance.

**2.3.3. Brixton Spatial Anticipation (Brixton; Shifting)—**The test consists of 10 different circles; one being blue while the rest are colorless. The circles appear in a 56-page booklet. The blue colored circle shifts position with some logical pattern after each page. This test measures the mechanism of shifting by asking participants to guess where the blue colored circle will appear on the next page. The total number of incorrect guesses are measured and the final scores are calculated (1 = impaired to 10 = superior).

**2.3.4. Color Trails (Shifting)—**This test comprises two different tasks in which participants connect different attributes, such as numbered and colored circles. In the first section, participants connect numbers from 1–25 within circles that are randomly organized on a page. In the second section, they connect the numbers in order but alternating between pink and yellow circles. Errors and latency scores are then computed to obtain the standard overall score.

### 2.4. Statistical Analysis

Structural equation modeling (SEM) was used to analyze all research questions (i.e., Mplus Version 7; Muthen & Muthen, 2012). All missing values for cognitive measures were assumed to be missing at random and handled using maximum likelihood. Missing predictor values were handled using list-wise deletion in Mplus. Only two participants with missing measures on all four EF tasks were lost due to list-wise deletion.

To test and establish a latent variable for EF, we used confirmatory factor analysis (CFA) to examine loadings of all four manifest variables (Stroop, Hayling, Brixton, and Color trails) on the predicted latent variable. The first model tested all observed variables on one latent EF factor. The best fitting model was determined by examining several fit statistics. The chi-

square test of model ($\chi^2$; $p > .05$) allowed for an overall indication of good model fit. Additional absolute/comparative fit indices were also examined to determine a good model fit to the data (Kline, 2011): the root mean square error of approximation (RMSEA ≤ .05), comparative fix index (CFI ≥ .95), and the standardized root mean square residual (SRMR ≤ .08). The one-factor parsimonious model provided good fit to the data and was used as the final CFA model for EF. Unstandardized regression coefficients for the expected EF latent variable will be examined to determine higher or lower performance.

For each research question, we used multiple linear regression analysis models (within Mplus). Our specific analyses are described below in the following convention. EF was regressed on all predictors simultaneously for all models. The interaction terms were calculated as product variables (gene x gene and gene x gene x age). The additive terms were calculated as sums of risk by adding the allelic risk (coded from 1–3 with three being the highest risk) and chronological age. Higher score represented higher genetic risk and older age. When testing for additive effects, only predictors absent from the additive term were added to covary for any remaining independent effects. For interactive effects, all three predictors (*COMT*, *BDNF*, and age) were always entered in the interactive model to covary for any independent effects. On the basis of two preliminary analyses, we did not include gender as a covariate. First, our tests of gender effects in allelic distributions for all three genotypes (*APOE*, *BDNF*, *COMT*) were not significant (see Table 1). Second, our test of gender differences in EF performance both overall and by each allelic group (within the three SNPs) also produced non-significant effects. We constrained our analysis plan to include the essential 13 models. By using the EF latent variable and testing only specific hypotheses, we set statistical significance threshold at $p < .05$.

For research question one, EF was regressed on *COMT*, *BDNF*, and age. For research question two, two models were tested for interaction and additive effects. Specifically, EF was simultaneously regressed on (a) *COMT* x *BDNF*, *COMT*, *BDNF*, age, and (b) *COMT* + *BDNF*, age. For research question three, six models were tested for interactive and additive effects with age. For interactive associations, EF was simultaneously regressed on (a) *COMT*, *BDNF*, age, *COMT* x age, (b) *COMT*, *BDNF*, age, *BDNF* x age, and (c) *COMT*, *BDNF*, age, *COMT* x *BDNF* x age. For additive effects, EF was regressed on (a) *BDNF*, *COMT* + age, (b) *COMT*, *BDNF* + age, (c) *COMT* + *BDNF* + age. For research question four, we first deleted all ε2/ε4 carriers ($n = 30$) and then stratified the groups by *APOE* risk (ε4+) versus reduced risk (ε4−) subgroups. Four models were then examined for interactive and additive effects by each subgroup, where EF was simultaneously regressed on (a) *COMT*, *BDNF*, age, *COMT* x *BDNF* x age and (b) *COMT* + *BDNF* + age.

## 3. Results

Descriptive characteristics by *COMT*, *BDNF*, and *APOE* alleles are displayed in Table 1. The best CFA model for EF was obtained with the one factor latent variable, which provided the best fit for all four EF tasks ($\chi^2$ (*df*) = 3.011 (2), $p = 0.222$; RMSEA (confidence interval) = 0.028 (0.000–0.089); CFI = 0.993; SRMR = 0.015). This latent variable was used in the analyses for all four research questions. Regarding the first research question, we observed that neither *COMT* (β = 0.114; standard error (SE) = 0.103; $p = 0.271$) nor *BDNF*

(β = 0.101; SE = 0.124; $p$ = 0.415) significantly predicted EF performance. However, as expected, a one-unit increase in age was associated with a significant decrease (β = −0.134; SE = 0.016; $p$ < .001) on EF performance.

Regarding the second research question, neither the *COMT* x *BDNF* interaction (β = 0.046; SE = 0.178; $p$ = 0.795) nor the *COMT* + *BDNF* (β = 0.109; SE = 0.079; $p$ = 0.169) additive effects model significantly predicted EF performance. Regarding the third research question, only age significantly predicted poorer EF performance in all three interactive models (see Table 2, rows 4, 8, 12 under research question three (interactive)). However, all three models examining additive effects with age significantly predicted EF performance in the expected direction. Specifically, a one-unit increase for additive effects of both *COMT* + age (β = −0.132; SE = 0.015; $p$ < .001) and *BDNF* + age (β = −0.132; SE = 0.015; $p$ < .001) predicted poorer EF performance (see Table 2, rows 1–4 under research question three (additive)). Moreover, the three-way model produced a one-unit increase in the additive effect, for which *COMT* + *BDNF* + age significantly predicted lower EF performance (β = −0.129; SE = 0.015; $p$ < .001) (see Table 2, row 5 under research question three).

Regarding the fourth research question, the *COMT* x *BDNF* x age interactive effect did not significantly predict EF performance as stratified by *APOE* allelic risk (ε4+) group (β = −0.297; SE = 0.214; $p$ < .166) and reduced risk (ε4−) group (β = −0.004; SE = 0.003; $p$ < .173). However, the corresponding three-way *COMT* + *BDNF* + age additive effect model significantly predicted EF performance as stratified by *APOE* groups. Although the difference between the *APOE* risk and reduced risk group was not significantly different (β = −0.039; SE = 0.165; $p$ = .811), we observed slightly lower EF performance in the *APOE* risk (ε4+) group (β = −0.136; SE = 0.024; $p$ < .001) than in the reduced *APOE* risk (ε4−) group (β = −0.131; SE = 0.020; $p$ < .001).

We then conducted a post hoc analysis to check the extent to which the age variable influenced the 3-way additive effect on EF. We dichotomized the sample into young-old (YO) (< 70 years old; $n$ = 296) and old-old (OO) (≥ 70 years old; $n$ = 338) groups. Arguably, if age was driving this effect on EF then we should expect to see similar patterns in both groups. Instead, we observed different patterns. Whereas in the YO group, the *COMT* + *BDNF* + age effect on EF was not significant (β = 0.013, p = .089), in the OO group the additive model was significant and in the expected direction (adults with additive allelic risk plus old age showed poorer performance) (β = −0.151, p < .001). The additive synergistic effects appear across a 40-year band of aging and are especially magnified with aging.

## 4. Discussion

We tested independent, interactive, and additive associations of *COMT* and *BDNF* risk alleles, along with age and effect modification by *APOE* allelic risk, in executive functioning for a large sample of normal older adults. Previous studies have reported results supportive of an interactive (Nagel et al., 2008) and additive (McIntosh et al., 2013 with schizophrenia related polymorphisms and cognition) aging-related magnification or intensification hypotheses (Fotuhi et al., 2009; Lindenberger et al., 2008; McClearn, 2006).

It was unknown how model-specific (interactive or additive) or generalizable these effects would be across samples, ages, and dimensions of executive functioning. For the present study, results consistent with this general hypothesis would be produced through interactive or additive gene risk (with synergistic effects of risk alleles associated with poorer performance) or age plus gene risk (with older age differences in genetic-cognition associations).

We observed a consistent age effect for a latent variable representing EF performance, as would be expected in the literature (de Frias et al., 2006; Luszcz, 2011). Although expected, this established at the outset the important precondition for age-specific genetic vulnerability hypotheses. Our sample featured a continuous 40-year band of older adults, thus testing genetic vulnerability for *COMT*, *BDNF*, and *APOE* within older adulthood and complementing the typically examined extreme group comparisons of young and old adults. In addition, the confirmed EF latent variable offers a more robust representation of EF than is typically available in single-indicator studies (Wishart et al., 2011), most of which employ different and single EF tests. The latent variable approach reduces the number of models tested and groups the shared variance among all EF tests. In addition, relatively few examples of genetic association studies have been conducted with multiple indicator latent variable representations (McFall et al., 2014). Given that age confers some vulnerability in EF, the next issue was whether the two polymorphisms were associated with EF. Notably, however, no corresponding independent associations with EF were observed for either the *BDNF* or *COMT* polymorphisms for our first research question. Therefore, we continued to test the two key aspects of this study, examining the two renditions of genetic and age risk that could convey cognitive vulnerability in normal older adults. Regarding the second research question, we found no interactive or additive associations with *COMT* and *BDNF* risk alleles on EF performance. From this perspective, there was no evidence of magnification effects of either genetic risk factor.

For our third research question, we observed systematically different results for the interactive versus additive models. Notably, only the additive model produced significant vulnerability associations with EF performance. Specifically, the additive associations with EF for *COMT* + age, *BDNF* + age, and *COMT* + *BDNF* + age were all significant. In contrast, the corresponding interactive models were not significant. The main evidence favoring the additive version of risk vulnerability and its potential for demonstrating associations with cognitive phenotypes in non-demented older adults was the three-way synergistic effect. This result showed exacerbated deficits for the vulnerability components of the allelic combinations, as they operated in a complementary and additive way that was associated with poorer EF performance. Arguably, this result pertains to general magnification or intensification hypotheses, extending earlier research with different polymorphisms and cognition (e.g., McIntosh et al., 2013; Verhaaren et al., 2013). In contrast to the not significant interaction effects, the small but significant additive effects remain neurobiologically interesting. Arguably, not significant traditional interaction effects may mask different mechanisms through which synergies can be transmitted (e.g., additive pathways and vulnerabilities of biomarker influence where eliminating one risk factor will not reduce the risk associated with other risk factors). Both models of synergistic biomarker

effects should continue to be studied. In addition, we note that the present study included adults along a continuous 40-year age range. This suggests that even within older adulthood, advancing chronological age may be an index along which researchers could detect evidence of increasing modulation of genetic, neurobiological, and environmental associations with neurocognitive functioning. The post hoc age-comparative analyses clarified these results. Whereas we observed no 3-way additive effect in the young-old group, the full significant additive effect was observed in the old-old group. Notably, the additive allelic risk for *COMT* and *BDNF* with very old age was associated with poorer EF performance. This implies that even within older adulthood chronological age is important and substantial in its influence on EF performance, but additive synergistic associations may be further magnified in very old adults. In terms of mechanisms, the additive model suggests that having only one protective factor (e.g., *COMT;* Met/Met allele) only reduces the risk associated with *COMT*, but does not affect the risk associated with *BDNF* risk allele or biological aging. In contrast, interactive effects (not observed here) may suggest that the moderation of *BDNF* risk allele factor on EF by *COMT* protective factor may dilute the risk associated with *BDNF* allelic risk. These results and the extant literature, however, do not yet provide specific guidance regarding the neurobiological underpinnings of these complex magnification effects (Harris et al., in press; Lindenberger et al., 2008; Savitz et al., 2006).

The role of aging in presumed aging-genetic magnification of neurocognitive deficits and impairment deserves further attention. Clearly, chronological age (and especially age groups) is not a causal factor but instead a proxy for to-be-determined underlying biological changes indexed by, but not tantamount to, age (MacDonald et al., 2011; Nakumura and Miyao, 2007). As theoretical and measurement advances continue, such concepts as biological vitality or biological age (e.g., Anstey, 2008; MacDonald et al., 2004) may enhance future efforts to examine aging-related vulnerability and magnification effects in the context of genetic polymorphisms, about which the underlying molecular mechanisms are becoming better understood. Systematic but unmeasured biological or health influences —indexed imperfectly by chronological aging—may be among the reasons that inconsistent associations have been observed for single candidate-gene links (including *BDNF* and *COMT*) with various cognitive phenotypes in older adults (Deary et al., 2004; Fotuhi et al., 2009; Mandelman and Grigorenko, 2012). Relatively recent literature reporting early tests of polygenic effects is small (but growing) and promising (but not yet strong)—and this too may benefit from stronger representation of biological aging. The present study is the first to examine additive effect models for genetic polymorphism associated with cognitive decline and impairment and it may therefore serve as a model for future studies testing additive effects. The approach and initial results have substantial promise for the development of panels of biomarker influences in non-demented aging.

For our fourth research question, we analyzed the *APOE* risk (ε4+) and reduced risk (ε4−) groups separately, with the expectation that we would observe a version of an antagonistic pleiotropy effect. Although not significant, we observed slightly lower EF performance for *APOE* risk (ε4+) group than the reduced risk (ε4−) group for additive effects of *COMT*, *BDNF*, and age. The potential magnification of *COMT* and *BDNF* allelic risk may be especially detectable and active in the context of older adults who are carriers of the most

prominent neurogenetic risk factor for cognitive decline. In the context of the powerful *APOE* (*ε4+*) risk factor among non-demented older adults, the additional risk provided by *COMT* and *BDNF* risk alleles may be more easily or differentially detectable. We note that older adults in the absence of the *APOE* (ε4+) risk factor may also be at risk for cognitive impairment from other risk factors in old age (e.g., stress, physical activity; see Fotuhi et al., 2009). In addition, individuals with allelic risk may not develop cognitive decline in old age (Henderson et al., 1995) or their allelic risk may be exacerbated in combination with other diseases or factors (i.e., cardiovascular disease; see Kang et al., 2005). As a follow-up, we tested the effect of *APOE* alone without separating the ε4+ and ε4− groups. The significant effect modification showed an effect size of −0.007. This implies that although the effect modification between the groups are not significantly different in value, the risk and reduced risk groups must be separated to observe the large effect modification present in an additive vulnerability model for genetic and age on EF. Future research may investigate the magnification hypothesis not only among genetic variants with known neurobiological underpinnings for specific cognitive phenotypes, but also in the context of prominent neurodegenerative-related or vulnerability genetic variants (especially *APOE*) with larger samples of ε4+ carriers. However, other neurobiological factors and genetic variants related to age are emerging in the literature. These include the afore-mentioned *ANKK1* (Wishart et al., 2011), several dopaminergic-related genes (e.g., Bellander et al., 2011), and insulin-related genes (e.g., McFall et al., 2013), as well as markers of aging-related brain resources (e.g., Lindenberger et al., 2008), emerging neurodegenerative conditions (e.g., MCI or Alzheimer's disease; Brainerd et al., 2011; Dixon et al., 2014; Dolcos et al., 2012), or aging-related health conditions with less proximal neurological implications (e.g., diabetes; Seaquist et al., 2012). In all cases, however, advances will be made with both substantial cross-sectional studies and emerging longitudinal or epidemiological studies.

Several strengths and limitations of the present study should be mentioned. First, although this study had no younger adult comparison group, it did feature a large sample of older adults representing a broad (40-year) band of age (from age 53–95 years). Given the heterogeneity of typical aging, this characteristic provided a unique opportunity to investigate a within-age genetic risk intensification hypothesis. This provided a conservative and unique test of the application of the phenomenon with this wide age range. Second, the tests used to measure EF phenotypes were four standard neuropsychological measures that contributed to a latent variable. The latent variable approach provides protection for shortcomings of single-test approaches and is preferred over typical composite variable formulations, thus extending knowledge of genetic associations with EF. Third, given some emerging research, other genetic variants, gender differences (e.g., Altmann et al., 2014), and neurobiological sources of vulnerability (e.g., vascular risk factors such as hypertension) should be considered in the future. Although this study investigated a range of EF phenotypes, further research could include additional domains such as neurocognitive speed and memory. For example, given the BDNF-hippocampus link and *APOE* and memory/AD risk, future research may examine *BDNF* allelic risk and *APOE* effect modification hypothesis for at least episodic memory, if not semantic and working memory (Mandelman and Grigorenko, 2012). Fourth, cross-sectional studies have well-known limitations in interpreting mechanisms and differences. Although these limitations apply to the present

study, the wide age range offers new and valuable information. Certainly, longitudinal studies of these phenomena are encouraged. Fifth, in our effort to explore the aging magnification hypothesis, we examined 13 regression models because of our clear and specific vulnerability hypotheses and our approach of using an EF latent variable, we set the statistical significance standard to $p < .05$. Our decision was informed by the expectation of subtle magnification effects within age (as compared with group designs) and specific interest in comparing two versions of vulnerability models.

In sum, genetic associations with complex cognitive phenotypes may confer exacerbated risk in selective polygenic (interactive and additive) combinations. We examined independent, additive, and interactive effects of *COMT* and *BDNF* alone and as stratified by *APOE* groups. Consistent with the specific expectations, we observed a synergistic effect (*BDNF* + *COMT* + Age) for EF performance, but selectively for the additive models. We note as an issue for future research that the overall and cognitive health of the present sample may be partly responsible for the systematically differential results between the two representations of magnified genetic-aging vulnerability. Future research can investigate the applicability of the interactive model for different phenotypes and samples (e.g., cognitively impaired). Nevertheless, as noted by recent observers, approaching the neurogenetics of normal aging from the perspective that incorporates independent, synergistic, and modifying risk (or protection) factors may yield further understanding of the cognitive neurobiology of aging.

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## Highlights

1. We have addressed all issues raised by the reviewers.
2. The report is improved and makes a novel contribution.
3. The role of APOE is clarified.
4. Importance of the unique 40-year band of aging is noted.
5. Novelty of testing additive vs interactive models is noted.
6. Role of age is discussed and better described.
7. Statistical questions are addressed and resolved.

**Table 1**

Participant characteristics by genotype

| | *COMT*[a] | | | | *BDNF*[b] | | | | *APOE*[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Characteristics** | **Met/Met** | **Met/Val** | **Val/Val** | ***p*[d]** | **Met/Met** | **Met/Val** | **Val/Val** | ***p*[d]** | **ε4−** | **ε4+** | ***p*[d]** |
| *n* | 146 | 338 | 150 | -- | 27 | 189 | 418 | -- | 452 | 148 | -- |
| Age (years) | 70.15 (8.86) | 70.85 (8.68) | 70.40 (8.40) | 0.69 | 68.54 (6.32) | 71.45 (8.52) | 70.32 (8.81) | 0.15 | 70.89 (8.84) | 69.81 (8.27) | 0.19 |
| Gender (F/M) | 101/45 | 226/112 | 96/54 | 0.64 | 18/9 | 128/61 | 277/141 | 0.94 | 305/150 | 93/56 | 0.35 |
| Education (years) | 14.92 (3.11) | 15.35 (2.80) | 15.36 (3.15) | 0.30 | 15.72 (2.70) | 15.13 (3.00) | 15.28 (2.96) | 0.59 | 15.20 (2.95) | 15.57 (3.07) | 0.19 |
| MMSE[e] | 28.74 (1.20) | 28.78 (1.11) | 28.53 (1.30) | 0.13 | 29.08 (0.76) | 28.72 (1.12) | 28.68 (1.23) | 0.27 | 28.71 (1.19) | 28.69 (1.20) | 0.88 |
| Absolute Health | 1.87 (0.74) | 1.83 (0.74) | 1.77 (0.77) | 0.47 | 1.89 (0.80) | 1.84 (0.72) | 1.82 (0.04) | 0.86 | 1.87 (0.73) | 1.68 (0.77) | 0.06 |
| Relative Health | 1.60 (0.66) | 1.62 (0.72) | 1.53 (0.71) | 0.42 | 1.41 (0.69) | 1.64 (0.69) | 1.59 (0.04) | 0.26 | 1.61 (0.70) | 1.56 (0.73) | 0.48 |
| Blood pressure (mmHg) (S/D)[f] | 128.30/76.06 | 127.71/75.71 | 125.36/75.06 | 0.51/0.92 | 128.19/78.78 | 127.05/74.06 | 127.36/76.15 | 0.97/0.96 | 127.14/75.78 | 127.11/75.42 | 0.99/0.86 |
| Bradburn Affect Balance Scale | 3.00 (1.87) | 3.24 (1.74) | 3.25 (1.81) | 0.42 | 3.48 (1.16) | 3.12 (1.90) | 3.19 (1.77) | 0.64 | 3.16 (1.84) | 3.34 (1.69) | 0.33 |
| Physical activities | 15.41 (4.66) | 15.89 (4.99) | 16.21 (4.84) | 0.44 | 16.28 (3.36) | 16.13 (4.91) | 15.69 (4.96) | 0.58 | 15.69 (4.93) | 16.11 (4.96) | 0.41 |
| Social activities | 23.00 (6.43) | 22.77 (6.79) | 22.49 (6.84) | 0.84 | 22.12 (7.40) | 23.23 (6.68) | 22.59 (6.67) | 0.54 | 22.94 (6.62) | 22.20 (6.95) | 0.29 |
| Integrative Information | 19.84 (9.69) | 18.90 (8.17) | 18.67 (9.09) | 0.52 | 19.28 (6.54) | 18.44 (8.29) | 19.33 (9.11) | 0.57 | 18.66 (8.56) | 20.47 (9.37) | 0.05 |

*Key: n*, total number. Standard deviations are in parentheses. Absolute health represents self-rating to a perfect state of health, and relative health is rated with respect to others ones' own age, both based on a 1–5 scale (1=very good, 5 = very poor). For the analyses involving the *APOE* genotypes, the ε2/ε4 carriers (*n* = 30) were deleted from the sample.

[a] COMT: Catechol-O-methyl transferase.

[b] *BDNF: Brain-derived neurotrophic factor.*

[c] *APOE: Apolipoprotein E.*

[d] $p < .05$.

[e] MMSE: Mini-mental State Exam.

[f] S/D; Systolic/Diastolic.

**Table 2**

Unstandardized regression coefficients and model fit indices by research question for all models examined on executive function.

| | Executive Function | | | | | | |
|---|---|---|---|---|---|---|---|
| | Model Fit Indicators | | | | | | |
| **Models** | **β** | **SE** | ***p*** | **$\chi^2_M(df_M)$** | **CFI** | **RMSEA (90% CI)** | **SRMR** |
| **Research question one** | | | | | | | |
| (a) *COMT*[a] | 0.114 | 0.103 | 0.271 | 20.83 (11); $p = 0.035$ | 0.974 | 0.038 (0.10–0.062) | 0.026 |
| *BDNF*[b] | 0.101 | 0.124 | 0.415 | | | | |
| Age | −0.134 | 0.016 | 0.000 | | | | |
| **Research question two** | | | | | | | |
| (a) *COMT* + *BDNF* | 0.109 | 0.079 | 0.169 | 20.08 (8); $p = 0.010$ | 0.968 | 0.049 (0.022–0.076) | 0.029 |
| Age | −0.134 | 0.016 | 0.000 | | | | |
| (b) *COMT* x *BDNF* | 0.046 | 0.178 | 0.795 | | | | |
| *COMT* | 0.049 | 0.268 | 0.854 | | | | |
| *BDNF* | 0.007 | 0.381 | 0.985 | | | | |
| Age | −0.134 | 0.016 | 0.000 | | | | |
| **Research question three** | | | | | | | |
| **Interactive** | | | | | | | |
| (a) *COMT* x age | 0.006 | 0.012 | 0.593 | 21.99 (14); $p = 0.079$ | 0.978 | 0.030 (0.000–0.053) | 0.028 |
| *BDNF* | 0.102 | 0.124 | 0.412 | | | | |
| *COMT* | −0.333 | 0.843 | 0.693 | | | | |
| Age | −0.147 | 0.029 | 0.000 | | | | |
| (b) *BDNF* x age | −0.016 | 0.016 | 0.319 | | | | |
| *COMT* | 0.112 | 0.104 | 0.281 | | | | |
| *BDNF* | 1.193 | 1.103 | 0.279 | | | | |
| Age | −0.114 | 0.025 | 0.000 | | | | |
| (c) *COMT* x *BDNF* x age | 0.000 | 0.002 | 0.909 | 26.03 (14); $p = 0.026$ | 0.968 | 0.037 (0.013–0.059) | 0.030 |

| Executive Function | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Model Fit Indicators** | | | | | | | |
| **Models** | **β** | **SE** | ***p*** | **$\chi^2_M(df_M)$** | **CFI** | **RMSEA (90% CI)** | **SRMR** |
| *COMT* | 0.140 | 0.249 | 0.575 | | | | |
| *BDNF* | 0.139 | 0.353 | 0.694 | | | | |
| Age | −0.134 | 0.017 | 0.000 | | | | |
| **Additive** | | | | | | | |
| (a) *COMT* + age | −0.132 | 0.015 | 0.000 | 12.54 (8); $p = 0.129$ | 0.987 | 0.030 (0.000–0.060) | 0.023 |
| *BDNF* | 0.105 | 0.123 | 0.396 | | | | |
| (b) *BDNF* + age | −0.132 | 0.015 | 0.000 | 17.66 (8); $p = 0.024$ | 0.974 | 0.044 (0.015–0.072) | 0.027 |
| *COMT* | 0.116 | 0.1036 | 0.259 | | | | |
| (c) *COMT* + *BDNF* + age | −0.129 | 0.015 | 0.000 | 9.24 (5); $p = 0.100$ | 0.988 | 0.037 (0.000–0.073) | 0.022 |
| **Research question four** | | | | | | | |
| ***APOE*$^C$ (ε4+)** | | | | | | | |
| (a) *COMT* x *BDNF* x age | 0.005 | 0.005 | 0.285 | 53.978 (31); $p = 0.007$ | 0.939 | 0.050 (0.026–0.071) | 0.046 |
| *COMT* | −0.474 | 0.502 | 0.346 | | | | |
| *BDNF* | −0.628 | 0.675 | 0.352 | | | | |
| Age | −0.154 | 0.030 | 0.000 | | | | |
| (b) *COMT* + *BDNF* + age | −0.136 | 0.024 | 0.000 | 18.539 (13); $p = 0.138$ | 0.984 | 0.038 (0.000–0.073) | 0.044 |
| ***APOE*$^C$ (ε4−)** | | | | | | | |
| (i) *COMT* x *BDNF* x age | −0.004 | 0.003 | 0.173 | 53.978 (31); $p = 0.007$ | 0.939 | 0.050 (0.026–0.071) | 0.046 |
| *COMT* | 0.554 | 0.318 | 0.081 | | | | |
| *BDNF* | 0.671 | 0.453 | 0.139 | | | | |
| Age | −0.127 | 0.022 | 0.000 | | | | |
| (ii) *COMT* + *BDNF* + age | −0.131 | 0.020 | 0.000 | 18.539 (13); $p = 0.138$ | 0.984 | 0.038 (0.000–0.073) | 0.044 |

*Key:* β, regression coefficient; SE, standard error; $\chi^2_M$, chi-square test of model fit; $df_M$, degrees of freedom for model fit; RMESA, root mean square error of approximation; CI, confidence interval; CFI, comparative fix index; SRMR, standardized root mean square residual.

[a] *COMT*: Catechol-O-methyltransferase.

[b] *BDNF*: Brain-derived neurotrophic factor.

[c] *APOE*: *Apolipoprotein E*.