Stress, *COMT* polymorphisms and depressive symptoms in older Australian women: An exploratory study

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Abstract

Objectives: This exploratory study examined the association between exposure to stressful life events (SLEs), polymorphisms (rs165774 and rs4680) in the catechol-O-methyltransferase *COMT* gene, and risk of depression in women. *Methods:* A cross-sectional design gathered information from 150 Australia women, aged 60-70 years, on socio-demographics, SLEs, and depressive symptoms. Participants also provided buccal cell swabs for genetic analysis. *Results:* Among women exposed to stressful life events, the odds of depressive symptoms increased by 20% with each additional exposure (95% CI 1.06–1.34, $p = 0.003$). Women who carried at least one 'A' allele (AA/AG) for both rs165774 and rs4680 SNPs were less likely to report depressive symptoms (compared with women with the GG genotype. Moderation analysis did not support the hypotheses of an interaction with SLEs (rs165774, OR = 1.13, 95% CI 0.87–1.46, $p = 0.347$; rs4680, OR = 1.15 95% CI 0.91–1.44, $p = 0.238$). *Conclusion:* Our research suggests that women with polymorphisms in *COMT* were less susceptible to depressive symptoms but polymorphism do not appear to influence susceptibility to depression in those exposed to life stressors. Further research should consider other genetic variants in catecholamine pathways and their potential impact on women's mental health.

Introduction

Studies on the susceptibility to depressive illness have linked polymorphisms in several genes with a range of neuropsychiatric conditions (Doornbos et al. 2009; Drachmann Bukh et al. 2009). One gene implicated in a number of mental health disorders including depression is catechol-O-methyltransferase (*COMT*), a gene which regulates catecholamine's like dopamine, epinephrine, and norepinephrine in the brain (Gogos et al. 1998). Among individuals with specific *COMT* genotypes and a history of stressful life events, polymorphisms might increase their risk of developing anxiety spectrum disorders, depressive illness, obsessive compulsive disorder, schizophrenia or alcohol dependence (Mandelli et al. 2007; Hettema et al. 2008; Wray et al. 2008; Voisey et al. 2011; Voisey et al. 2012). Our understanding of these interactions is limited despite the growing evidence that inter-individual genetic variability can moderate an individual's vulnerability or resilience to the effects of stressful life events (SLEs) and subsequent mental health problems (Kendler et al. 2010). In this exploratory study, we examine the influence of two SNPs encoded on the *COMT* gene (rs165774 and rs4680) and risk of depressive symptoms in older Australian women with and without a history of past SLE exposure.

Methods

Sample

This paper presents the 2012 cross-sectional data from 150 women from the Healthy Aging of Women (HOW) study, recruitment strategies, response rates and methodological considerations have been detailed elsewhere (Seib et al. 2013a; Seib et al. 2013b). Briefly, a random sample of women aged 45 to 60, from high- and low- income, rural and urban areas of

South-East Queensland participated in a postal survey in 2001 and were followed up every 5 years. Ethics approval for the project was obtained from the Human Research Ethics Committee of the Queensland University of Technology (Ethical approval number 1100000171).

Measurement

Self-administered postal questionnaires collected data on socio-demographic characteristics, lifestyle exposure to stressful life events (LSC-R, range 0-30; (Wolfe and Kimerling 1997)) and depressive symptoms (CES-D; (Radloff 1977)). For this analysis, the CESD was also categorized to form a dichotomous variable indicating the presence of depressive symptoms (scores 0-15 representing no or few depressive symptoms and scores ≥ 16 suggesting some depressive symptoms; (Radloff 1977)).

DNA was extracted from buccal cell swabs using a Gentra Puregene Buccal Cell Kit, (QIAGEN, Hilden, Germany) and DNA integrity and concentration in each sample was analyzed using a Nanodrop 1000 spectrophotometer (Thermo Fisher, Scoresby, VIC, Australia). DNA samples were sent to the Australian Genome Research Facility for genotyping using a homogeneous MassEXTEND (hME) Sequenom assay (Oeth et al. 2009). The hME assay is based on the annealing of an oligonucleotide primer (hME primer) adjacent to the SNP of interest. The addition of a DNA polymerase along with a mixture of terminator nucleotides allows extension of the hME primer through the polymorphic site and generates allele-specific extension products, each having a unique molecular mass. The resultant masses of the extension products are then analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and a genotype is assigned in real time. The hME assay was performed in multiplex with up to 36 reactions in a single well.

The genotyping failure rate was 1% for rs165774 (n = 2) and 0% for rs4680 (n = 0) for all included women. Genotypes were determined by investigators who were unaware of concurrent health status, or exposure to stressors.

Statistical analysis

This analysis used SPSS version 19 to examine the odds of depressive symptoms in women of different genotypes with and without a history of stressful life events(Statistics 2010). The adequacy of regression models were explored using several criteria: (1) a non-significant Hosmer-Lemeshow (H-L) goodness-of-fit $\chi 2$ test; a high predicted probability percentage for correct group classification, and; (3) Nagelkerke's $R^2 \geq .5$; and, the likelihood ratio $\chi 2$ test (LR test statistic) which estimated the addition of a variable (or in this instance the interaction term) significantly contributed to the model (p <0.05).

For the genetic analysis ethnicity needs to be considered and typically ethnically diverse samples would be analyzed in separate ethnic groups. The small sample size prevented these women from being analyzed separately and so 13 women removed from the analysis. The final sample included only Australian born, non-Indigenous women (N = 148). Hardy-Weinberg equilibrium (HWE) was computed using Utility Programs for Analysis of Genetic Linkage (Ott 1988). Genotype frequencies indicated that the *COMT* polymorphisms (rs165774 and rs4680) were in Hardy-Weinberg equilibrium (χ^2 = 0.001, p = 0.997 and χ^2 = 0. 521, p = 0.481 respectively).

Due to the high correlation between rs165774 and rs4680 (i.e., D' = 1.0, r^2 = 0.5 [www.HapMap.org, CEU]), the tests were non-independent and thus a Bonferroni correction could not be applied.

Results

Women with depressive symptoms also reported more life stressors compared with women with few or no depressive symptoms (mean ± SD: 6.6 ± 3.2 and 4.5 ± 3.2 respectively; t = -3.42, *p* =0.001) and these results suggested that for every additional stress exposure, the odds of depressive symptoms increased by 20% (*95% CI* 1.06–1.35, *p*= 0.002). Odds ratios (OR) and 95% confidence intervals (95% CI) were used to examine the relationship between depressive symptoms, exposure to life stressors, and SNPs rs165774 and rs4680 encoded on the *COMT* gene (see model 1, table 1). Results suggested that women who carried at least one 'A' alleles for rs165774 and rs4680 had a lower odds of depressive symptoms (*OR* = 0.36, *95% CI* 0.16–0.85, *p* = 0.019 and *OR* = 0.41, *95% CI* 0.18–0.95, *p* = 0.037 respectively) compared with women with the GG genotype.

Moderation analysis was performed to explore potential interactions between *COMT* polymorphisms, stress exposure, and risk of depressive symptoms (see model 2, table 1). In this sample, no significant interactions were noted and LR test statistics suggested that the addition of the interaction terms did not contribute to the model (rs165774, χ^2 =0.91, *p* = 0.341; rs4680, χ^2 =1.35, *p* = 0.244). On this basis model 1, the more parsimonious model, was determined to be the better fit for the data. Moreover, fit indices suggested the data were an adequate fit; The overall classification percentage suggested that model 1 correctly classified 73.8% of cases (probability of correct classification) for rs165774 and 75.2% of cases for rs4680 and the H-L goodness-of-fit tests were non-significant (rs165774: χ^2 = 46.95, *p* = 0.434; rs4680: χ^2 = 10.32, *p* = 0.243). In contrast, the models provided Nagelkerke's R^2 of 0.156 and 0.123 respectively, suggesting only a modest relationship between prediction and grouping.

[Insert table 1 about here]

Discussion

This exploratory study examined the correlations between stressful life events, depressive symptoms, and two polymorphisms on the *COMT* gene, a gene associated with dopamine activity. Our results suggest that two polymorphisms on *COMT* might the decrease risk of depression adding support to the notion that inter-individual variability could influence an individual's propensity towards depressive symptoms. These findings are supported by others who have suggested that although depression is frequently associated with environmental causes, the heterogeneity in individuals' responses suggests differences in depressive symptoms are possibly attributable to genetic variation (Caspi and Moffitt 2006).

Interestingly, results from this study did not support the hypothesis that genetic variations on *COMT* might influence vulnerability to depressive symptoms in those exposed to stressful life events. Indeed, moderation analysis suggested that though polymorphisms encoded on the *COMT* gene might be protective against depressive symptoms, the variations do not appear to influence the strength of the relationship between variables. Results also suggest that the polymorphisms on rs4680 and rs165774 are perhaps less influential in the face of adversity. These results are in contrast with others who have suggested that SNPs encoded on the *COMT* gene are associated with resilience in response to stress (Heinz and Smolka 2006; Feder et al. 2009).

Several limitations associated with this exploratory study should be mentioned. First, the cross-sectional study design was unable to examine cause and effect but did allow correlations to be explored. Second, although participants were obtained through random sampling in 2001, a proportion of women were lost-to-follow-up over time. Attrition may have impacted on the study

participant's characteristics over time (discussed elsewhere, Seib 2013a, 2013b) and prevented more detailed subgroups and effect modification analyses.

Despite these limitations, genotype frequencies of our available sample were similar to the HapMap CEU (European) data showed no significant differences between observed and expected genotype frequencies among participants. Moreover, our preliminary research suggests that variation in the *COMT* gene is associated with resilience to depressive symptoms but polymorphism do not appear to influence susceptibility to depression in women exposed to life stressors. Future research is needed to validate our findings and should also examine possible interactions between other genetic variations, exposure to stress and risk of depression in women, specifically in relation to catecholamine pathways and their potential impact on women's mental health.

Author Disclosure Statement

The authors have no conflicts of interest.

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TABLE 1. ODDS RATIOS AND CONFIDENCE INTERVALS FOR DEPRESSIVE SYMPTOMS BY EXPOSURE TO STRESS AND GENOTYPE

	Model 1		*Model 2*	
	OR (95% CI)	*p- value*	*OR (95% CI)*	*p- value*
rs165774				
LSC-R	1.20 (1.06–1.35)	0.003	1.15 (1.00–1.32)	0.045
AA/AG	0.36 (0.16–0.85)	0.019	0.17 (0.03–1.05)	0.056
rs165774 x LSC-R			1.13 (0.87–1.46)	0.347
Likelihood ratio $\chi 2$ test			0.91, df = 1	0.341
rs4680				
LSC-R	1.20 (1.06–1.35)	0.003	1.10 (0.92–1.31)	0.313
AA/AG	0.41 (0.18–0.95)	0.037	0.19 (0.04–0.89)	0.035
rs4680 x LSC-R			1.15 (0.91–1.44)	0.238
Likelihood ratio $\chi 2$ test			1.35, df = 1	0.244

LSC-R, Life Stressor Checklist - Revised (Wolfe and Kimerling 1997); OR, Odds Ratio; CI, Confidence Interval; rs165774 HapMap release #28 CEU (European) genotype frequencies are 0.115 (AA), 0.398 (AG), 0.487 (GG); rs4680 HapMap CEU (European) genotype frequencies are 0.248 (AA), 0.460 (AG), 0.292 (GG).