Interactive Effect of the Serotonin Transporter 5-HTTLPR Genotype and Chronic Stress on Depressive Symptoms in Postmenopausal Women

THESIS

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Abstract

The short (s) allele of the serotonin transporter gene length polymorphism (5-HTTLPR) is associated with depression when combined with stress, in a gene by environment interaction model. This research examined the relationship between the 5-HTTLPR gene and caregiving stress on symptoms of depression in postmenopausal women. The sample included 105 female controls and 79 female dementia caregivers. Depressive symptomatology was assessed with the Center for Epidemiologic Studies Depression Scale (CES-D). Results showed that the gene by environment interaction was not significant ($p=0.167$) in postmenopausal women, although there was a main effect for caregiver status, such that caregivers showed higher CES-D scores than controls ($p<0.001$). When comparing premenopausal women to postmenopausal women, the three-way gene by environment by menopausal status interaction was not significant ($p=0.711$), nor was the gene by environment interaction ($p=0.140$). However, there was a main effect for caregiver status such that caregivers exhibited higher CES-D scores compared to controls ($p=0.003$). Results suggest that postmenopausal women undergoing caregiving stress exhibit higher levels of depressive symptomatology regardless of genotype, and that female caregivers are more depressed than female controls regardless of menopausal status.
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Background & Significance

The short \((s)\) allele of the serotonin transporter gene length polymorphism (\(5-HTTLPR\)) is associated with depression when combined with stressful life events, in a gene by environment interaction model \((Brummett, Boyle, et al., 2008; Caspi, et al., 2003; Cicchetti, Rogosch, & Sturge-Apple, 2007; Eley, et al., 2004; Gunthert, et al., 2007; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Taylor, et al., 2006; K. Wilhelm, et al., 2007)\). The gene \((SLC6A4)\) coding for the serotonin transporter (SERT) protein contains a repeat length polymorphism in its promoter region, known as the 5-HT transporter gene-linked promoter region \((5-HTTLPR)\). This polymorphism affects transcriptional activity, such that the short \((s)\) allele is less transcriptionally active, resulting in production of less serotonin transporter protein \((Heils, et al., 1995)\).

Depression is associated with decreased density of SERT sites. This is observed in postmortem brain tissue of depressed suicide victims \((Stanley, Virgilio, & Gershon, 1982)\), and in postmortem hippocampus and occipital cortex tissue of depressed patients \((Leake, Fairbairn, McKeith, & Ferrier, 1991; Perry, Marshall, Blessed, Tomlinson, & Perry, 1983)\). Reduced SERT sites have also been demonstrated in platelets of drug-free currently-depressed patients \((human platelets serve as a model for the transport, metabolism and release of serotonin by serotonergic neurons)\) \((Briley, Langer, Raisman, Sechter, & Zarifian, 1980; Langer, Zarifian, Briley, Raisman, & Sechter, 1981)\).
Clinical studies have shown that the short allele (s) of 5-HTTLPR is associated with myriad psychological features, including increased vulnerability toward the depressogenic effects of stressful life events (Brummett, Muller, et al., 2008; Caspi, et al., 2003; Cicchetti, et al., 2007; Eley, et al., 2004; Gunthert, et al., 2007; Kendler, et al., 2005; K Wilhelm, et al., 2006). Individuals homozygous for the s allele (s/s) exhibited more symptoms of depression, higher prevalence of diagnosed depression, and increased suicidality following significant life stressors (Caspi, et al., 2003). Young women carrying the s allele exhibited more depressive symptomatology in response to stress than did s-carrying men (Brummett, Boyle, et al., 2008; Kendler, et al., 2005; Taylor, et al., 2006). Women with the s/s genotype exhibited more depressive symptomatology in response to tryptophan depletion, regardless of family history of depression (Neumeister, et al., 2002). In men, it was the l/l genotype which was associated with depression in the presence of stress (Brummett, Boyle, et al., 2008; Sjoberg, et al., 2006; Surtees, et al., 2006). However, a recent meta-analysis failed to reveal a sex difference for this gene by environment interaction. This meta-analysis also revealed that when using a model based on number of stressful life events, there is overall no interaction effect between genotype and stressful life events on the phenotype depression (Risch, et al., 2009b). This meta-analysis used a wide age range (12 - 80 years) and did not account for hormonal status (e.g. post-pubertal, post-menopausal). Further, it focused only on studies employing recent stressful life events as stressors, as opposed to chronic stress, such as caregiving.

Whether this sex difference exists in later life, and with chronic stress, has not been examined. Replications of this gene by environment interaction have largely been in
young adults (Caspi, et al., 2003; Chipman, et al., 2007; Eley, et al., 2004; Kendler, et al., 2005; Taylor, et al., 2006; K. Wilhelm, et al., 2007); age ranges included, for instance, 21-25 years old (Caspi, et al., 2003), 20-24 years old (Chipman, et al., 2007), and 18-29 years old (Taylor, et al., 2006). There are important differences between younger and older populations, making it difficult to translate results from younger samples to an older population. For instance, in the aging human brain, SERT availability is decreased as compared to younger individuals, with a decrease of nearly 30% from the age of 18 to 88, or 4.2% per decade (van Dyck, et al., 2000). Additionally, with aging comes a drop in sex steroid hormones in both men and women, which may influence the gene by environment interaction. Given the relationship between sex steroid hormones and the serotonergic system (Epperson, et al., 2007; Lasiuk & Hegadoren, 2007), the serotonin transporter may be an important target in exploring changes in stress response between men and women as they age.

The following sections will describe the 5-HTTLPR gene by environment interaction, the effects of aging on the serotonergic system in both animal and clinical studies, and the influence of sex steroid hormones on serotonergic function, particularly in the context of aging. It will also discuss a possible role for sex steroid hormone modulation of the gene by environment interaction.

*The 5-HTT Gene by Environment Interaction*

Genetic variation in serotonin-related genes may pose a significant risk for depression vulnerability. According to a gene by environment interaction model, the
short (s) allele of the serotonin transporter gene length polymorphism (5-HTTLPR) is
associated with depression when combined with an environmental stressor, such as
childhood abuse, caregiving stress, or recent stressful life events.

Recent stressful life events are the most commonly used stressor in the 5-HTT
gene by environment interaction model. However, results of a recent meta-analysis have
called this interaction into question (Risch, et al., 2009b). The meta-analysis pooled 14
studies, six of which replicated Caspi's original work. However, the pooled studies
combined a large range of ages (12 - 80 years), a variety of stressors, and varied means of
assessing depression (e.g. DSM diagnosis, Beck Depression Inventory, or other rating
scale). A second meta-analysis, which pooled an even greater range of studies (age
ranges 5 years to 75 years; stressors ranging from childhood abuse to unemployment)
also found, overall, no gene by environment interaction (Munafo, Durrant, Lewis, &
Flint, 2009). However, grouping such a diverse range of ages and stressors may mask
effects that are age-specific, or occur only in response to a particular type of stress.
Indeed, attempts to replicate Caspi's original work have yielded mixed results. In
addition, some studies have shown a sex difference, while others have not. Lack of
replication has been attributed to failure to account for confounds such as age, gender,
family history of depression, marital status, and social support (Drachmann Bukh, et al.,
2009). Menopausal status is a confound that has not been accounted for in replication
attempts, and may influence the gene by environment interaction.

Sex Difference in the 5-HTTLPR Gene by Environment Interaction
Some studies examining the interaction between stress and 5-HTTLPR have found a sex difference in the 5-HTTLPR gene by environment interaction. The two studies specifically addressing sex difference suggested that: 1) the pattern of male versus female s-carriers is in the opposite direction; while female s-carriers tend to develop depressive symptoms, male s-carriers are protected from depression (Sjoberg, et al., 2006). 2) In females, the s allele combined with caregiving stress or low childhood SES is associated with increased depressive symptomatology relative to non-stressed individuals or l/l homozygotes, while in males, l/l genotype combined with stress is associated increased depressive symptomatology (Brummett, Boyle, et al., 2008).

In a study of depressed teenagers, significant risk for depression was associated with the s/s genotype, but only for adolescent women, and only those who had experienced high family adversity in childhood or adverse life events in the past six months (Eley, et al., 2004). In a group of 16 to 19 year olds, s/s genotype and low childhood socioeconomic status resulted in higher likelihood to develop depression in the adolescent women. In males, the pattern was reversed, so that s/s genotype had a protective effect on the depressogenic effects of childhood stress (Sjoberg, et al., 2006). Consistent with this, the l/l genotype was associated with depression in response to childhood adversity in adult men (Surtees, et al., 2006). In adults with a mean age of 34, low childhood socioeconomic status or caregiving stress was associated with depression in women carrying the s allele, but not s-carrying men (Brummett, Boyle, et al., 2008). A study examining the moderating effect of gender on the association between 5-HTTLPR genotype and another mood disorder, bipolar disorder, found that gender moderated the
relationship between genotype and manic symptoms, such that women with an *l* allele had less severe manic symptoms than men with the same genotypes, while women with the *s/s* genotype had the same level of manic symptoms as *s/s* men (Rucci, et al., 2009). Some studies examining the interaction between stress and 5-HTTLPR have found the gene by environment interaction, but no gender effect. In a study of young adults aged 18 to 29, while poor early family environment and *s/s* genotype interacted to produce depression, this did not vary by gender (Taylor, et al., 2006).

**Recent Stressful Life Events**

In Caspi's original work, in a sample of adults aged 26, recent stressful life events interacted with the *s* allele of 5-HTTLPR to produce depression; this did not vary by gender (Caspi, et al., 2003). Among the first replication attempts, Gillespie et al found no gene by environment interaction, when using stressful life events in the past year, in a sample of adults aged 19 to 78 years (Gillespie, Whitfield, Williams, Heath, & Martin, 2005). In a similar sample with a broad age range (18 to 78 years, with a mean of 39 years), a gene by environment interaction with the *s* allele did occur for recent stressful life events, but, similar to Caspi's results, a gender effect was not detected (Kendler, et al., 2005). Another sample with a mean age of 47.7 years (range unknown) showed that the *s* allele was associated with depression in individuals who had experienced negative life events in the preceding five years; no gender difference was found (K Wilhelm, et al., 2006). If hormonal status influences the gene by environment interaction, then broad age
ranges which include older adults with lower sex steroid hormone levels may mask sex differences.

However, studies using narrower age ranges have found similarly mixed results. Using a racially homogenous sample of Caucasian men and women aged 20-24 years, Chipman et al. assessed both influence of childhood adversity and stressful events in the preceding 6 months to examine the 5-HTTLPR gene by environment interaction. The authors found no gene by environment interaction, although there were significant main effects for sex, recent stressful life events, and childhood adversity on depression (Chipman, et al., 2007). Similarly, a sample of 19 year olds showed no 5-HTTLPR gene by environment interaction for recent stressful life events, however, l/l individuals who were exposed to childhood adversity (including experiences such as a one parent family, low educational level of parent, or marital discord) showed higher rates of depression and more depressive symptoms than s carriers, demonstrating a reverse relationship than found by Caspi (Laucht, et al., 2009). Another study in a sample of female twins ages 13 to 23, in which at least one twin had a history of depression, also found a reverse relationship, in which the high-activity l(a) allele (l(a) is functionally equivalent to the l allele; the l(g) allele is low-activity and functionally equivalent to the s allele) combined with lifetime history of any traumatic event was associated with increased risk of depression (Chorbov, et al., 2007). However, the authors do not specify what qualifies as a traumatic event, and when this event occurred (e.g. early childhood versus past year); strikingly, 46.2% of the sample reported a traumatic life event. Further, the authors do not
report whether they controlled for pubertal status, which would be important consideration in a sample of this age range.

Severity of stressor is also an important consideration. While Caspi’s group used a range of recent stressful life events, other studies, particularly those examining childhood stress, use more severe or traumatic events as stressors. One study in adults (mean age 49) restricted "stress" to include only severe events occurring in the past six months; severe events included death of a parent or child, onset of a serious illness, or marital separation, for instance (Cervilla, et al., 2007). These were categorized into no stressful events, one stressful event, or two or more stressful events. The researchers found that the s allele significantly modified risk for depression as level of exposure to severe stressful events increased. The model fit improved when adjustments were made for age, gender, and family psychiatric history. The authors acknowledge, however, that the influence of sex on this gene by environment interaction is puzzling, as some studies have reported a sex difference, while others have not.

*Caregiving Stress*

An established chronic stressor, dementia family caregiving has been associated with increased risk of depression and anxiety (J. Dura, Stukenberg, & Kiecolt-Glaser, 1990), impaired wound healing (J. K. Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995), more frequent infections (J. K. Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991), poor response to influenza and pneumonia vaccinations (Glaser, Sheridan, Malarkey, MacCallum, & Kiecolt-Glaser, 2000; J. K. Kiecolt-Glaser, Glaser,

Family dementia caregiving is a potent stressor, often outstripping caregiver resources. After initiation of caregiving, dementia family caregivers exhibited greater incidence of depressive disorders, regardless of personal or family psychiatric history, compared to controls (J. R. Dura, Stukenberg, & Kiecolt-Glaser, 1991). That is, caregivers were prone to develop depression, even with no apparent vulnerability toward depression. When compared to sociodemographically similar controls, caregivers have fewer people in their support networks, less frequent social contact, and less closeness and emotional support (J. K. Kiecolt-Glaser, et al., 1991). Indeed, less satisfaction with social contacts was associated with greater depression among caregivers (Tompkins, Schulz, & Rau, 1988). This underscores the importance of a supportive social network in buffering the effects of caregiving stress against depression (Light & Lebowitz, 1989). In fact, several factors protect against caregiver burden, including support from others (Barusch & Spaid, 1989) use of adaptive coping strategies (Ott, Sanders, & Kelber, 2007), and caregiver’s educational level (Graham, Ballard, & Sham, 1997). Coping
strategies may include venting, reframing, or humor; these strategies were used more often by parental caregivers than by spousal caregivers (Ott, et al., 2007).

Caregiver burden refers to the demands made upon the caregiver’s resources (e.g. time, money) as well as the caregiver’s emotional state and health (Black & Almeida, 2004; George & Gwyther, 1986; Vitaliano, Russo, Young, Teri, & Maiuro, 1991). While burden and depression are correlated (Clyburn, Stones, Hadjistavropoulos, & Tuokko, 2000; Sherwood, Given, Given, & von Eye, 2005), depression as an emotional reaction to caregiving represents just one facet of burden (Baumgarten, et al., 1992; Bedard, Molloy, Pedlar, Lever, & Stones, 1997; Black & Almeida, 2004). Persistently elevated burden may increase risk for depression in caregivers (Epstein-Lubow, Davis, Miller, & Tremont, 2008).

Increases in dementia care recipient’s behavioral and psychological symptoms of dementia, such as an increase in disruptive behavior, have been associated with increased caregiver burden (Claus, Teunisse, Walstra, & van Gool, 1998; Gaugler, Davey, Pearlin, & Zarit, 2000). Over the course of caregiving, when dementia care recipient’s behavioral and psychological symptoms of dementia decrease, the caregiver’s burden also decreases (Grafstrom & Winblad, 1995). A meta-analysis revealed that among several studies, behavioral and psychological symptoms of dementia explained 30% to 49% of variance in caregiver psychological distress. This meta-analysis revealed a robust correlation between dementia care recipient’s behavioral and psychological symptoms of dementia and caregiver burden (r=0.57), but a weaker correlation between dementia care recipient’s behavioral and psychological symptoms of dementia and caregiver depression
(r=0.3) (Black & Almeida, 2004). This interrelationship has been referred to as the depression-stress-burden constellation (Ott, et al., 2007). However, some have called for abandonment of the concept of burden in favor of more clinically relevant measures, such as caregiver depression (Black & Almeida, 2004).

Studies on the influence of dementia symptoms on caregiver depression show mixed results. In one study, behavioral problems in the care recipient predicted caregiver depression (Ballard, Eastwood, Gahir, & Wilcock, 1996). However, behavioral and psychological symptoms of dementia did not predict change in caregiver depression (Schulz & Williamson, 1991). In another study, baseline depression in the care recipient predicted caregiver depression at follow-up, and increasing care recipient depression over the course of caregiving correlated with elevated rate of change of caregiver depression (Neundorfer, et al., 2001).

One view proposes that caregiver appraisals of the caregiving process or of the care recipient are more important predictors of caregiver burden and depression than dementia severity or physical symptoms (Steadman, Tremont, & Davis, 2007). That is, the relationship between dementia symptoms and caregiver may not be direct, but may be mediated by the caregiver’s appraisals of how stressful the situation is (Goode, Haley, Roth, & Ford, 1998). Caregiver perception of current problems, as well as caregiver self-efficacy, predicted caregiver depression and perceived burden, as did caregiver social support, resources, and coping skills (Coen, Swanwick, O'Boyle, & Coakley, 1997; Deimling & Bass, 1986; Mangone, et al., 1993).
Caregiver depression at onset of caregiving is also an important predictor of depression during caregiving; many caregivers are depressed at baseline, and maintain this level of depression throughout caregiving (Alspaugh, Stephens, Townsend, Zarit, & Greene, 1999; Schulz & Williamson, 1991).

Specific aspects of caregiving stress have been examined to determine whether its depressogenic effects vary by setting (e.g. caregiving in-home versus a nursing home), relationship (e.g. spousal versus parental caregiving), or temporal factors (e.g. hours per week, duration of caregiving). Among seven objective aspects of caregiving stress (hours spent caregiving per week, duration of caregiving, and patient self-care problems, behavioral problems, physical symptoms, or emotional symptoms), the temporal factors, hours spent caregiving per week and duration of caregiving, did not influence level of depression in the caregiver (Haley, LaMonde, Han, Burton, & Schonwetter, 2003). Thus, those who began caregiving more recently were no more likely to be depressed than those who had been caregiving for longer time periods. Setting, that is community versus nursing home, did not influence depression levels of caregivers (Stephens, Kinney, & Ogrocki, 1991). Although caregiving at home was associated with more hours of caregiving per week than caregiving at a nursing home, this did not influence depression levels (Haley, et al., 2003). Rather, caregiver depression levels tend to vary with care recipient symptomatology, including both dementia symptoms and medical symptoms (Grant, et al., 2002), and thus may peak and ebb at different points over the course of caregiving (Haley & Pardo, 1989).
In fact, there are two bodies of thought on caregiver function over the course of caregiving. The wear and tear hypothesis posits that caregiver functioning will progressively deteriorate as dementia progresses, while the adaptation hypothesis suggests that caregivers adapt over time to the stresses of caregiving (Schulz & Williamson, 1991; Townsend, Noelker, Deimling, & Bass, 1989).

While relationship with care recipient (spousal versus parental, i.e. caring for a spouse versus caring for a parent) did not affect depression levels (Fisher & Lieberman, 1994), among parental caregivers, adult children with high levels of filial obligation, a culturally-influenced factor, tended to continue care for a parent in the community, as opposed to placing the parent in a home, even when experiencing great amounts of burden and stress (Stiens, Maeck, & Stoppe, 2006). Spousal caregivers may have fewer coping resources due to age-associated losses (Pinquart & Sorensen, 2003). However, parental caregivers may have competing roles to fill (e.g. caregiving, work, family) thus increasing likelihood that resources will be strained (Moen, Robison, & Dempster-McClain, 1995).

Further, quality of relationship with the care recipient before dementia onset does contribute to caregiver stress. Low premorbid marital intimacy (Morris, Morris, & Britton, 1988) and longstanding interpersonal problems (Teusink & Mahler, 1984) were both associated with increased caregiver burden in spousal caregivers. In both spousal and parental caregivers, an emotionally distant premorbid relationship was associated with greater caregiver burden, but not with depression (Gold, et al., 1995; Williamson & Schulz, 1990). In both spousal and parental caregivers, low premorbid relationship
satisfaction was associated with higher caregiver burden; this was independent of length
of caregiving, disease severity, care recipient’s daily functioning, or relationship type
(Steadman, et al., 2007).

Attachment style may also influence caregiver response to caregiving. Parental
caregivers with a secure attachment style reported lower level of burden (Carpenter,
2001; Crispi, Schiaffino, & Berman, 1997). Those with an avoidant attachment style were
more likely to engage in poor methods of coping, expressed higher levels of caregiver
burden, and were more prone to anxiety during caregiving (Cooper, Owens, Katona, &
Livingston, 2008).

Further, personality traits of the caregiver may influence reactions to caregiving.
Personality traits and coping technique (e.g. problem-focused versus emotion-focused)
impact the caregiver’s perception of the caregiving experience (Goode, et al., 1998;
Such personality traits may serve as a diathesis toward depression in the face of
caregiving, or serve as a protective buffer against it.

Caregiving stress provides an ideal means of conceptualizing stress from a gene
by environment interaction standpoint, as opposed to stressful life events. Research
suggests that the relationship between stressful life events and depression is bidirectional;
that is, individuals who are at risk for depression may select themselves into high risk
situations (Kendler, Neale, Kessler, Heath, & Eaves, 1993). In older adults, there was a
significant genetic influence, up to 40% of variance, on reported stressful life events,
particularly for events within the individual’s control (Plomin, Lichtenstein, Pedersen, McClearn, & Nesselroade, 1990).

From a genetic standpoint, this has been termed “nature via nurture;” genetic predisposition drives an individual to select or create certain environments, which results in the mistaken appearance of a purely genetic effect (Scarr & McCartney, 1983). Up to one third of the relationship between depression and stressful life events may be explained by this bidirectional “nature via nurture” relationship in which high risk environments are sought out (Kendler, Karkowski, & Prescott, 1999). In terms of this gene by environment interaction model, it may be that s-allele carriers are more likely to put themselves into high-risk, depression-inducing environments. Thus, caregiving is ideal as a stressor in a gene by environment interaction model because it removes the bidirectional influence; providing care for a spouse or parent is not likely to reflect genetic influences, as, particularly for spousal caregivers, marriage occurred before the development of the dementia disorder. Caregiving has therefore been termed a “natural experiment,” in which exposure to events is not related to underlying genetic diathesis (Kessler, 1997). Determining whether older individuals with a genetic diathesis are more vulnerable to the depressogenic effects of current chronic stress, according to the 5-HTTLPR gene by environment interaction model, will be key.

5-HTTLPR Gene by Environment Interaction in Older Adults

A small number of studies examined the 5-HTTLPR gene by environment interaction in older adults. In a study of Korean elders, s allele carriers facing stressful
life events in the past year had higher rates of diagnosed depression (Kim, et al., 2007). In individuals aged 60 and older who had experienced the stressful event of hip fracture, s allele carriers showed higher levels of depressive symptomatology up to 14 weeks after the event, compared to l/l homozygotes (Lenze, et al., 2005). In a sample with mean age 51.6 years, unemployment stress, in combination with the s/s genotype, predicted psychological and physical distress (e.g. back pain, irritability, nervousness, palpitations, stomach ache, as measured by the BL-38, the von Zerssen Complaints Scale) in women, but not in men (Grabe, et al., 2005). There have also been nonreplications in older adults. In a study of European adults aged 65 and older, there was no interaction between 5-HTTLPR and past-year stressful events for depressive symptomatology (Power, et al., 2008).

Serotonin transporter gene by environment studies in older populations have not focused on postmenopausal sex differences. Two of the three abovementioned studies (Kim, et al., 2007; Lenze, et al., 2005) failed to reveal sex differences in the gene by environment interaction, with both sexes exhibiting a female-typical pattern, in which s allele and stress interacted to produce depressive symptoms. It should be noted that the two studies showing the female-typical pattern operationalized stress as discrete stressful events. Whether type of stress influences the gene by environment interaction pattern is unknown; the current study will employ chronic stress. Also, menopausal status was not included as a variable in these models; if the study samples included premenopausal, perimenopausal, and postmenopausal women, the differences in hormonal status may have skewed the results.
Indeed, studies in older populations have focused on discrete stressful life events (Kim, et al., 2007; Lenze, et al., 2005; Power, et al., 2008), as opposed to chronic stress. Chronic stress, such as caregiving, may be more representative of the typical stress experienced by older adults (Monroe & Reid, 2008). To date, only one study has examined caregiving stress and 5-HTTLPR. In female caregivers, those with a copy of the s allele exhibited higher self-reported depressive symptomatology than non-stressed controls. The reverse was true in males, in whom l/l genotype was associated with increased depressive response to current chronic stress (Brummett, Boyle, et al., 2008). This study, however, used a large age range (22-88 years) and did not control for menopausal status. Thus, although sex differences in depressive symptoms and health complaints have begun to emerge in these chronically stressed groups (Brummett, Boyle, et al., 2008; Grabe, et al., 2005), research to date has not focused explicitly on the influence of menopausal status in this gene by environment interaction.

5-HTTLPR Gene by Environment Interaction in Older Women

Among the few existing studies on the 5-HTTLPR gene by environment interaction in older populations, findings on sex differences have not accounted for menopausal status. There is some evidence suggesting that expression of 5-HTTLPR may change with menopause. In a study of women who had attempted suicide, postmenopausal attempters had a higher frequency of the l allele, reflecting a pattern consistent with that exhibited by younger men (Baca-Garcia, et al., 2003). This study, however, simply examined associations between genotype and suicide attempt; it did not
involve a gene by environment interaction. While this work suggests that post-menopausal women may exhibit a more male-like pattern in 5-HTTLPR expression, whether this holds for the gene by environment interaction remains to be determined. If the 5-HTTLPR gene by environment interaction is modulated by sex steroid hormones, then older adults, with waning sex steroid hormone levels, are likely to show a different relationship between 5-HTTLPR genotype and depressive phenotype than younger adults. Whether older males will exhibit a more premenopausal female-like pattern (s/s genotype x stress associated with depression), or whether postmenopausal women will show a more male-like pattern (l/l genotype x stress associated with depression) is unknown.

*Interactions Between Sex Steroid Hormones and the Serotonergic System*

*Hormones and the Serotonin Transporter Gene*

Recent evidence shows that 5-HTT polymorphisms interact with the endocrine system. Individuals homozygous for the s allele showed elevated cortisol reactivity to acute stress (Alexander, et al., 2009). Importantly, the promoter region of the 5-HTT gene contains a glucocorticoid response element, meaning that glucocorticoids may regulate expression of 5-HTT (Glatz, Mossner, Heils, & Lesch, 2003). Extending this gene-hormone interaction to sex steroid hormones, if ovarian hormones interact with serotonin to influence mood, then serotonin-related genetic polymorphisms, such as 5-HTTLPR, may be an important piece in this puzzle. The menopausal drop in ovarian hormone levels may influence the serotonin gene by environment interaction, via several possible pathways.
First, cyclic shifts in ovarian hormones enhance stress sensitivity in women (Seeman, 1997); postmenopausally, low ovarian hormone levels are associated with greater reactivity to acute stress (Lindheim, et al., 1992; Saab, Matthews, Stoney, & McDonald, 1989; Stoney, Owens, Guzick, & Matthews, 1997), as well as chronic stress (Steffen, Thompson, Gallagher-Thompson, & Koin, 1999). How postmenopausal heightened stress sensitivity presents in women with a putative genetic diathesis toward depression, such as \textit{5-HTTLP}Rs allele, is unknown. Further, how this extends to chronic, as opposed to acute stress, is not well characterized.

Second, in addition to influencing stress sensitivity, there is evidence that ovarian hormones influence depression. Rates of depression are similar between boys and girls (Birmaher, et al., 1996), but increase in women after puberty. This sex difference in depression prevalence persists through adulthood, then wanes following menopause (Jorm, 1987), indicating hormonal influence on mood. However, how this operates in women with a possible genetic diathesis toward depression is unknown.

Indeed, it has been proposed that genetic factors, in combination with hormonal factors, mediate vulnerability toward depression in women (Kendler, 1998). Specific genes have not been implicated, but, as the \textit{5-HTTLPR} has been tied to a sex difference in depressive response to stress, it is an excellent candidate. As interactions between ovarian hormones and the serotonergic system in depression have been proposed (Epperson, et al., 2007; Janowsky, 1996; Lasiuk & Hegadoren, 2007; Poirier, Loo, Dennis, Le Fur, & Scatton, 1985), examining how genetic vulnerabilities in the serotonergic system change with waning ovarian hormones is warranted.
Animal Research: Estrogens and Serotonergic Function

Animal research has demonstrated sexual dimorphism in the density of 5-HT innervation to forebrain structures (Gorski, 1985), differences in brain serotonin turnover rates (Carlsson, Svensson, Eriksson, & Carlsson, 1985) and higher serotonin synthesis and serotonin brain levels in females (Dickinson & Curzon, 1986). Males show a higher turnover rate, indicating that, in rats, serotonin may be processed differently in male versus female brains (Reznikov & Nosenko, 1996). Importantly, in rats, 5-HT receptor and transporter activity is differentially regulated by gender and estrous cycle, such that during proestrus, when estrogen levels are highest, 5-HT levels are also highest (Maswood, Truitt, Hotema, Caldarola-Pastuszka, & Uphouse, 1999). With the surge of estradiol during the proestrous phase in female rats, there is a notable increase in 5-HT\textsubscript{2A} receptors in the forebrain (Henderson, Baker, & Fink, 1977).

At the molecular level, sex steroid hormones act as transcription factors, acting to increase or decrease gene expression (Katzenellenbogen, 1996). In fact, sex steroid hormones alter expression of serotonin-related genes (Pecins-Thompson, Brown, & Bethea, 1998; Pecins-Thompson, Brown, Kohama, & Bethea, 1996). Estrogen and progesterone administration in rats alters expression of the 5-HT\textsubscript{2A} receptor (Sumner & Fink, 1998), SERT (McQueen, Wilson, & Fink, 1997), and the vesicular monoamine transporter (Rehavi, Goldin, Roz, & Weizman, 1998). SERT gene expression is regulated, in both males and females, by estrogen (Sumner & Fink, 1998). Importantly, estrogens may modulate activity of the serotonin transporter. Administration of estrogen
induced an increase in serotonin transporter mRNA within 16 hours, accompanied by an approximately 50% increase in SERT sites in forebrain areas involved with emotion and behavior, including the basolateral amygdala (McQueen, et al., 1997).

In female mice, ovariectomy, with its concomitant decrease in estradiol, resulted in reduced SERT expression (Bertrand, et al., 2005). In rats, females in a low estrogen state also showed decreased SERT gene expression (Maswood, et al., 1999). Expression of the SERT gene was significantly increased when female rats were induced into a hormone-stimulated pseudopregnancy (ovariectomy followed by implantation of estradiol, progesterone); SERT mRNA levels declined to baseline levels after 4 days of hormone withdrawal (Suda, Segi-Nishida, Newton, & Duman, 2008).

Animal Research: Androgens and Serotonergic Function

In the male, testosterone largely exerts its influence on brain and behavior via conversion to estrogen by the aromatase enzyme (Selmanoff, Brodkin, Weiner, & Siiteri, 1977). In rats, castration and its concomitant decrease in testosterone results in a decrease in number of cells expressing SERT mRNA in the dorsal raphe (McQueen, et al., 1997), and a decrease in 5-HT$_{2A}$ receptor sites in the frontal cortex, cingulate cortex, olfactory nucleus, and nucleus accumbens (Fink, Sumner, Rosie, Wilson, & McQueen, 1999). Treatment of castrated male rats with testosterone or estradiol brought the number of 5-HT$_{2A}$ receptors and SERT expression back to normal levels (Fink, Dow, McQueen, Bennie, & Carroll, 1999). These decreases are likely due to the decrease in testosterone, which subsequently is not available to be converted to estrogen. In areas of the brain
where the aromatase enzyme is not present, testosterone had no effect on the 5-HT$_{2A}$ receptor, underscoring the fact that it is not testosterone itself, but its conversion to estrogen, that impacts serotonergic function in males (Fink, Dow, et al., 1999). In intact male rats, testosterone administration increased SERT mRNA expression level in the dorsal raphe, as well as the amygdala, an area of the brain critical to mood (McQueen, Wilson, Sumner, & Fink, 1999). However, 5α-dihydrotestosterone (5α-DHT), a potent androgen that is not aromatized to estradiol 17-β, does not affect SERT mRNA expression (McQueen, et al., 1999). This is further evidence that it is estrogens, not androgens themselves, influencing SERT expression.

In sum, animal research on the interactions between sex steroid hormones and the serotonergic system reveal that in both females and males, high levels of sex steroid hormones result in high levels of SERT mRNA and protein, while low levels of sex steroid hormones (via ovariectomy or castration) cause decreased levels of SERT mRNA and protein. Animal research has indicated direct effects of sex steroid hormones on SERT expression, which is apparently estrogen-dependent, as testosterone's effect on SERT expression is due to its conversion to estradiol.

Animal Research: Aging and Serotonergic Function

Animal research shows that aging alters the 5-HT system. In older animals, 5-HT receptor density is decreased (Davidoff & Lolova, 1991), 5-HT turnover is reduced (I. R. Cohen & Wise, 1988; Simpkins, 1984), and metabolism and receptor expression are also affected (Krajnak, Rosewell, Duncan, & Wise, 2003). There is also global age-related
deterioration of the 5-HT system (van Luijtelaar, Steinbusch, & Tonnaer, 1989); ascending and descending tracts of the 5-HT system show reduced axon fibers and terminals, and increased aberrant fibers (Davidoff & Lolova, 1991; Meister, Johnson, & Ulfhake, 1995).

Aging is associated with decreased SERT density as suggested by a decrease of approximately 20% in the maximal density ($B_{\text{max}}$) of tritiated ($3\text{H}$) paroxetine binding sites in the dorsal raphe nucleus of hamsters ($3\text{H}$-paroxetine is a radiolabeled ligand which binds to SERT and serves as a measure of SERT function and density) (Duncan & Hensler, 2002). In female rats, aging and its concomitant changes in estradiol level affect SERT levels; reproductively senescent female rats showed diminished ability of estrogen to regulate binding of $3\text{H}$-paroxetine compared to younger females (Krajnak, et al., 2003). In aging male rats, there is increased SERT mRNA in the raphe nucleus as compared to younger male rats, which may serve to compensate for the global degradation of 5-HT function that occurs with age (Meister, et al., 1995). Thus, aging is associated with both a decrease in sex steroid hormone levels, and with decreased SERT expression and density.

Interactions Between Estrogenic Function and Serotonergic Function in Women

During fetal development, estrogen acts on the brain, influencing differentiation of the serotonergic system (McCarthy, 2008) and producing sexual dimorphism in serotonergic system structure and function (Carlsson, et al., 1985; Dickinson & Curzon, 1986; Gorski, 1985). In adult mammals, estrogen acts in the brain in several manners. It
increases monoamine oxidase degradation (Luine & McEwen, 1977), increases free tryptophan availability in the brain (Aylward & Maddock, 1973) and enhances 5-HT transport (Sherwin & Suranyi-Cadotte, 1990). Estrogen also upregulates 5-HT synaptic responsivity, and is associated with increased 5-HT concentration at the synapse (Halbreich, 1997). These features are consistent with the idea that estrogen acts as a 5-HT agonist, enhancing serotonergic function. In women, serotonin synthesis rates are 10-20% higher than in men (Chugani, Muzik, Chakraborty, Mangner, & Chugani, 1998). Compared to men, women show lower dissociation constant ($K_d$; a measure of receptor affinity) of 5-HT transporter (Marazziti, et al., 1988) and lower density ($B_{\text{max}}$) of 5-HT$_{1A}$ receptors in the parietal cortex and hippocampus (Palego, et al., 1997). Depression is associated with a decrease in density of SERT binding sites in the hippocampus and occipital cortex (Leake, et al., 1991; Perry, et al., 1983).

Some researchers suggest that in humans, estrogen-induced changes in SERT binding sites in areas such as the amygdala could result in changes in mood (McQueen, et al., 1999), such as depression during the perimenopause (McQueen, et al., 1997). An estrogen-induced increase in serotonin transporter may enhance reuptake of 5-HT, thus reducing extracellular 5-HT (Maswood, et al., 1999), which may also have implications for mood.

Hormonal Influence on Serotonergic Function in Post-Menopausal Women

Menopause is defined as cessation of menstrual period for greater than one year; with menopause comes a drop in ovarian hormone levels in women, including estradiol-
17β, the major circulating estrogen in humans (Broekmans, Soules, & Fauser, 2009). Platelet serotonin content is positively correlated with plasma estradiol concentrations in perimenopausal and postmenopausal women, supporting the relationship between the serotonergic and ovarian systems (Guicheney, et al., 1988). In menopausal and ovariectomized women, estrogen replacement therapy increased serum 5-HT levels (G. F. Gonzales & Carrillo, 1993). Further, treatment of postmenopausal women with estrogen produced increased urinary secretion of 5-HIAA, a serotonin metabolite (Lippert, Filshie, Muck, Seeger, & Zwirner, 1996). These results mirror animal research, in which high levels of estrogens are associated with high serotonin levels, and low levels of estrogen, e.g. after menopause, are associated with low serotonin levels.

Postmenopausally, women show lower 5-HT uptake capacity, measured by imipramine binding, as compared to premenopausal women (Halbreich, et al., 1995). Postmenopausal women also showed blunted response to stimulation of the serotonergic system with a serotonin agonist when compared to premenopausal women; estrogen replacement therapy augmented the response to the serotonergic agonist in postmenopausal women (Halbreich, et al., 1995).

Hormonal Influence on Mood in Post-Menopausal Women

Research suggests important interactions between ovarian hormones and serotonin in modulating mood. Women exhibit increased psychiatric symptoms at times of hormonal shift, i.e. at puberty (Angold, Costello, Erkanli, & Worthman, 1999), during the premenstruum (Abramowitz, Baker, & Fleischer, 1982; Endicott, 1993), postpartum,
and in the perimenopause (Freeman, et al., 2004). A key factor may be fluctuations in level of hormone, or hormone withdrawal, as opposed to absolute level of ovarian steroids. In fact, fluctuations in neuroactive steroids such as estrogen are thought to be involved in a range of psychiatric symptomatology; postmenopausal women are at increased risk, compared to premenopausal women, for the onset of schizophrenic episodes (Hafner, et al., 1993). Also, postmenopausal women showed greater hostility during times of chronic stress, as compared to postmenopausal women receiving hormone replacement therapy (Steffen, et al., 1999). In women receiving HRT, abrupt cessation of estrogen treatment can induce onset of a depressive episode in women with a history of depression (Stewart, Rolfe, & Robertson, 2004). These women reported rapid onset of depression (mean within 3 weeks) subsequent to discontinuation of hormone therapy; it was unclear whether depression resulted from absolute absence of hormone, or the sudden fluctuation, or rate of change.

Estrogen Therapy: Further support for a link between serotonergic function and estrogen is found in studies examining treatment of depression with estrogen replacement therapy (ERT), or a combination of ERT and selective serotonin reuptake inhibitors (SSRIs). Efficacious treatment of depression with ERT has been demonstrated, particularly in perimenopausal women (Soares, Almeida, Joffe, & Cohen, 2001). In fact, during the perimenopause, estrogen alone was effective in improving mood in women, independent of vasomotor symptoms, history of depression, and baseline estradiol level (Saletu, et al., 1995; Schmidt, et al., 2000). Perimenopausal depressed women were more responsive to
the effects of ovarian steroid hormones on mood than postmenopausal depressed women (Montgomery, et al., 1987).

Meta-analysis has shown that in postmenopausal women, estrogen was associated with a moderate to large effect size for improvement in depressed mood (Zweifel & O'Brien, 1997). However, some studies have shown that in postmenopausal women over age 60, estrogen is ineffective in improving mood; a study in women aged 70 years and older, in which estrogen was administered for 20 weeks at relatively high doses, showed that the treatment did not improve mood (Almeida, et al., 2006). In a prospective, double-blind cross-over study in surgically menopausal women, estrogen treatment produced an increase in the number of tritiated (3H) imipramine binding sites (imipramine is a tricyclic antidepressant which enhances 5-HT reuptake inhibition; 3H-imipramine serves as a measure of serotonin binding), and an improvement of mood. When placebo was administered, this effect was reversed (Sherwin, 1999).

Estrogen & Antidepressant Combination Therapy: Estrogen enhances antidepressant effectiveness, particularly in premenopausal (Martenyi, Dossenbach, Mraz, & Metcalfe, 2001) and perimenopausal women (e.g. within 12 months of their last menstrual period) (Soares, et al., 2001), and younger postmenopausal women (Zanardi, et al., 2007). Older postmenopausal women (those who have been in the menopause for ten years or more) who were depressed also showed better response to ERT plus SSRI than those receiving SSRI alone, who showed minimal improvement in mood, similar to those treated with placebo (Schneider, et al., 1997).
Aging, Hormonal Function, and Mood in Men

With aging, men exhibit a gradual decrease in sex steroid hormone levels. This is often referred to as the male climacterium, or "andropause" (Vermeulen, 1993). Total testosterone levels decline by 0.5–2% per year, beginning as early as the fourth decade (Vermeulen, 2001); in a longitudinal study, incidence of free testosterone levels that had diminished to hypogonadal levels occurred in about 9% of men in their 50s, but jumped to 34% of men in their 60s, 68% in their 70s, and 91% in their 80s (Harman, Metter, Tobin, Pearson, & Blackman, 2001). Compared to men between the ages of twenty and forty years, men aged sixty to eighty show nearly 6 nmol/L less plasma testosterone (Mitchell, Hollis, Rothwell, & Robertson, 1995).

While estrogen supplementation improves mood in peri and postmenopausal women, it is not clear that testosterone exerts similar mood effects in older men. In a longitudinal study of older men, testosterone levels were not associated with prevalence or incidence of depression (Ponholzer, et al., 2009). A large community study of healthy older men also showed no relationship between low testosterone and low mood (Hall, et al., 2008). In terms of androgen supplementation, a recent review determined that testosterone treatment is no more efficacious than placebo for major depressive disorder, although testosterone supplementation in older men may lead to some improvement in mood for those with milder depressive symptoms (Amore, Scarlatti, Quarta, & Tagariello, 2009). Also, androgen deprivation therapy in healthy adult men (Schmidt, et al., 2004), or used for the treatment of prostate cancer (Pirl, Greer, Goode, & Smith, 2008) is not associated with increased risk of depression.
The Present Study

This research examined interactions among menopausal status, stress and 5-\textit{HTTLPR} genotype in an older adult population. The sample consisted of family dementia caregivers and sociodemographically similar noncaregiving controls, drawn from an ongoing study. The aim of the proposed study was to examine the effects of caregiving stress on the gene by environment interaction model of depression in older adults, particularly postmenopausal women.

In examining the literature on the 5-\textit{HTTLPR} gene by environment interaction in older adults, two studies (Kim, et al., 2007; Lenze, et al., 2005) failed to reveal sex differences in the gene by environment interaction. Theoretically, as women appear to be more vulnerable to the effects of hormone fluctuations on mood, a low-hormone state in late life might abolish sex differences in the gene by environment interaction, if these interactions are, in fact, influenced by hormonal status. Postmenopausal women show circulating estrogen levels similar to men (Cornil, Ball, & Balthazart, 2006). Thus, we hypothesize that postmenopausal women will show a male-like gene by environment interaction pattern.

Some have hypothesized that 5-\textit{HTTLPR} acts more globally, to modulate the serotonergic response to stress (Uher & McGuffin, 2008). Given that the serotonergic system is influenced as well by sex steroid hormones, it would stand that the 5-\textit{HTTLPR}
and steroid hormones could interact to influence mood. Thus, if this gene by environment interaction is, in fact, influenced by hormonal status, particularly in women, then premenopausal women, with higher sex steroid hormone levels, should show a different gene by environment interaction pattern than postmenopausal women, with lower hormone levels.

Hypotheses

The following hypotheses were tested: (1) postmenopausal women will exhibit a more male-like pattern in gene by environment interaction, $l/l$ genotype associating with depression in response to stress, (2) the pattern exhibited by postmenopausal women will differ from that exhibited by premenopausal women.

Research Design and Methods

Research Protocol

Subjects

Control subjects were recruited from newspaper advertisements, notices posted in senior citizen centers, newsletters, church groups, and referrals from other participants. Caregiving subjects were recruited from support groups, respite care programs, and governmental caregiver support programs. Caregivers were defined as providing at least 5 hours per week of care for a spouse or a parent with dementia. Those who were interested in participation completed a screening questionnaire.
Study Protocol

Eligible, interested individuals were scheduled for an appointment at the General Clinical Research Center (GCRC). Participants met with study personnel to review the consent form and study procedures, and sign informed consent. Participants then had their blood drawn (60 mL). Self-report questionnaires about mood, health behaviors, and socio-demographic characteristics were completed. The entire protocol took between 3 and 4 hours to complete.

Self-Report Measures

Both caregivers and controls were assessed with the same self-report measures. A background questionnaire collected sociodemographic and basic information including age, highest level of education completed, and ethnicity.

Older Americans Resources and Services Program (OARS) Multidimensional Functional Assessment Questionnaire

To assess medication use, including hormone replacement therapy and antidepressants, questions from the Older Americans Resources and Services Program (OARS) Multidimensional Functional Assessment Questionnaire were used (Fillenbaum & Smyer, 1981). The OARS, a self-report measure, provides a list of 26 illnesses or impairments. It asks the subject to indicate which ailments they have, and which medications they take, if any. The OARS has been used in community, emergency care, and primary care settings to assess physical health and medication use in older adults; it
has high internal consistency, with Cronbach’s $\alpha$ greater than 0.9 in community samples (Haywood, Garratt, & Fitzpatrick, 2004).

Center for Epidemiological Studies Depression Scale

The Center for Epidemiological Studies Depression Scale (CES-D) has been used extensively as a brief measure of depressive symptomatology (Basco, Krebaum, & Rush, 1997; Radloff, 1977). The CES-D is a 20 item self-report measure. Four of the items are worded in a positive direction to control for response bias. Subjects are asked to rate each item on a scale from 0 to 3 on the basis of “how often you have felt this way during the past week”; 0 = rarely or none of the time (less than 1 day), 1 = some or a little of the time (1–2 days), 2 = occasionally or a moderate amount of time (3–4 days), and 4 = most or all of the time (5–7 days). CES-D scores range from 0 to 60; higher scores indicate more severe depressive symptoms. A score of 16 or higher is used as a cutoff, as this score was identified in early studies as demarking subjects with depressive illness (Weissman, Sholomskas, Pottenger, Prusoff, & Locke, 1977).

The CES-D has good reliability in assessing the number, types, and duration of depressive symptoms (R. G. Knight, Williams, McGee, & Olaman, 1997; Radloff, 1977). It has high internal consistency, with Cronbach’s $\alpha$ of 0.85 in community samples and 0.9 in clinical samples (Radloff, 1977). Construct validity is good based on clinical and self-report data (Radloff, 1977). The CES-D has distinguished depressed from non-depressed individuals in community and clinical samples, indicating good discriminative validity (Basco, et al., 1997).
Further, the CES-D is a valid measure in both younger and older adult populations. The CES-D provides similar results in younger and older adults, with reliability coefficients of 0.85 to 0.91 (Radloff, 1977). Although older adults often have increased somatic symptoms relative to younger adults, research shows that the CES-D is not affected by increased somatic complaints (Gatz & Hurwicz, 1990), and that its somatic subscale was relatively unbiased by the respondent's somatic complaints in a sample of adults aged fifty-five and older (Foelker & Shewchuk, 1992).

In fact, the CES-D is frequently used in dementia caregiving research (Adams, 2008; Gallicchio, Siddiqi, Langenberg, & Baumgarten, 2002; Pruchno & Resch, 1989; Roth, Ackerman, Okonkwo, & Burgio, 2008). A large-scale study with over 1000 participants, the Resources for Enhancing Alzheimer's Caregiver Health Study, employed the CES-D as a measure of depressive symptoms in caregivers, and found a mean CES-D score of 15.4 (SD=11.5), with 41% of the sample having a CES–D score of 16 or higher (Wisniewski, et al., 2003).

Diagnostic Interview for Genetic Studies

The DIGS is a clinical interview developed through the National Institute of Mental Health’s Genetics Initiative (Nurnberger, et al., 1994), and can be used to provide Axis I and II diagnoses in multiple systems, including the DSM. The DIGS showed excellent diagnostic reliability with the DSM-III (0.73 to 0.95), and excellent inter-rater reliability in a multi-site study, with overall kappas ranging from 0.66 to 0.86.
(Nurnberger, et al., 1994). The DIGS is used frequently in genetic studies of mood disorders (Goes, et al., 2007; Potash, et al., 2007).

Blessed Dementia Scale

To assess function of the care recipient, caregivers responded to the Blessed Dementia Scale (BDS). The BDS is a 22-item scale which measures changes in a dementia patient’s function across several factors: daily living, self-care, and personality. Higher scores indicate greater decrements in function. It shows excellent sensitivity (90%) and specificity (84%) for dementia diagnosis (Erkinjuntti, Haltia, Palo, Sulkava, & Paetau, 1988). While caregiving recipient’s function is correlated with caregiver distress in some studies, correlative studies have not specifically linked the BDS to caregiver distress levels.

Caregiving Status

Caregivers in this sample were defined as providing care for a spouse or same-sex partner, biological parent, or sibling affected by Alzheimer’s or another form of dementia. The caregiver must provide more than 5 hours per week of direct care (e.g. assistance with routine caregiving tasks, such as dressing) or indirect care (e.g. time spent arranging activities); caregiving may be for individuals who live with the caregiver, or who live in a separate home or nursing home. A questionnaire assessed caregiving setting (e.g. in home versus nursing home), whether the caregiver cared for a spouse or parent, duration of caregiving, and hours per day spent caregiving.
Menopausal Status

To assess menopausal status in this sample, study participants responded to the question, “Are you currently post-menopausal?” by answering “Yes” or “No.” If a participant replied “No,” she was then prompted “Please list start date of most recent menstrual cycle.” Participants were also asked “Have you ever been pregnant?” and “If Yes, have you ever had any severe emotional problems during a pregnancy or within a month of childbirth?”, as well as “Have you ever noticed regular mood changes in the premenstrual or menstrual period?” These questions were administered as part of the Diagnostic Interview for Genetic Studies (DIGS; version 3.0/B).

5-HTT Polymorphism

The 5-HTT gene was genotyped for the allelic polymorphism occurring upstream of the transcriptional start site. This polymorphism is characterized by the presence (long, or \( l \)) or absence (short, or \( s \)) of a 44 base pair (bp) repeat, and influences expression of the 5-HTT such that individuals with the \( l/s \) or \( s/s \) genotypes (~68% of the population) express lower levels of 5-HTT than those with the \( l/l \) genotype (Heils, et al., 1996). The 5-HTT genotype is determined from genomic DNA isolated from one ml of EDTA-treated peripheral blood leukocytes. Briefly, 50 ng of DNA was amplified via polymerase chain reaction (PCR) using a sense primer from -1416 to -1397 (5' GGCGTTGCGCTCTGAATGC) and an antisense primer from -888 to -910 (5' GAGGGACTGAGCTGGACAACCAC), relative to the transcriptional start site. The
amplification conditions consisted of 2.5 mM dNTPs, 2 μM of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1 U Taq polymerase. The cycling conditions were 60°C annealing for 30 sec, 72°C extension for 1 min, and 95°C denaturing for 30 sec, for 35 cycles. The products were analyzed via gel electrophoresis in 2.5% agarose and detected by ethidium bromide staining. The l allele was identified by the presence of a 528 bp band, and the s allele was identified by the presence of a 484 bp band.

Statistical Analyses

The main analysis techniques to assess the hypotheses were General Linear Models (GLM). For each hypothesis, I used GLM with an outcome of depressive symptomatology as measured by CES-D score, a continuous variable. Main effects were tested for the effect of the 5-HTT allele (l/l vs. s/l and s/s), and stress (caregiver status), as well as interaction between genotype and stress. As the s allele confers reduced transcriptional efficiency, s-carriers are considered the low efficiency group and are thus grouped together (Heils, et al., 1996), following convention in the literature (Caspi, et al., 2003). Thus, I analyzed 5-HTT genotype as a bivariate variable (l/l vs. s/l and s/s). Confounds included as covariates were antidepressant use and socioeconomic status (operationalized as highest level of education completed). Women taking hormone replacement therapy were excluded. All races were included in these analyses due to cell size limitations.

To test hypothesis 1, I examined the interaction between genotype and stress in postmenopausal women, with depressive symptomatology as the outcome. Predictors
were genotype and caregiving status; covariates were antidepressant use and socioeconomic status (SES); SES was operationalized as highest level of education completed. As the outcome (depressive symptomatology, assessed via CES-D) is a continuous variable outcome, ANOVA was used.

To test hypothesis 2, ANOVA was also performed, with predictors as menopausal status, genotype, and caregiving status; covariates were antidepressant use and SES (highest level of education completed); interaction terms were genotype x caregiving status, and menopausal status x genotype x caregiving status. Depressive symptomatology (assessed via CES-D) was the outcome.
Results

Characteristics of the Sample

Excluding participants with missing data for genotype or menopausal status, and postmenopausal women taking HRT, the final sample consisted of 184 females: 79 family dementia caregivers and 105 sociodemographically-similar noncaregiving controls, drawn from an ongoing study. Of the women, 41 were premenopausal and 143 were postmenopausal. The age range was 30 to 93 years (mean 63.5, S.D. 14.5).

Demographics

Data on age, race and education level per group (e.g. controls, caregivers; premenopausal, postmenopausal) are included in Table 1. One-way analysis of variance (ANOVA) revealed that control versus caregivers were not significantly different in terms of age ($p=0.283$); Chi square tests revealed that control versus caregiver groups were not significantly different in terms of highest level of education completed ($p=0.723$), but were different in racial composition ($p=0.018$); the control population was comprised of 88.6% Caucasian, 5.7% African-American, 3.8% Asian, and 1.9% multi-racial individuals. The caregiver population was 78.5% Caucasian, 17.7% African-American, and 3.8% multi-racial. In terms of genotype, one-way ANOVA revealed that $s$-carriers versus $l/l$ were not significantly different in terms of age ($p=0.16$); Chi square
tests revealed that that s-carriers versus l/l were not significantly different in terms of highest level of education completed ($p=0.496$), nor racial composition ($p=0.656$).

Finally, one-way ANOVA revealed that premenopausal versus postmenopausal women were significantly different in terms of age ($p<0.01$); Chi square tests revealed that premenopausal versus postmenopausal women were not significantly different in terms of highest level of education completed ($p=0.453$), but were different in racial composition ($p=0.006$), with the premenopausal group being comprised of 68.3% Caucasian, 19.5% African-American, 7.3% Asian, and 4.9% multi-racial individuals, while the postmenopausal group was comprised of 88.8% Caucasian, 8.4% African-American, 2.1% multi-racial, and 0.7% Asian individuals.

Caregiver Characteristics

In this sample, the majority of caregiving took place in the caregiver’s home (60.2%), followed by a nursing home (26.5%), the dementia patient’s own home (7.1%), or another family member’s home (5.1%). Caregivers were about equally split in terms of parental (49%) versus spousal (51%) caregiving. Time spent caregiving per day ranged from 1 hour to 24 hours, with a mean of 7.8 hours per day (S.D. 7.15). On average, caregivers in this sample had been caregiving for 60.4 months (5 years), ranging from 8 months to 16 years.

The mean CES-D score among caregivers in this sample was 11.86 (S.D. 8.55), compared to a mean of 7.33 in controls (S.D. 8.18). Using the CES-D cutoff of 16 or above as indicating clinically significant depressive symptoms, 26.6% (n=21) of the caregivers in this sample scored at or above 16, while among controls, 12.4% (n=13)
scored at or above 16. Compared to studies of caregivers with similar demographic features, this is a relatively low proportion (Wisniewski, et al., 2003). In a gene-environment interaction study in female caregivers, those homozygous for the $s$ allele had a mean CES-D score of 17.8, and $l/s$ and $l/l$ women had a mean CES-D of approximately 11.5 (Brummett, Boyle, et al., 2008).

Among caregivers in this sample, depressive symptoms (as measured by CES-D total score) did not vary caregiving setting ($p=0.507$). Further, there was no difference in CES-D total score between spousal versus parental caregivers ($p=0.686$), nor did CES-D score differ by hours per day caregiving ($p=0.182$). Finally, there was no difference in CES-D score based upon length of time spent caregiving ($p=0.540$).

*Analyses of Primary Hypotheses*

**5-HTTLPR Gene by Environment Interaction in Postmenopausal Women (Hypothesis 1)**

This hypothesis compared postmenopausal women to the male-typical pattern of gene by environment interaction found in the literature, in which $l/l$ genotype interacts with stress to produce depression. Postmenopausal women were selected from the total sample from which HRT users were excluded yielding a final sample of 143 (63 caregivers) (Table 2) with an age range of 47.32 - 93.24 (mean 68.73, S.D. 10.92).

ANOVA, controlling for current antidepressant use and SES, showed that the gene by environment interaction was not significant ($p=0.167$, $\eta^2 = 0.014$, $1-\beta_{obs} = 0.293$). However, there was a main effect for group such that caregivers exhibited higher CES-D scores compared to controls (control mean = 6.9198, S.D. = 7.16795; caregiver mean =
11.5973, S.D. = 7.92727) (p<0.001) (Table 3). In sum, in postmenopausal women, those undergoing caregiving stress exhibited higher levels of depressive symptomatology regardless of genotype (Figure 1).

5-HTTLPR Gene by Environment Interaction in Pre versus Postmenopausal Women

(Hypothesis 2)

This hypothesis compared premenopausal and postmenopausal women (Table 4). For premenopausal women, the age range was 30.99 - 54.62 (mean 44.07, S.D. 6.28); for postmenopausal women, the age range was 47.32 - 93.24 (mean 68.73, S.D. 10.92).

Analyses showed that the gene by environment by menopausal status interaction was not significant (p=0.711, \( \eta^2 = 0.001, 1-\beta_{\text{obs}} = 0.066 \)), nor was the gene by environment interaction (p=0.140). However, there was a main effect for caregiver status such that caregivers exhibited higher CES-D scores compared to controls (control mean = 7.24, S.D. = 7.99; caregiver mean = 11.92, S.D. = 8.42) (p=0.003) (Table 5). In sum, caregivers were more depressed, regardless of genotype or menopausal status; there was no gene by environment interaction, nor was there an interaction moderated by menopausal status (Figure 2).

Exploratory Analyses

In the absence of major findings in the main hypotheses, I conducted additional exploratory analyses.
Analyses for the main hypotheses were repeated using a dichotomous outcome variable, a CES-D score of 16 and greater indicating clinically significant depressive symptoms. Results were the same; there was no gene by environment interaction between the serotonin transporter linked polymorphic region (5-HTTLPR) and caregiving stress \( (p=0.144) \). Further, there was no gene by environment interaction moderated by menopausal status \( (p=0.993) \). Thus, analyses were repeated again, this time using a rater-administered diagnosis (DIGS) of current depression. Within the sample, 17.7% of caregivers received a diagnosis of depression, compared to 9.5% of controls. Results still showed no significant gene by environment interaction, nor moderation by menopausal status \( (p=0.32) \).

Genetic influences were also examined. To test the possibility that \( s \)-carriers are more likely to expose themselves to the stressful event of caregiving, Fisher's exact test was used to test differences in genotype by caregiving status. (While a Chi square test would typically be used to test this difference, the test becomes inaccurate with n’s less than five per cell. Fisher’s exact test is not subject to such inaccuracies.) Fisher’s exact test revealed no difference in caregiving status based on genotype \( (p=0.160) \). Thus, \( s \)-carriers were no more likely to undertake the stress of caregiving than \( l/l’ \)’s. Further analyses revealed that \( s \)-carriers did not spend more hours per day caregiving than \( l/l’ \)’s \( (p=0.917) \), nor had they acted as caregivers for longer than \( l/l’ \)’s \( (p=0.570) \). Further, analyses revealed that \( s \)-carriers were no more likely to take on in-home caregiving versus placing their relative in a nursing home \( (p=0.15) \), again indicating that \( s \)-carriers were not more likely to take on the burden of in-home caregiving.
I tested to determine whether depressive symptoms in this sample were influenced by factors related to the caregiving stressor. Among caregivers in this sample, depressive symptoms (as measured by CES-D total score) did not vary caregiving setting ($p=0.507$), relationship with care recipient (spousal versus parental) ($p=0.686$), hours per day caregiving ($p=0.182$), or length of time spent caregiving ($p=0.540$). One factor which influenced level of depression in caregivers was the dementia symptomatology of the person for whom they cared. Linear regression revealed that higher scores on the Blessed Dementia Scale were significantly correlated with CES-D scores among caregivers ($p=0.009$) meaning that caregivers of individuals presenting more dementia symptoms were more depressed.

In this sample, caregivers showed a trend toward being significantly more likely than controls to have a history of depression ($p=0.076$); among caregivers, nearly 60% had a history of depression, while among controls, only 44.8% had a history of depression, as assessed by the DIGS. However, in this sample, $s$-carriers were not more prone to major depression; Chi square analyses of this sample revealed that $s$-carriers were no more likely to have a history of depression than $l/l$ individuals ($p=0.762$), nor were they more likely to use antidepressants ($p=0.182$). Further, while $s$-carriers were no more likely than $l/l$ women to report severe emotional changes during the premenstruum ($p=0.758$) or at menopause ($p=0.592$), $s$-carriers were marginally more likely to report severe emotional changes associated with pregnancy or postpartum ($p=0.054$). Of $s$-carriers, 20.2% reported severe emotional changes during pregnancy, while 8.9% of $l/l$s reported severe emotional changes during pregnancy.
Discussion

No gene by environment interaction was found between the serotonin transporter linked polymorphic region (5-HTTLPR) and caregiving stress in this sample. Postmenopausal women undergoing caregiving stress exhibited higher levels of depressive symptomatology regardless of genotype. Further, female caregivers were more depressed than controls, regardless of menopausal status; there was no gene by environment interaction, nor was there a gene by environment interaction moderated by menopausal status.

These analyses were repeated using a dichotomous outcome variable, a CES-D score of 16 and greater indicating clinically significant depressive symptoms. Results were the same; there was no gene by environment interaction was found between the serotonin transporter linked polymorphic region (5-HTTLPR) and caregiving stress. Further, there was no gene by environment interaction moderated by menopausal status. Given the low CES-D score relative to other samples of caregivers (Brummett, Boyle, et al., 2008; Wisniewski, et al., 2003), it is possible that this sample was underreporting depressive symptoms on the CES-D, a self-report measure. Thus, analyses were repeated using a rater-administered diagnosis (DIGS) of current depression. Within the sample, 17.7% of caregivers received a diagnosis of depression, compared to 9.5% of controls.
Results still showed no significant gene by environment interaction, nor moderation by menopausal status.

Although this study failed to detect a gene by environment interaction, the results of these analyses results speak to the virulence of caregiving stress. Caregivers were more depressed, regardless of menopausal status or genotype. While studies employing stressful life events have demonstrated a gene by environment interaction, this study, using caregiving stress, found that caregiving stress alone was enough to result in increased depressive symptomatology; all genotypes were equally vulnerable to the effects of this chronic stressor.

While these results do not replicate a gene by environment interaction for caregiving stress, it is important to consider how variables in replication attempts are operationalized. As described in a recent meta-analysis which failed to find a 5-HTTLPR gene by environment interaction across 14 studies, the fact that samples, study designs, depression measures, and analyses are highly heterogeneous across studies limits evidence regarding replication (Risch, et al., 2009a). A similar meta-analysis suggests that not only are replication attempts qualitatively different, perhaps studies thus far have been underpowered (Munafo, et al., 2009). Indeed, not only is stress operationalized in different ways across different studies (e.g. caregiving stress, stressful life events, etc.), but the genotype itself can be characterized differently across studies (e.g. biallelic versus triallelic categorization, \(l(a)\) versus \(l(g)\) alleles). Further, depression outcome may be measured in different ways across studies, and covariates such as age or menopausal status may also exert influence.
Thus, there are several possible reasons for this nonreplication, engendered in each of the independent variables: stress, genotype, and menopausal status. Each will be discussed in turn, as a possible contributor to the nonreplication.

First, the way in which stress is operationalized may be a factor. Caregiving stress is a chronic stressor; most studies examining the 5-HTTLPR gene by environment interaction have operationalized stress as stressful life events. An important difference between caregiving stress and stressful life events is that caregiving stress is not brought on by the individual undergoing the stress, while stressful life events may be, for instance, by poor decisionmaking or impulsive behavior (King, Molina, & Chassin, 2008). Thus, it may be that when operationalizing stress as stressful life events, s-carriers are more prone to expose themselves to stressful life events, thus producing an interaction. Further, research shows individuals carrying an s allele had a higher mean score for perceived stress than l/l individuals (Otte, McCaffery, Ali, & Whooley, 2007). Thus, it may be that s-carriers are more likely to perceive a situation as stressful, and develop a depressive response to it, than l/l homozygotes.

Such increased exposure to stressful events, when moderated by genetic factors, is an example of “nature via nurture.” To test the possibility that s-carriers are more likely to expose themselves to the stressful event of caregiving, I tested differences in genotype by caregiving status. Analysis revealed no difference in caregiving status based on genotype, indicating that s-carriers were no more likely to undertake the stress of caregiving than l/l’s. Further analyses revealed that s-carriers did not spend more hours per day caregiving than l/l’s, nor had they acted as caregivers for longer than l/l’s. This
suggests that s-carrying caregivers did not expose themselves to greater amounts of stress (time per day, or time over the course of caregiving), nor did they avoid stress, when compared to l/l caregivers. Further, analyses revealed that s-carriers were no more likely to take on in-home caregiving versus placing their relative in a nursing home, again indicating that s-carriers were not more likely to take on the burden of in-home caregiving.

Research shows that hours spent caregiving per week and duration of caregiving do not influence level of depression in the caregiver (Haley, et al., 2003), nor does caregiving setting (Haley, et al., 2003; Stephens, et al., 1991), or relationship to care recipient (spousal versus parental) (Fisher & Lieberman, 1994; Moen, et al., 1995; Pinquart & Sorensen, 2003). I tested to determine whether depressive symptoms of caregivers in this sample were influenced by these factors, which are independent of genotype. Among caregivers in this sample, depressive symptoms (as measured by CES-D total score) did not vary caregiving setting, relationship with care recipient (spousal versus parental), hours per day caregiving, or length of time spent caregiving.

One factor which influenced level of depression in caregivers was the status of the person for whom they cared; higher scores on the Blessed Dementia Scale were significantly correlated with CES-D scores among caregivers. This is reflected to some degree in the literature, in which degree of impairment of care recipient is associated with caregiver distress in some studies. However, caregiver distress is a broad construct; more specific constructs include caregiver burden or caregiver depression. When examining these specific aspects of caregiver distress, the literature is rich yet mixed. Some studies
show that an increase in care recipient’s dementia symptoms is associated with increased caregiver burden (Claus, et al., 1998; Gaugler, et al., 2000; Grafstrom & Winblad, 1995). Studies examining caregiver depression reveal that depression is less dependent on dementia symptomatology (Black & Almeida, 2004; Steadman, et al., 2007) and more dependent upon factors such as caregiver’s perceptions, personality, or coping skills (Black & Almeida, 2004; Goode, et al., 1998; Hayslip, et al., 2008; Hooker & McAdams, 2003; Steadman, et al., 2007).

Although caregiving stress is supposed to be free of bidirectionality (e.g. individuals who are at risk for depression select themselves into stressful situations), it is possible that such environment selection in caregivers occurs. For instance, in the case of parental caregiving, there may be several potential adult children caregivers. Of these adult children, perhaps one is more likely to take on the stress of caregiving than others. This could be independent of genotype, and may instead be rooted in, for instance, personality features or family environment. Interestingly, in this sample, caregivers showed a trend toward being significantly more likely than controls to have a history of depression; among caregivers, nearly 60% had a history of depression, while among controls, only 44.8% had a history of depression, as assessed by the Diagnostic Interview for Genetic Studies. Thus, disregarding genotype, perhaps individuals who become caregivers are more likely to take on stressful situations, which in turn, result in depression. Therefore, perhaps there is some characteristic of caregivers, independent of genotype, which makes them more likely to partake in or be exposed to stressful or depression-inducing situations. Or, it is possible that caregivers who are more depressed,
as opposed to “seeking” stress, simply have poorer coping skills. In fact, depression may indicate poor coping skills, a passive coping style, or insufficient coping resources (Williamson & Schulz, 1993). Alternatively, caregivers who are more depressed may be more likely to recall past episodes of depression, or to appraise past reactions to stressful events as more severe.

Racial differences in stress and depression may also be a factor in this lack of replication. While there are racial differences in 5-HTTLPR expression, there may also be racial differences in coping style, availability of coping resources in managing stress, or other factors which influence vulnerability to depression. Due to cell size limitations, race was not covaried in these analyses. However, there were racial differences in group composition. For controls versus caregivers, there was a higher proportion of Caucasians in the control group (88.6%), compared to the caregiver group (78.5%), and a higher proportion of African American women in the caregiver group (17.7%), versus the control group (5.7%).

As mentioned, there may be cultural or family environment differences which make African American women more likely to take on the caregiver role, as compared to Caucasian women. In African-American culture, women often take on the role of caregiving, and are often unlikely to solicit or receive help from male relatives or children (Wells-Wilbon & Simpson, 2009).

There may be differences in access to coping resources, or differences in coping styles, based on cultural factors or race. African American female caregivers often lack access to resources, or fail to seek services for cultural reasons such as stigma (Gutierrez,
1990). However, studies including both sexes have shown no difference between Caucasian and African-American dementia caregivers in terms of use of formal or informal support services, or social support (Cox, 1999; Haley, et al., 1996). A meta-analysis revealed that while Caucasian caregivers appraised caregiving as more stressful than African American caregivers, minority caregivers had less support available than Caucasians (Janevic & Connell, 2001). Despite low support, African-American caregivers are more likely than Caucasian caregivers to appraise the caregiving process as meaningful, and to report deriving benefit from the experience (Farran, Miller, Kaufman, & Davis, 1997; E. W. Gonzales, 1997; Haley, et al., 1996; B. G. Knight & McCallum, 1998).

Regarding stress management skills, Lazarus and Folkman define two basic coping styles: emotion-focused and problem-focused (Lazarus & Folkman, 1984). African American caregivers rely more often on emotion-focused coping and less on problem-focused coping (B. G. Knight, Silverstein, McCallum, & Fox, 2000). Emotion-focused coping in African-American caregivers is associated with increased levels of psychological distress (B. G. Knight, et al., 2000). Further, African American women are often more vulnerable to health, social, and economic threats (Williams, Lavizzo-Mourey, & Warren, 1994); these multiple vulnerabilities (Williams, Yu, Jackson, & Anderson, 2004), coupled with caregiving stress, may result in a sense of powerlessness, in some cases leading to depression (Chadiha, Adams, Biegel, Auslander, & Gutierrez, 2004).
However, several studies comparing African-American and Caucasian caregivers show that African-American caregivers are less depressed (Farran, et al., 1997; Haley, et al., 1996; B. G. Knight & McCallum, 1998). In this sample, female African-Americans as a whole had more symptoms of depression, compared to female Caucasians, as measured by the CES-D and controlling for SES and antidepressant use ($p = 0.001$); the mean CES-D score for Caucasian women was 8.4 (S.D. 7.5), and for African-American women was 13.9 (S.D. 12.1). Among female caregivers, however, there no significant difference in depressive symptomatology between Caucasians and African-Americans ($p = 0.066$). Although this was not a significant difference, African-American female caregivers did trend toward higher CES-D scores than Caucasian female caregivers (mean 14, S.D. 11.8, versus mean 11.07, S.D. 7.5).

Second, subtleties in genotype, or gene expression, may have contributed to the nonsignificant interaction. 5-HTTLPR polymorphisms are quite complex, with at least 14 alleles of the 5-HTTLPR (El Hage, Powell, & Surguladze, 2009). Thus, looking at a single allele may be too limited. This could explain why results in replicating Caspi’s original finding of gene by environment interaction for 5-HTTLPR have been inconsistent; it is perhaps not a single polymorphism influencing the interaction, but an array of genes. Further, it may not be 5-HTTLPR itself that is associated with a predisposition toward depression, but that 5-HTTLPR rather modulates the serotonergic response to stress (Uher & McGuffin, 2008). For instance, tryptophan depletion response (Neumeister, et al., 2006; Roiser, et al., 2006), SSRI response (Smeraldi, et al., 1998), or HPA response (Alexander, et al., 2009; Gotlib, Joormann, Minor, & Hallmayer, 2008),
which show variation by 5-HTTLPR genotype, may be biological intermediate phenotypes indicating a general poor reactivity to stress, which results in the psychological phenotype of proneness to depression. However, in this sample, s-carriers were not more prone to major depression; s-carriers were no more likely to have a history of depression than l/l individuals, nor were they more likely to use antidepressants.

Finally, using menopausal status as a proxy for hormone levels in this model may have contributed to the nonsignificant results. Evidence suggests that hormones do interact with the serotonin system, particularly with the 5-HTTLPR. However, which hormones are influential (e.g. sex steroid hormones versus glucocorticoids) is unclear. Some hypothesize that cortisol response is the mechanism underlying the association between the 5-HTTLPR gene by environment interaction (Gotlib, et al., 2008). In terms of sex steroid hormones, there are a number which may play a role, including testosterone, estradiol, lutenizing hormone, progesterone, and follicle-stimulating hormone, all of which wane with age. In women, lowered sex steroid hormone levels following the menopausal transition may influence both psychological and physiological reaction to stress. As described, postmenopausal dementia caregivers not receiving HRT reported higher levels of hostility and depression than postmenopausal caregivers receiving HRT (Steffen, et al., 1999), and some results have indicated postmenopausal women have heightened cardiovascular reactivity to stress compared to postmenopausal women on HRT (Saab, et al., 1989). Thus, women with a genetic vulnerability, who also have low sex steroid hormone levels due to menopause, may be especially vulnerable to the psychological and health effects of stress. If such a gene-hormone interaction is in
fact present, then one would also expect to see increased vulnerability to depression at other points involving hormonal fluctuation, such as premenstrually or postpartum, in genetically vulnerable women.

In this sample, a questionnaire was used to determine self-reported history of severe emotional changes in the premenstruum, postpartum, and menopause. Although $s$-carriers were no more likely than $l/l$ women to report severe emotional changes during the premenstruum or at menopause, $s$-carriers were marginally more likely to report severe emotional changes associated with pregnancy or postpartum. Of $s$-carriers, 20.2% reported severe emotional changes during pregnancy, while 8.9% of $l/l$s reported severe emotional changes during pregnancy. The mechanism of this association is unknown – while it may be due to genetic and hormonal factors, it may just as well be due to personality factors, perceived stress, or likelihood to report an event as stressful. Future research in gene-hormone interactions will aid in delineating the interplay of such factors.

In sum, while there was no gene-environment interaction in this sample, I found that that $s$-carriers were no more likely to have a history of depression than $l/l$ individuals, but that caregivers trended toward a higher likelihood of a history of depression than controls. This speaks to the conjecture that in this case, genetics are being trumped by some other factor, perhaps some personality feature that promotes stress-seeking. That is, perhaps caregivers have in the past sought stressful, depressogenic situations, and continue to do so with caregiving.

Limitations

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There are several limitations to this study. First, small cell sizes limited power, particularly in comparing premenopausal versus postmenopausal women. Ideally, a larger sample would have been used. Although our overall sample size was consistent with what has been used by other groups in studying the 5-HTTLPR gene by environment interaction, some argue that sample sizes for such studies ought to be larger (Munafo, et al., 2009). Additionally, we did not assess hormone levels directly, and instead used self-reported menopausal status as a proxy for hormonal status. Ideally, a study examining the influence of hormones on the 5-HTTLPR gene by environment interaction would assess hormone level directly, by saliva or plasma assays.

Future Directions

If this study were to be repeated, a number of improvements could be made. First, a larger sample would be ideal. This would increase power, and also allow analysis of 5-HTTLPR genotype in a triallelic manner, giving a more fine-grained picture of the genotype aspect of the gene by environment interaction. Second, an ideal study would examine the gene by environment interaction in each racial group (e.g. Caucasian, Asian, etc.), as there may be differences in 5-HTT expression among racial groups (Ng, et al., 2006). Finally, to examine the influence of hormones on the 5-HTTLPR gene by environment interaction, one would ideally assess hormone level directly. Such an assay would confirm menopausal status biologically, as opposed to relying on self-report. It would also allow researchers to explicitly examine influence of relative hormone levels, i.e. group women according to estradiol levels (high, moderate, low), progesterone levels,
etc., to examine the influence of specific hormones on the gene by environment interaction.

Some women may be more vulnerable to the effects of these hormonal shifts than others, particularly those with a history of depression (Stewart & Boydell, 1993). In this vein, future research might examine whether women with the s allele of 5-HTTLPR are more susceptible to develop depression during times of hormonal shift (e.g. premenstrually, postpartum, or at menopause) as compared to women who are l homozygotes. Further, the pattern of interaction between 5-HTTLPR genotype and hormonal status (premenopausal female, postmenopausal female, pre-climacteric male, post-climacteric male) may serve as a starting point for further examination of precise hormone levels and genotype.

Finally, future work might consider whether factors, such as personality traits, developmental features, family environment, or cultural influences make those who undertake caregiving also more likely to take on other stressful circumstances. For instance, traits such as optimism (Marquez-Gonzalez, Losada Baltar, Penacoba Puente, & Romero-Moreno, 2009) or self-efficacy (Rabinowitz, Mausbach, & Gallagher-Thompson, 2009) may moderate the impact of caregiving stress on depression, as opposed to genotype. To look at this, one might examine family environment, personality features, or stress-seeking qualities in spousal caregivers (who presumably have little choice in becoming a caregiver) versus parental caregivers (who, if they have siblings, may have some choice in whether or not they take on caregiving responsibility).
Results of this study indicate that family dementia caregiving is a powerful stressor, overriding genetic factors in producing depression. Determining which aspects of the caregiving experience contribute to depression, be they characteristics of the caregiver, characteristics or of the person receiving care, or facets of dementia progression, will be important continuing work for researchers.
Table 1.
Sample Characteristics According to Caregiver Status

<table>
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<th>Demographic</th>
<th>Controls (n=105)</th>
<th>Caregivers (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (Mean, S.D.)</td>
<td>64.54 (15.51)</td>
<td>62.22 (12.95)</td>
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<tr>
<td>Race (n)</td>
<td></td>
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<tr>
<td>Caucasian</td>
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<tr>
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<td>3</td>
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<tr>
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<td>Some high school</td>
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<tr>
<td>l/l</td>
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Table 2.
Sample Characteristics According to Menopausal Status

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<td><strong>Genotype (n)</strong></td>
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<tr>
<td>l/l</td>
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<td>57</td>
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Table 3.

5-HTTLPR Allele Frequency Distributions According to Caregiving Status in Postmenopausal Women (Hypothesis 1)

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<tr>
<th>Genotype</th>
<th>Stress (Caregiving Status)</th>
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<td>Controls</td>
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<tr>
<td>l/l</td>
<td>28</td>
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Table 4.

Analysis of Variance for Hypothesis 1

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<th>Source</th>
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<th>F</th>
<th>η²</th>
<th>p</th>
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<tbody>
<tr>
<td>Corrected Model</td>
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<td>5</td>
<td>4.460</td>
<td>.140</td>
<td>.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>583.584</td>
<td>1</td>
<td>10.662</td>
<td>.072</td>
<td>.001</td>
</tr>
<tr>
<td>Education Level</td>
<td>20.466</td>
<td>1</td>
<td>.374</td>
<td>.003</td>
<td>.542</td>
</tr>
<tr>
<td>Antidepressant Use</td>
<td>195.943</td>
<td>1</td>
<td>3.580</td>
<td>.025</td>
<td>.061</td>
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<tr>
<td>5HTT Genotype (5HTT Gene)</td>
<td>95.176</td>
<td>1</td>
<td>1.739</td>
<td>.013</td>
<td>.189</td>
</tr>
<tr>
<td>Caregiving Status (Stress)</td>
<td>794.317</td>
<td>1</td>
<td>14.512</td>
<td>.096</td>
<td>.000*</td>
</tr>
<tr>
<td>5HTT Gene x Stress</td>
<td>105.543</td>
<td>1</td>
<td>1.928</td>
<td>.014</td>
<td>.167</td>
</tr>
<tr>
<td>Error</td>
<td>7498.668</td>
<td>137</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20164.001</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>8719.218</td>
<td>142</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* $R^2 = 0.138$, Adjusted $R^2 = 0.107$.

* indicates statistical significance.
Table 5.

5-HTTLPR Allele Frequency Distributions According to Caregiving Status in Women (Hypothesis 2)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Stress (Caregiving Status)</th>
<th>Controls</th>
<th>Caregivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Premenopausal</td>
<td>Postmenopausal</td>
</tr>
<tr>
<td>s/s, s/l</td>
<td></td>
<td>18</td>
<td>52</td>
</tr>
<tr>
<td>l/l</td>
<td></td>
<td>7</td>
<td>28</td>
</tr>
</tbody>
</table>
Table 6.

Analysis of Variance for Hypothesis 2

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III SS</th>
<th>df</th>
<th>F</th>
<th>$\eta^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>1442.533</td>
<td>9</td>
<td>2.300</td>
<td>.106</td>
<td>.018</td>
</tr>
<tr>
<td>Intercept</td>
<td>909.597</td>
<td>1</td>
<td>13.050</td>
<td>.070</td>
<td>.000</td>
</tr>
<tr>
<td>Education Level</td>
<td>34.324</td>
<td>1</td>
<td>.492</td>
<td>.003</td>
<td>.484</td>
</tr>
<tr>
<td>Antidepressant Use</td>
<td>159.664</td>
<td>1</td>
<td>2.291</td>
<td>.013</td>
<td>.132</td>
</tr>
<tr>
<td>5HTT Genotype (5HTT Gene)</td>
<td>14.445</td>
<td>1</td>
<td>.207</td>
<td>.001</td>
<td>.650</td>
</tr>
<tr>
<td>Caregiving Status (Stress)</td>
<td>614.168</td>
<td>1</td>
<td>8.812</td>
<td>.048</td>
<td>.003*</td>
</tr>
<tr>
<td>Menopausal Status (Meno)</td>
<td>124.277</td>
<td>1</td>
<td>1.783</td>
<td>.010</td>
<td>.184</td>
</tr>
<tr>
<td>5HTT Gene x Stress</td>
<td>153.067</td>
<td>1</td>
<td>2.196</td>
<td>.012</td>
<td>.140</td>
</tr>
<tr>
<td>5HTT Gene x Meno</td>
<td>18.110</td>
<td>1</td>
<td>.260</td>
<td>.001</td>
<td>.611</td>
</tr>
<tr>
<td>Stress x Meno</td>
<td>.073</td>
<td>1</td>
<td>.001</td>
<td>.000</td>
<td>.974</td>
</tr>
<tr>
<td>5HTT Gene x Stress x Meno</td>
<td>9.631</td>
<td>1</td>
<td>.138</td>
<td>.001</td>
<td>.711</td>
</tr>
<tr>
<td>Error</td>
<td>12127.911</td>
<td>174</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29264.001</td>
<td>184</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>13570.444</td>
<td>183</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* $R^2 = 0.140$, Adjusted $R^2 = 0.109$.

* indicates statistical significance.
Figure 1. (Hypothesis 1.) Mean CES-D score for s-carrier and l/l genotype groups, in postmenopausal control and caregiving subjects. Error bars indicate 95% confidence intervals.
Figure 2. (Hypothesis 2.) Mean CES-D score for s-carrier and l/l genotype groups, in pre- and postmenopausal control and caregiving subjects. Error bars indicate 95% confidence intervals.
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88*(1), 91-124.


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Appendix A: Abbreviations

5α-DHT: 5α-dihydrotestosterone
5-HIAA: 5-hydroxyindoleacetic acid
5-HT: 5-hydroxytryptamine; serotonin
5-HT$_{2A}$: serotonin$_{2A}$ receptor
5-HTT: serotonin transporter
5-HTTLPR: serotonin transporter linked polymorphic region
ANOVA: analysis of variance
$B_{\text{max}}$: maximal density
bp: base pair
CES-D: Center for Epidemiologic Studies Depression Scale
DNA: deoxyribonucleic acid
DSM: Diagnostic and Statistical Manual of Mental Disorders
ERT: estrogen replacement therapy
GLM: General Linear Models
ml: milliliter
mRNA: messenger ribonucleic acid
OARS: Older Americans Resources and Services Program
mM: millimolar
ng: nanogram

PCR: polymerase chain reaction

SERT: serotonin transporter

SES: socioeconomic status

SSRI: selective serotonin reuptake inhibitor

S.D.: standard deviation

μM: micromolar
Appendix B: Measures

Background Questionnaire

Please list your date of birth:
  month _ _ day _ _ year _ _ _ _

What is your gender?:
  male / female

Are you Hispanic or Latino?
  No / Yes / Don't know/ Not sure

Which of the following would you say is your race? (Please mark ALL that apply):
  White
  Black or African American
  Asian
  Native Hawaiian or Other Pacific Islander
  American Indian, Alaska Native
  Other: (please specify):
  Don't know/Not sure

What is the highest level of education you have completed?
  Graduate or professional training (Masters, JD, MD, PhD, etc.)
  College or University Graduate
  Some college
  High School
  Some high school
  Junior high school
  Less than 7 years
Older Adults Resource Center Scale (OARS) Multidimensional Functional Assessment Questionnaire

Please answer the following questions regarding your health history. If you select yes for any question, please give a brief description of the condition (when you were diagnosed, current symptoms, etc.), and any important dates in regards to the condition:

Have you had rheumatic fever or rheumatic heart disease?
   No / Yes; please explain and provide date:

Have you had any heart or blood vessel disease such as a heart attack or stroke?
   No / Yes; please explain and provide date:

Do you have or have you had a heart murmur or mitral valve prolapse?
   No / Yes; please explain and provide date:

Have you been told that your blood pressure is too high?
   No / Yes; please explain and provide date:

Have you ever had respiratory failure?
   No / Yes; please explain and provide date:

Have you ever had a bronchospasm?
   No / Yes; please explain and provide date:

Have you ever had chest pain or angina?
   No / Yes; please explain and provide date:

Have you been treated for a seizure disorder (convulsions or epilepsy)?
   No / Yes; please explain and provide date:

Have you had a tumor or disease that required x-ray, radium or cobalt treatments?
   No / Yes; please explain and provide date:

Have you had excessive or prolonged bleeding following a cut, tooth extraction, or injury?
   No / Yes; please explain and provide date:

Have you had any allergic or unusual reactions to any drugs, medications, bandages, or plastic?
   No / Yes; please explain and provide date:

Are you currently taking any:
   antibiotics?   No / Yes
   cortisone?    No / Yes
medications for glaucoma?  No / Yes
medication for depression?  No / Yes
blood thinners?  No / Yes
tranquilizers or other medications for your nerves?  No / Yes

Have you ever been hospitalized or had surgery (including chemotherapy and radiation)?
  No / Yes (If yes, please explain and include date)

To the best of your knowledge, do you have or have you ever had any hormone or immunological problems?
  No / Yes
  (If yes), please explain and include date.

Are you pregnant or nursing at the present time?
  No / Yes / Not applicable

Below is a short list of illnesses and physical problems. Please indicate if you have any of these illnesses. If you do, please indicate how much the illness or physical problem interferes with your day to day life, and whether or not you take medication as treatment.

Rheumatoid Arthritis
  N / Y
  (If yes) Interferes?  not at all / some / a lot
  Medications:

Asthma
  N / Y
  (If yes) Interferes?  not at all / some / a lot
  Medications:

Emphysema
  N / Y
  (If yes) Interferes?  not at all / some / a lot
  Medications:

High Blood Pressure
  N / Y
  (If yes) Interferes?  not at all / some / a lot
  Medications:

Heart Trouble
  N / Y
  (If yes) Interferes?  not at all / some / a lot
  Medications:
Diabetes
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Liver Disease
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Kidney Disease
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Hormone Problems
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Cancer
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Stroke
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Thyroid Problems
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Muscle Disorder, e.g. MS, Post-Polio
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:

Depression
N / Y
(If yes) Interferes? not at all / some / a lot
Medications:
Anxiety
 N / Y
 (If yes) Interferes? not at all / some / a lot
 Medications:

Have you ever had a skin cancer removed?  N / Y
Medications

Please list in the box below any medications (either prescription or over-the-counter) you are currently taking. If you are not currently taking medications, please write "none" in the box below: ________________________________.
**Center for Epidemiologic Studies Depression Scale (CES-D)**

Please read each statement and then indicate how many days you felt or behaved this way in the past week by filling in the corresponding circle:

<table>
<thead>
<tr>
<th>Statement</th>
<th>less than 1 day</th>
<th>1-2 days</th>
<th>3-4 days</th>
<th>5-7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I was bothered by things that usually don't bother me.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. I did not feel like eating; my appetite was poor.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. I felt that I could not shake off the blues even with help from my family or friends.</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4. I felt that I was just as good as other people.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. I had trouble keeping my mind on what I was doing.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. I felt depressed.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. I felt that everything I did was an effort.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. I felt hopeful about the future.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. I thought my life had been a failure.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. I felt fearful.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. My sleep was restless.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. I was happy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. I talked less than usual.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. People were unfriendly.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. I enjoyed life.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. I had crying spells.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. I felt sad.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. I felt that other people dislike me.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Blessed Dementia Scale (BDS)

The BDS is a caregiver interview during which questions about the patient are asked. The goal of the interview is to detect how much change has occurred in the memory, behavior, and personality of the patient over the course of the disease. The Interviewer is to fill in the circle beside the number corresponding to the description of the patient. At the end, a total score is calculated.

A. Changes in Patient Memory
1. Ability to perform household tasks: Large Some None
2. Ability to cope with small sums of money: Large Some None
3. Ability to remember a short list of items: Large Some None
   (for example, shopping) : Large Some None
4. Ability to find way about indoors: Large Some None
5. Ability to find way about familiar streets: Large Some None
6. Ability to interpret surroundings (for example, recognize whether in a hospital or at home; to discriminate between people, relatives, doctors, friends) : Large Some None
7. Ability to recall recent events: Large Some None
8. Tendency to dwell in the past: Large Some None

B. Changes in Patient Hygiene
9. Eating: please select one:
   Patient eats with proper utensils, can use knife and fork effectively
   Patient needs some assistance in eating (for example, needs to have food cut, etc.)
   Patient rarely uses utensils, eats with fingers, can only eat certain foods
   Patient cannot feed self, has to be fed

10. Dressing: please select one:
    Patient dresses self unaided
    Patient has problems with buttons, zippers
    Patient needs to have clothes laid out, forgets items (socks, underwear), puts items on in wrong order or inside out
    Patient unable to dress self, needs to be dressed

11. Bladder and Bowel Control: please select one:
    Patient has normal complete control
    Patient occasionally wets self (1-4 times per month)
    Patient frequently wets self (once a week or more)
    Patient lacks bladder and bowel control, wets self daily

C. Changes in Patient Personality and Interests
12. Increased rigidity, diminished flexibility (for example, patient deals with matters in a fixed or stereotyped manner)
    Change Present / Change Absent
13. Increased self-concern, self-focus, or self-centeredness
   Change Present / Change Absent

14. Impaired or diminished regard for the feelings of others
   Change Present / Change Absent

15. Coarsening of emotion (being rude, rough, unrefined)
   Change Present / Change Absent

16. Impairment of emotional control (bouts of crying, bursts of anger, or any loss of control)
   Change Present / Change Absent

17. Laughing or smiling at inappropriate times
   Change Present / Change Absent

18. Diminished emotional responsiveness, patient reacts little or not at all emotionally (never smiles, etc.)
   Change Present / Change Absent

19. Sexual misbehavior or inappropriateness (undressing in front of others, making inappropriate advances or comments)
   Change Present / Change Absent

20. Hobbies given up because patient can no longer do them or has lost interest in them
   Change Present / Change Absent

21. Diminished initiative, growing apathy (sitting around, not starting or doing anything)
   Change Present / Change Absent

22. Purposeless hyperactivity, excessive activity
   Change Present / Change Absent

23. When was the person you are caregiving for first diagnosed with dementia?
   Month: __ Year: ___

24. When did symptoms of dementia first start to appear?
   Month: __ Year: ___

25. When did you first begin the role of a caregiver for this particular person?
   Month: __ Year: ___

26. Did you ever stop this role of caregiving and then start again? No Yes
If YES, for how long? __ months

27. Up through the present, how long have you been caregiving for this particular person?
Years: __ __ __ Months: __
Questions Regarding Caregiving

Where does your loved one currently live?
   - Your home
   - Home of another family member
   - Alone
   - Nursing home
   - Hospital

Is the caregiver a spousal or parental caregiver?
   - Spousal
   - Parental

At present, how much time do you spend each day in activities related to caring for your loved one (on average)? This can include direct care, such as direct assistance with routine caregiving tasks, or indirect care, such as time spent arranging activities such as respite or other outside help?
   - Mon-Fri hours/day: ___
   - Weekend hours/day: ___
   - Average hours/day: ___
Diagnostic Interview for Genetic Studies (DIGS)

B. Medical History

Are you currently post-menopausal?
No / Yes

If NO, please list start date of most recent menstrual cycle:
Month __ Day __ Year __ __ __

If YES, have you had any severe emotional problems associated with menopause?
No / Yes

If YES, specify: ________________________________

Have you ever been pregnant?
No / Yes

If YES, how many times (including if pregnancy didn't result in live birth)?
__ times

If YES, how many live births (children)?
__ births

If YES, have you ever had any severe emotional problems during a pregnancy or within a month of childbirth?
No / Yes

If YES, specify: ________________________________

Have you ever noticed regular mood changes in the premenstrual or menstrual period?
No / Yes

If YES, specify: ________________________________

F. Major Depression

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you ever had a period of at least one week when you were bothered most of the day, nearly every day, by feeling depressed, sad, down, low?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>1.a) By feeling irritable?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>1.b) By feeling anxious?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>1.c) Have you ever had a period of at least one week when you did not enjoy most things, even things you usually like to do?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>
2. If 1–1.c are all NO:

INTERVIEWER: Do you suspect a past or current episode from subject’s responses, behavior, or other information?

If yes: Specify:

______________________________________________

Skip to G. Mania/Hypomania

3. Have you been feeling that way recently (i.e., for at least one week during the past 30 days)?

3.a) If yes: How long have you felt this way?

4. Think about the most severe period in your life when you were feeling this way.
When did it begin?

4.a) INTERVIEWER: Compute age.

4.b) How long did that period last?

4.c) Did you feel depressed, sad, down, or low?

4.d) Did you feel irritable?

4.e) Did you feel anxious?

5. INTERVIEWER: Is the most severe episode also the current episode?

6. Did you have a loss of appetite or did your appetite greatly increase?

0. No
1. Yes, decreased
2. Yes, increased
3. Yes, mixture
9. Unknown/No information

6.a) *Did you lose/gain weight when you were not trying to?*  

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

**If yes:**

6.b) *What was your weight before the loss/gain?*  

<table>
<thead>
<tr>
<th>Pounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

6.c) *What was your weight after the loss/gain?*  

<table>
<thead>
<tr>
<th>Pounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

6.d) *Over what period of time did you lose/gain this amount of weight?*  

<table>
<thead>
<tr>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

7. *Did you have trouble sleeping or were you sleeping more than usual?*  

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

**If yes:**

7.a) *Were you unable to fall asleep?*  

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

7.b) **If yes:** *Was this for at least one hour?*  

<p>| | | |</p>
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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

7.c) *Were you waking up in the middle of the night and having trouble going back to sleep?*  

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

7.d) *Were you waking up too early in the morning?*  

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

7.e) **If yes:** *Was this at least one hour earlier than usual?*  

<p>| | | |</p>
<table>
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</table>

7.f) *Were you sleeping much more than usual?*  

<p>| | | |</p>
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8. *Were you so fidgety or restless that other people could have noticed (e.g., pacing or wringing hands)?*  

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9. *Were you moving or speaking so slowly that other people could have noticed?*  

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<td>0</td>
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</table>
10. *Were you much less able to enjoy sex and other pleasurable activities?*  
   0 1 9

10a. *Did you lose interest in nearly all of your usual activities?*  
   0 1 9

11. *Were you feeling a loss of energy or more tired than usual?*  
   0 1 9

12. *Were you feeling guilty or that you were a bad person?*  
   0 1 9

13. *Were you feeling that you were a failure or worthless?*  
   0 1 9

14. *Were you having difficulty thinking, concentrating, or making decisions?*  
   0 1 9

15. *Were you frequently thinking about death, or wishing you were dead, or thinking about taking your life?*  
   0 1 9

16. *Did you actually try to harm yourself?*  
   0 1 9

17. **INTERVIEWER:** Enter number of boxes with at least one YES response in questions 6–16  
   **TOTAL BOXES**

   **INTERVIEWER:** If less than three, probe for other potentially severe episodes. If necessary, recode questions

18. **(INTERVIEWER: Review symptoms in questions 6–16 plus depressed mood or hand subject Depression Tally Sheet to review):**  
   During this episode was there a two-week period when these symptoms were present nearly every day (at least four symptoms plus depressed mood)?  
   0 1 9

19. *Did you tend to feel worse in the morning or in the evening?*  
   0 1 2

   0. A.M.  
   1. P.M.  
   2. No difference
20. *During this episode, did you have beliefs or ideas that you later found out were not true?*  
   **Probe:** Like believing you had committed a crime or sin? Or that God was punishing you? Or that some terrible thing was going to happen? Or that someone was trying to harm you, or was talking about you? Or that something had gone wrong with your body?  
   **How certain were you?**  
   **INTERVIEWER:** If delusions are suspected, probe further to determine the content and whether the beliefs were held with certainty. Code on the basis of this information and describe below:

If yes to question 20:

20.a) *Did these beliefs occur either just before this depression or after it cleared?*  

20.b) **If yes:** *How long were they present before the depression began?*  

20.c) **If yes:** *How long did they last after your mood returned to normal?*  

20.d) **INTERVIEWER:** Does this total more than 14 days?  

21. *Did you see or hear things that other people could not see or hear?*  
   **Probe:** Like voices talking or noises, or visions? Or have unusual tastes, smells, or physical sensations?  
   **If yes:** *Specify:*  

If yes:

21.a) *Did these (refer to experiences) occur either just before this depression or after it cleared?*  

21.b) **If yes:** *How long were they present before the depression began?*  

21.c) **If yes:** *How long did they last after your mood returned to normal?*
21.d) **INTERVIEWER:** Does this total more than 14 days?

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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<tbody>
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<td></td>
<td>0</td>
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<td>9</td>
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</table>

22. **If yes to questions 20 or 21:**

**INTERVIEWER:** Did psychotic symptoms have content that was inconsistent with depressive themes such as poverty, guilt, illness, personal inadequacy or catastrophe?

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<thead>
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<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>9</td>
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</tbody>
</table>

22.a) **If yes:** **INTERVIEWER:** Was the subject preoccupied with psychotic symptoms to the exclusion of other symptoms or concerns?

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
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<td>9</td>
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</table>

23. **Did you seek or receive help from a doctor or other professional for this period of depression?**

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>9</td>
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</table>

24. **Were you prescribed medication for depression?**

**If yes:** **Specify:**

---

25. **During this episode were you admitted to the hospital for depression (including day hospital)?**

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

25.a) **If yes:** **For how long (inpatient)?**

| Days |     |     |

25.b) **If yes:** **For how long (day hospital)?**

| Days |     |     |

26. **Did you receive ECT (shock treatments)?**

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>9</td>
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</tbody>
</table>

**INTERVIEWER:** If the patient was hospitalized two days or more, had ECT, or had psychotic symptoms, skip to question 29 and code incapacitation.
27. **Was your major responsibility during this episode job, home, school, or something else?**

   1. Job
   2. Home
   3. School
   4. Other

   **If other:** Specify:

   ________________________________

<table>
<thead>
<tr>
<th>Code Response</th>
</tr>
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<tr>
<td>1</td>
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</table>

28. **Was your functioning (in this role) affected?**

   **If yes:** Specify:

   ________________________________

   **28.a)**  *Did something happen as a result of this (such as marital separation, absence from work or school, loss of a job, or lower grades)?*

   **If yes:** Specify:

   ________________________________

   **28.b)**  *Did someone notice a change in your functioning?*

   **No** | **Yes** | **Unk**
   -------|---------|-------
   0      | 1       | 9     

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29. **INTERVIEWER:** Code based on answers to questions 20, 21 and 25–28

<table>
<thead>
<tr>
<th>Code Response</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impairment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incapacitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
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</table>

**Modified RDC Impairment:** A decrease in quality of the most important role performance (noticeable to others). This usually requires a decrease in the amount of performance; it may be manifested by a person taking ten hours to do what normally may require five hours.

**Modified RDC Incapacitation:** Includes complete inability to carry out principal role at home, school or work for 2 days in a row
- OR Hospitalization for 2 days.
- OR ECT treatment.
- OR Presence of hallucinations or delusions.

**If impaired or incapacitated:** Specify:

30. **RDC Minor Role Dysfunction**

**If no change in question 29:** *Was your functioning in any other area of your life affected?*

**If yes:** Specify:

30.a) **INTERVIEWER:** If no to questions 25–30, is there any other evidence of clinically significant distress?

**If yes:** Specify:
INTERVIEWER: If MALE or NEVER PREGNANT, skip to question 32

<table>
<thead>
<tr>
<th>Question</th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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</thead>
<tbody>
<tr>
<td>31. Did this episode occur during pregnancy (code 1) or just after childbirth (code 2)?</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>31.a) If yes: What was the date of childbirth?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>–</td>
<td>Year</td>
<td></td>
</tr>
<tr>
<td>32. Did this episode occur during or shortly after a serious physical illness?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

INTERVIEWER: The following illnesses, among others, may be relevant: Hypothyroidism, CVA, MS, Mono, Hepatitis, Cancer, Parkinson's, HIV, Cushing's or other endocrine illnesses.

If yes: Specify:

____________________________________________________

<table>
<thead>
<tr>
<th>Question</th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
</thead>
<tbody>
<tr>
<td>33. Did this episode begin shortly after you started taking any prescribed medication?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

INTERVIEWER: Aldomet, Inderal (propranolol), reserpine, interferon, and steroid medications (Prednisone, etc.) are important precipitants. Probe to distinguish precipitants from drugs actually prescribed to treat early symptoms of depression, such as hypnotics given for insomnia.

If yes: Specify medications:

____________________________________________________

<table>
<thead>
<tr>
<th>Question</th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
</tr>
</thead>
<tbody>
<tr>
<td>34. Did this episode begin while you were using street drugs?</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

INTERVIEWER: The following drugs, among others, may be relevant: Amphetamines, Barbiturates, Cocaine, "Downers", Tranquilizers

If yes: Specify drug and quantity:

____________________________________________________

<table>
<thead>
<tr>
<th>Question</th>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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<tbody>
<tr>
<td>35. Did this episode follow increased use of alcohol?</td>
<td>0</td>
<td>1</td>
<td>9</td>
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</tbody>
</table>

If yes: Specify:

____________________________________________________
35.a) Did this episode follow decreased use of alcohol?  
   If yes: Specify: _______________________

36. Did this episode follow the death of someone close to you?  
   36.a) If yes: Specify relationship: _______________________
   36.b) Date of death  
         Month    Year

37. During this episode of depression did you have a week or more during which your mood frequently changed between sadness and irritability or even elation?  
   37.a) During this episode of depression did you also experience any of these symptoms?  
       37.a.1) Overactivity—Running around, many projects, or physically agitated?  
       37.a.2) More talkative than usual, speech pressured?  
       37.a.3) Thoughts racing, jumping from topic to topic?  
       37.a.4) Feeling grandiose - more important, special, powerful?  
       37.a.5) Needing less sleep - energetic after little or no sleep?  
       37.a.6) Attention distracted by unimportant things?  
       37.a.7) Doing risky things for pleasure - spending, sex, reckless driving, etc.?  
   37.a.8) INTERVIEWER: Enter number of YES responses in 37.a.1-7: TOTAL
   
   If total in 37.a.8 is less than 3, skip to question 38

   37.a.9) How long were these symptoms present?  
            Days  Weeks
   OR
38. Did you have at least one other episode when you were depressed for at least one week and had several of the symptoms you described? [0 1 9]

**If yes:** When was the most recent time that you had depression that was almost as severe as the time we just talked about?

**INTERVIEWER:** Based on the overview or additional probing, identify the most recent severe episode that the subject remembers well. Avoid episodes with probable organic precipitants and episodes that occurred less than 2 months before or after the Most Severe Episode. A Current Episode should be rated here if it meets these criteria.

Briefly describe the subject’s response:
________________________________________

38.a) Is the selected episode also the current episode (in the past 30 days)? [0 1]

38.b) When did it begin? [Month] [Year]

38.c) **INTERVIEWER:** Compute age.

38.d) How long (did that period last/has it lasted)? [No Yes Unk]
38.e) Did you feel depressed, sad, down, or low? 0 1 9
38.f) Did you feel irritable? 0 1 9
38.g) Did you feel anxious? 0 1 9

During the selected episode…:

39. Did you have a loss of appetite or did your appetite greatly increase?
    0. No
    1. Yes, decreased
    2. Yes, increased
    3. Yes, mixture
    9. Unknown/No information

39.a) Did you lose/gain weight when you were not trying to? 0 1 2 9
    0. No
    1. Loss
    2. Gain
    9. Unknown

If yes:

39.b) What was your weight before the loss/gain? Pounds
39.c) What was your weight after the loss/gain? Pounds
39.d) Over what period of time did you lose/gain this amount of weight? Weeks

40. Did you have trouble sleeping or were you sleeping more than usual? 0 1 9

If yes:

40.a) Were you unable to fall asleep? 0 1 9
40.b) If yes: Was this for at least one hour? 0 1 9
| 40.c) | **Were you waking up in the middle of the night and having trouble going back to sleep?** | No | Yes | Unk |
| 40.d) | **Were you waking up too early in the morning?** | 0 | 1 | 9 |
| 40.e) | **If yes:** **Was this at least one hour earlier than usual?** | 0 | 1 | 9 |
| 40.f) | **Were you sleeping much more than usual?** | 0 | 1 | 9 |
| 41. | **Were you so fidgety or restless that other people could have noticed (e.g., pacing or wringing hands)?** | 0 | 1 | 9 |
| 42. | **Were you moving or speaking so slowly that other people could have noticed?** | 0 | 1 | 9 |
| 43. | **Were you much less able to enjoy sex and other pleasurable activities?** | 0 | 1 | 9 |
| 43.a) | **Did you lose interest in nearly all of your usual activities?** | 0 | 1 | 9 |
| 44. | **Were you feeling a loss of energy or more tired than usual?** | 0 | 1 | 9 |
| 45. | **Were you feeling guilty or that you were a bad person?** | 0 | 1 | 9 |
| 46. | **Were you feeling that you were a failure or worthless?** | 0 | 1 | 9 |
| 47. | **Were you having difficulty thinking, concentrating, or making decisions?** | 0 | 1 | 9 |
| 48. | **Were you frequently thinking about death, or wishing you were dead, or thinking about taking your life?** | 0 | 1 | 9 |
| 49. | **Did you actually try to harm yourself?** | 0 | 1 | 9 |
| 50. **INTERVIEWER:** | Enter number of boxes with at least one **YES** response in questions 39–49 | | | |
| 51. **INTERVIEWER:** | If less than three, probe for other potentially severe episodes. If necessary, recode questions | | | |
| 51. **INTERVIEWER:** | Review symptoms in questions 39–49 plus depressed mood or hand subject Depression Tally Sheet to review:** | 0 | 1 | 9 |
52. Did you tend to feel worse in the morning or in the evening?
   0. A.M.
   1. P.M.
   2. No difference

   Code Response
   No  Yes  Unk
   0   1    2

53. During this episode, did you have beliefs or ideas that you later found out were not true?  
   Probe: Like believing you had committed a crime or sin? Or that God was punishing you? Or that some terrible thing was going to happen? Or that someone was trying to harm you, or was talking about you? Or that something had gone wrong with your body? How certain were you?
   INTERVIEWER: If delusions are suspected, probe further to determine the content and whether the beliefs were held with certainty. Code on the basis of this information and describe below:

   If yes to question 53:

   53.a) Did these beliefs occur either just before this depression or after it cleared? 0 1 9
   Days

   53.b) If yes: How long were they present before the depression began?

   53.c) If yes: How long did they last after your mood returned to normal?

   53.d) INTERVIEWER: Does this total more than 14 days? 0 1 9

54. Did you see or hear things that other people could not see or hear?  
   Probe: Like voices talking or noises, or visions? Or have unusual tastes, smells, or physical sensations?
   If yes: Specify:

   If yes:

   54.a) Did these (refer to experiences) occur either just before this depression or after it cleared? 0 1 9
   Days

123
54.b) **If yes:** How long were they present before the depression began?

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<tr>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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Days

54.c) **If yes:** How long did they last after your mood returned to normal?

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<th>No</th>
<th>Yes</th>
<th>Unk</th>
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</table>

54.d) **INTERVIEWER:** Does this total more than 14 days?

0 1 9

55. **If yes to questions 53 or 54:**

**INTERVIEWER:** Did psychotic symptoms have content that was inconsistent with depressive themes such as poverty, guilt, illness, personal inadequacy or catastrophe?

55.a) **If yes:** **INTERVIEWER:** Was the subject preoccupied with psychotic symptoms to the exclusion of other symptoms or concerns?

0 1 9

56. **Did you seek or receive help from a doctor or other professional for this period of depression?**

0 1 9

57. **Were you prescribed medication for depression?**

0 1 9

**If yes:** Specify:

________________________________________________________________________

58. **During this episode were you admitted to the hospital for depression (including day hospital)?**

0 1 9

58.a) **If yes:** For how long (inpatient)?

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<tr>
<th>No</th>
<th>Yes</th>
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</table>

Days

58.b) **If yes:** For how long (day hospital)?

<table>
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<tr>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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</thead>
</table>

Days

59. **Did you receive ECT (shock treatments)?**

0 1 9

**INTERVIEWER:** If the patient was hospitalized two days or more, had ECT, or had psychotic symptoms, skip to question 62 and code incapacitation.
60. Was your major responsibility during this episode job, home, school, or something else?

1. Job
2. Home
3. School
4. Other

If other: Specify:

61. Was your functioning (in this role) affected?

If yes: Specify:

61.a) Did something happen as a result of this (such as marital separation, absence from work or school, loss of a job, or lower grades)?

If yes: Specify:

61.b) Did someone notice a change in your functioning?

INTERVIEWER: If MALE or NEVER PREGNANT, skip to question 65

64. Did this episode occur during pregnancy (code 1) or just after childbirth (code 2)?

64.a) If yes: What was the date of childbirth?

Month: – Year:
65.  Did this episode occur during or shortly after a serious physical illness?

INTERVIEWER: The following illnesses, among others, may be relevant: Hypothyroidism, CVA, MS, Mono, Hepatitis, Cancer, Parkinson's, HIV, Cushing's or other endocrine illnesses.

If yes: Specify:
____________________________________________________

66.  Did this episode begin shortly after you started taking any prescribed medication?

INTERVIEWER: Aldomet, Inderal (propranolol), reserpine, interferon, and steroid medications (Prednisone, etc.) are important precipitants. Probe to distinguish precipitants from drugs actually prescribed to treat early symptoms of depression, such as hypnotics given for insomnia.

If yes: Specify medications: ______________________

67.  Did this episode begin while you were using street drugs?

INTERVIEWER: The following drugs, among others, may be relevant: Amphetamines, Barbiturates, Cocaine, "Downers", Tranquilizers

If yes: Specify drug and quantity: ______________________

68.  Did this episode follow increased use of alcohol?

If yes: Specify: ____________________________________________

68.a) Did this episode follow decreased use of alcohol?

If yes: Specify: ____________________________________________

69.  Did this episode follow the death of someone close to you?

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69.a) **If yes:** Specify relationship: __________________________

69.b) **Date of death**

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
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70. *During this episode of depression did you have a week or more during which your mood frequently changed between sadness and irritability or even elation?*

0  1  9

70.a) *During this episode of depression did you also experience any of these symptoms?*

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>70.a.1) Overactivity—Running around, many projects, or physically agitated?</td>
<td>0 1 9</td>
</tr>
<tr>
<td>70.a.2) More talkative than usual, speech pressured?</td>
<td>0 1 9</td>
</tr>
<tr>
<td>70.a.3) Thoughts racing, jumping from topic to topic?</td>
<td>0 1 9</td>
</tr>
<tr>
<td>70.a.4) Feeling grandiose - more important, special, powerful?</td>
<td>0 1 9</td>
</tr>
<tr>
<td>70.a.5) Needing less sleep - energetic after little or no sleep?</td>
<td>0 1 9</td>
</tr>
<tr>
<td>70.a.6) Attention distracted by unimportant things?</td>
<td>0 1 9</td>
</tr>
<tr>
<td>70.a.7) Doing risky things for pleasure - spending, sex, reckless driving, etc.?</td>
<td>0 1 9</td>
</tr>
</tbody>
</table>

70.a.8) **INTERVIEWER:** Enter number of **YES** responses in 70.a.1-7: **TOTAL**

If total in 70.a.8 is **less than 3**, skip to

<table>
<thead>
<tr>
<th>Days</th>
<th>Weeks</th>
</tr>
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<tbody>
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</table>

70.a.9) *How long were these symptoms present?*

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
<th>Unk</th>
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</table>

71. **INTERVIEWER:** Has there been at least one “clean” episode? A “clean” episode is one WITHOUT prior physical illness, drug or alcohol abuse, organic precipitants, or bereavement.

0  1  9
If yes:

72. How many like this have you had?

72a. How old were you the first time you had an episode of depression like this? (Review requirements for clean episode above)

72b. How old were you the last time you had an episode of depression like this? (Review requirements for clean episode above)

73. If no clean episodes:

73.a) How many episodes like this have you had?

73.b) How old were you the first time you had an episode like this?

73.c) How old were you the last time you had an episode like this?

74. What was the duration of your longest episode of depression in weeks?

75. How many times were you hospitalized for an episode of depression? (inpatient)

75.a) How many times were you hospitalized for an episode of depression? (day hospital)

76. How many courses of ECT have you had for depression?

77. Did you ever feel high or were you overactive following medical treatment for depression?

If yes: Describe: ________________________________

78. Do your depressions tend to begin in any particular season?

  0. No pattern

<table>
<thead>
<tr>
<th>Code Response</th>
</tr>
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<tbody>
<tr>
<td>0 1 2 3 4 9</td>
</tr>
</tbody>
</table>

128
62. **INTERVIEWER:** Code based on answers to questions 53, 54 and 58–61

<table>
<thead>
<tr>
<th>Code</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

- No change
- Impairment
- Incapacitation
- Unknown

**Modified RDC Impairment:** A decrease in quality of the most important role performance (noticeable to others). This usually requires a decrease in the amount of performance; it may be manifested by a person taking ten hours to do what normally may require five hours.

**Modified RDC Incapacitation:** Includes complete inability to carry out principal role at home, school or work for 2 days in a row OR Hospitalization for 2 days OR ECT treatment OR Presence of hallucinations or delusions.

**If impaired or incapacitated:** Specify:

63. **RDC Minor Role Dysfunction**

If no change in question 62: *Was your functioning in any other area of your life affected?*

**If yes:** Specify:
63.a) INTERVIEWER: If no to questions 58–63, is there any other evidence of clinically significant distress?

If yes: Specify: