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Longitudinal Loneliness and Cognitive Aging in Mid and Late Life: Patterns of Associations and Epigenetic Pathways

A Dissertation submitted in partial satisfaction
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ABSTRACT OF THE DISSERTATION

Longitudinal Loneliness and Cognitive Aging in Mid and Late Life: Patterns of Associations and Epigenetic Pathways

by

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Dr. Chandra A. Reynolds, Chairperson

The aims of this dissertation were to compare associations between baseline and longitudinal loneliness and performance and change in four specific cognitive abilities and to explore whether DNA methylation at specific locations in blood leukocytes may play a role in the association between loneliness and cognition. In Study 1, we assessed effects of baseline loneliness and two measures of longitudinal loneliness (time-varying loneliness and geometric means for loneliness across waves) on cognitive performance and change across up to 28 years of follow-up in a large pooled sample from the Consortium on Interplay of Genes and Environment Across Multiple Studies (IGEMS). Results showed small effects of loneliness on cognition that varied across cognitive domains, with faster processing speed at age 65 and faster decline in processing speed and spatial ability. In Study 2, we evaluated loneliness and longitudinal methylation and cognitive data in a subsample from the Swedish Adoption/Twin Study of Aging (SATSA) to evaluate associations between loneliness and methylation at 1,586 CpG sites within genes linked with the conserved transcriptional response to adversity (CTRA)

using both phenotypic and co-twin control approaches. For sites with associations between loneliness and methylation, regression models were used to explore relations between loneliness, methylation, and cognition. Results showed associations between loneliness and methylation level at age 70 at cg00403457 in *PTPN12* and change in methylation with age at cg00619097 in *CPT1B* and cg26661481 in *IL10RA*, with partial confounding of these relations by genetic or common environmental factors indicated by co-twin control results. Although direct effects of loneliness on cognition were not significant, indirect associations of perceived loneliness to methylation of cg00403457 in *PTPN12* to change in processing speed were observed, indicative of a potential role of methylation at this site in the loneliness—cognition relation. Overall, across study1 and study 2, results indicate that feelings of loneliness predict faster cognitive decline with small albeit meaningful effects that play out across age with hints of indirect mediation via methylation pathways that may be partly genetically moderated. Additional work is needed to further clarify how loneliness relates to cognition change.

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GENERAL INTRODUCTION

In the context of a rapidly growing population of individuals over the age of 65, which is expected to nearly triple worldwide by the year 2050, the number of individuals with declining cognitive functioning is expected to rise significantly over the next few decades (World Health Organization, 2011). Consequently, research aimed at elucidating potentially modifiable factors which contribute to or detract from healthy cognitive aging has the potential to simultaneously impact the lives of an unprecedented number of individuals. A growing body of work points to perceived loneliness, defined as emotional suffering which arises from the perception that one's social relationships are inadequate (especially in terms of quality) (Hawkley & Cacioppo, 2010), as one such potentially modifiable factor shown to detract from healthy cognitive aging (e.g., Boss, Kang, & Branson, 2015; Wilson et al., 2007).

As loneliness is more strongly linked with relationship quality than quantity, it is distinct from objective social isolation, or having little to no interaction with others, and often occurs outside of the context of social isolation (Hawkley & Cacioppo, 2010). However, perceived loneliness and measures of objective social isolation have been consistently found to be moderately positively associated (e.g., Wilson et al., 2007), which indicates that with growing social isolation, there is an increasing likelihood one will experience loneliness, as it becomes less likely that one will perceive their social relationships and interaction as being adequate as objective social isolation increases (Boomsma, Cacioppo, Muthén, Asparouhov, & Clark, 2007). Consequently, shifting trends in living arrangements, social network structure, and social interaction in many

modern countries also indicate the importance of elucidating the negative association between loneliness and healthy cognitive aging. For example, in the U.S. a rapidly growing number of individuals are living alone, with fewer individuals living with a spouse than ever before (U.S. Census Bureau, 2016). Moreover, reductions in the number of confidants an individual has on average have been observed, along with diminishing time spent interacting with members of one's social network (Boomsma et al., 2007). Such changes to social networks and individuals' behavioral patterns related to social interaction may contribute to an increase in the prevalence of stable loneliness and increasing vulnerability of individuals in the growing aging population to experiencing loneliness.

Loneliness and Cognition

Several studies of loneliness and cognition in older individuals have linked feelings of loneliness with poorer performance on measures of global cognition (O'Luanaigh et al., 2012; Wilson et al., 2007), faster decline in global cognitive performance over time (Holwerda et al., 2012; Tilvis et al., 2004; Wilson et al., 2007), and an increased risk of developing Alzheimer's disease (AD; Holwerda et al., 2012; Rafnsson et al., 2017; Wilson et al., 2007). However, limited work assessing associations between loneliness and performance on tasks assessing individual domains of cognition suggests that the relationship between loneliness and cognition is not consistent across domains, although further study is warranted and replication of these initial findings is needed. Loneliness has been negatively associated with performance on tasks which assess executive function (Shankar et al., 2013), working memory (Wilson et al., 2007)

episodic memory (Shankar et al., 2013; Wilson et al., 2007), semantic memory (Wilson et al., 2007), visual memory (O'Luanaigh et al., 2012), visuospatial ability (Wilson et al., 2007), and processing speed (O'Luanaigh et al., 2012; Wilson et al., 2007). Limited longitudinal work also suggests that of these domains, loneliness predicts subsequent decline in performance on measures of visuospatial ability, semantic memory, and processing speed (Wilson et al., 2007). Although it has been asserted that change with age rather than time is more relevant in studies of cognition (c.f. Grimm, Ram, & Estabrook, 2017; Reynolds et al., 2005), prior work on loneliness and domain-specific cognition has primarily used cross-sectional or time-based analyses to explore these associations. Consequently, further work is needed to clarify how loneliness relates to cognitive performance at particular ages and cognitive change across age.

Exploring how longitudinal loneliness relates to these cognitive outcomes is also important to consider. Studies of loneliness and physical health outcomes and mortality suggest that the effects of loneliness appear to accumulate over time, such that individuals who experience longer periods of loneliness have a greater risk of experiencing particular negative health outcomes including cardiovascular disease (Caspi, Harrington, Moffitt, Milne, & Poulton, 2006) and a greater risk of mortality (Shiovitz-Ezra & Ayalon, 2010) than individuals who experience shorter periods of loneliness. It has been proposed that it is unlikely that short-term loneliness has lasting physiological effects, as these effects are thought to dissipate once loneliness has resolved (Hawkley & Capitanio, 2015). It has also been proposed that physiological

changes associated with longer-term loneliness may also resolve once loneliness is overcome (Hawkley & Capitanio, 2015).

Although most of the work on loneliness and late-life cognitive outcomes has assessed how cross-sectional loneliness relates to cognition, longitudinal loneliness has been considered in two prior studies. Zhong, Chen, and Conwell (2016) examined relations between patterns of loneliness across two waves of data collected three years apart and global cognition 6 years after the baseline wave, and reported that loneliness was associated with global cognition regardless of whether it was experienced at one or both waves, with a stronger effect of loneliness for those who were lonely at both waves compared to that for those who were lonely at one wave only. Findings from another study in which the association between one's long-term propensity to feel lonely (operationally defined as participants' average loneliness scores across four years of follow-up) and AD risk was assessed showed that a greater propensity to feel lonely was strongly linked to accelerated decline in global cognitive performance and increased AD risk (Wilson et al., 2007). The results of these studies support the importance of consideration of chronicity/duration of loneliness in assessing links between loneliness and cognitive outcomes. In addition to focusing on whether baseline loneliness predicts subsequent cognitive performance and decline (which is often a main focus given the potential for reverse causality for these associations), future work should also aim to further align the investigation of the association between loneliness and cognitive outcomes with empirically supported theoretical positions on loneliness through

additional exploration of associations between longitudinal loneliness and these outcomes.

Potential Mechanisms of the Associations between Loneliness and Cognition: An Epigenetic Approach

Loneliness and Gene Expression

While a growing body of work suggests an association between loneliness and cognitive performance and decline, the mechanisms of these associations remain largely unknown. However, work which has shown that loneliness may moderate expression of genes associated with immune function and inflammation in human blood leukocytes (Cole et al., 2007; Creswell et al., 2012) and genes associated with emotional functioning, mental health disorders, social behavior, inflammation, and various diseases including AD in human brain tissue (Canli et al., 2016; Canli et al., 2018) suggests that one way by which loneliness may contribute to poorer cognitive performance or accelerated cognitive decline may be by altering the expression of these genes.

Loneliness and gene expression in blood leukocytes.

Recent work has shown that gene expression in human blood leukocytes and brain tissue (examined post-mortem) differs in chronically lonely and chronically non-lonely individuals (Canli et al., 2016; Canli et al., 2018; Cole et al., 2007; Creswell et al., 2012). In blood leukocytes, expression has been found to vary for more than 200 genes in lonely compared to non-lonely individuals, with estimates ranging from 209 (Cole et al., 2007) to 256 genes (Creswell et al., 2012). Differential gene expression in leukocytes of lonely and non-lonely individuals has been shown to involve increased expression of genes

associated with inflammation and reduced expression of genes associated with fighting viral infections in lonely compared to non-lonely persons (Cole et al., 2007; Creswell et al., 2012), an expressional pattern which has been referred to as a '*conserved transcriptional response to adversity*' (CTRA) and has also been linked with other circumstances involving objective and perceived stress, including perceived socioeconomic status (SES), and to a lesser extent, objective SES measures and objective social isolation (Cole, 2013). Importantly, findings also suggest that the altered gene expression in leukocytes of lonely individuals is, at least in part, reversible (Cole, 2013; Creswell et al., 2012). In a randomized controlled trial, Creswell et al. (2012) showed attenuation of the increased expression of genes associated with inflammation observed in lonely individuals following an intervention that focused on teaching mindfulness to help participants reduce stress over a two-month period. Such potential reversibility of negative physiological changes associated with loneliness speaks to the importance of considering longitudinal patterns of loneliness in studies of loneliness and epigenetic biomarkers and other associated outcomes and provides further support that chronic loneliness may be predictive of worse outcomes than shorter-term loneliness.

Loneliness and gene expression in brain tissue.

In human brain tissue, loneliness has been associated with altered expression of genes in the nucleus accumbens and the dorsolateral prefrontal cortex (Canli et al., 2016; Canli et al., 2018). In line with findings on loneliness and gene expression in leukocytes, the genes found to be differentially expressed in brain tissue were clustered into categories representing specific physiological responses and behaviors. In nucleus

accumbens tissue, these included anxiety, emotional functioning, frequency of social interaction, and various disease processes, including AD (Canli et al., 2016). Findings were similar for the dorsolateral prefrontal cortex, with altered expression observed for genes associated with AD, cancer, inflammation, and mental health disorders in individuals who reported feeling lonely at baseline (Canli et al., 2018). Importantly, however, it is unknown whether a causal association exists between loneliness and such altered gene expression (Canli et al., 2016) and future work should use longitudinal designs to test competing hypotheses with regards to directionality of this association and explore potential mechanisms.

Loneliness and Stress

As the CTRA pattern of expression has been linked with other stressors, it is reasonable to hypothesize that heightened stress and perception of threat experienced by lonely individuals may contribute to altered gene expression observed in lonely persons. Specifically, in contrast to the attenuation of the stress response in response to stressors linked with high levels of social support (Carroll, Roux, Fitzpatrick, & Seeman, 2013), feelings of loneliness have been proposed to promote or exacerbate perceptions of threat, thereby amplifying the magnitude of the stress response (Cacioppo, Hawkley, & Berntson, 2003). Three hypotheses linking loneliness with heightened levels of stress have received empirical support: the '*added stress hypothesis*', the '*differential reactivity hypothesis*', and the '*differential stress buffering hypothesis*'. The '*added stress hypothesis*' posits that heightened perception of social threat experienced by lonely individuals is associated with ongoing heightened activation of processes associated with

the stress response (Cacioppo et al., 2003). The '*differential reactivity hypothesis*' states that lonely individuals experience stronger activation of the stress response than non-lonely individuals in response to similar stressors (Cacioppo et al., 2003). These two hypotheses are supported by the observation that individuals who are lonely tend to perceive their lives as being more stressful than non-lonely individuals despite reporting exposure to similar stressors in daily life at similar frequencies (Cacioppo et al., 2000). Moreover, findings suggest that positive emotional responses to similar daily events tend to be lower in magnitude in lonely compared to non-lonely individuals whereas negative emotional responses (e.g., feeling like things are a hassle) tend to be higher in response to similar daily events for those who are lonely (Cacioppo et al., 2000). The '*added stress hypothesis*' is further supported by the finding that those who are lonely tend to experience social interactions as being less enjoyable and view them less positively than non-lonely individuals (Cacioppo et al., 2003).

The '*differential stress buffering hypothesis*' proposes that lonely individuals tend to perceive stressors as being more stressful because they are less likely to have others they can rely on for social support (Cacioppo et al., 2003). This hypothesis is empirically supported by the finding that lonely and non-lonely individuals did not vary with respect to how often they interacted with others, but lonely individuals had social interactions that were less intimate, supportive, comforting, and were associated with lower levels of positive feelings in general in comparison to those of non-lonely persons (Hawkley, Burleson, Berntson, & Cacioppo, 2003). Collectively, these hypotheses and the limited findings that support them suggest that lonely individuals may not only experience

'added stress' (i.e., heightened perception of social threat), but they also appear to perceive such stress as being more severe than do non-lonely individuals, an observation which, at least in part, seems to result from a reduced likelihood of having others they can depend on for support (Cacioppo et al., 2003).

Loneliness and Inflammation

One pathway by which elevated exposure to stressors and heightened perception of stress in lonely individuals may affect health and cognition over time is through promotion of chronic inflammation. Loneliness has been linked with greater production of inflammatory markers following a stressful experience, with greater IL-1β, IL-6, and TNF-α levels observed following such an experience for those with higher loneliness scores compared to those who reported lower loneliness (Jaremka et al., 2013). As discussed above, loneliness may affect gene expression in ways that promote inflammation. Those who feel lonely have also been found to have higher cortisol levels than those who do not feel lonely, suggesting that the functioning of the hypothalamic-pituitary-adrenal (HPA) axis is dysregulated in lonely individuals, a factor which has been shown to contribute to inflammatory processes (Carroll et al., 2013; Hawkley & Capitanio, 2015; Lin, Epel, & Blackburn, 2012). The link between loneliness and inflammation is further supported by studies of loneliness and health that have shown associations between loneliness and multiple health conditions linked with inflammation, including poor immune function and higher blood pressure, weight, and cholesterol (Hawkley & Capitanio, 2015). Making matters worse, loneliness has also been linked

with poor quality sleep—an important mechanism of physiological restoration (Cacioppo et al., 2002).

Inflammation and Cognition

Inflammation has also been linked with dementia risk, cognitive performance, and cognitive change, although some studies have produced conflicting findings with respect to the link between particular inflammatory markers and cognitive outcomes (e.g., Schram et al., 2007; Teunissen et al., 2003; Trollor et al., 2012; Yaffe et al., 2003). Two inflammatory markers which have been linked with both cognitive function and cognitive decline are C-reactive protein (CRP) (Komulainen et al., 2007; Ravaglia et al., 2005; Schram et al., 2007; Teunissen et al., 2003) and interleukin-6 (IL-6) (Elwan et al., 2003; Rafnsson et al., 2007; Schram et al., 2007). High serum concentrations of CRP have been linked with poorer cross-sectional performance on measures of executive functioning (Schram et al., 2007) and global cognitive performance (Ravaglia et al., 2005; Schram et al., 2007), poorer performance on measures of memory 6 years (Teunissen et al., 2003) and 12 years later (Komulainen et al., 2007), decline in performance on memory tasks (Schram et al., 2007), and heightened dementia risk (Engelhart et al., 2004; Schmidt et al., 2002). High serum concentrations of IL-6 have been linked with poorer performance on measures of executive function (Schram et al., 2007), sensory memory, attention (Elwan et al., 2003), and global cognitive performance (Schram et al., 2007), faster decline in performance on measures of memory (Schram et al., 2007) and speed of processing (Rafnsson et al., 2007), elevated risk of dementia (Engelhart et al., 2004), and active dementia (Eriksson et al., 2011).

Other inflammatory markers that have been linked with such cognitive outcomes include intercellular adhesion molecule-1 (ICAM-1) (Rafnsson et al., 2007), haptoglobin (Teunissen et al., 2003), and α1-antichymotrypsin (Engelhart et al., 2004). While numerous studies point to a relation between inflammatory markers and cognition, it is important to consider that several studies have found no association between these inflammatory markers and such cognitive outcomes (e.g., Alley, Crimmins, Karlamangla, Hu, & Seeman, 2008; Dik et al., 2005; Trollor et al., 2012; Yaffe et al., 2003). However, findings linking loneliness with increased inflammatory processes and those showing a relation between elevated inflammatory marker concentrations and cognitive performance and decline suggest that exploration of epigenetic changes which may contribute to altered gene expression and heightened inflammation in lonely individuals as potential mediators of the association between loneliness and these cognitive outcomes could lead to important insights regarding biological processes that mediate this link.

DNA Methylation and Social Context

DNA methylation is one mechanism by which environmental influences such as social context affect human physiology and behavior by altering the expression of particular genes (Szyf, 2011). DNA methylation is an epigenetic process; a process that involves changes to DNA expression in response to environmental influences that do not involve modification of the nucleotide sequence itself but are maintained for some duration as cells divide and under some circumstances may be passed on to offspring (Feinberg, 2013; Meloni, 2014). In DNA methylation, methyl (CH_3) groups are added to cytosine nucleotides in DNA strands (Blaze & Roth, 2015). DNA methylation most often

interferes with transcription factors' ability to bind to promotor regions of DNA, thereby impeding transcription of a gene. Sometimes, however, it can result in the expression of a gene that was previously not expressed (Blaze & Roth, 2015; Goossens et al., 2015).

While the association between loneliness and DNA methylation has yet to be assessed, findings from both the human and animal literatures provide evidence of an association between social context and epigenetic changes both early and later in life (e.g., Fraga et al., 2005; McGowan et al., 2009; Siuda et al., 2014). Human research, for example, has shown that exposure to a neglectful or abusive environment in childhood is linked with altered expression of the glucocorticoid receptor gene in hippocampal tissue (McGowan et al., 2009). Moreover, research on epigenetic differences between monozygotic (MZ) twins has shown that while these twins have identical or highly similar epigenetic profiles in early life, these profiles may diverge across time and have been shown to be quite different by late life, resulting in differential gene expression in these twins which contribute to divergent phenotypes (Fraga et al., 2005; Talens et al., 2012; van Dongen et al., 2016). Findings linking epigenetic changes with specific environmental exposures (e.g., McGowan et al., 2009) suggest that such divergence in epigenetic profiles arises as a result of different environmental exposures or perceptions of environmental circumstances (Cole, 2013) for each twin within a monozygotic twin pair.

Research with mice has linked both poor maternal care in early life (Weaver et al., 2004) and persistent social isolation in adulthood (Siuda et al., 2014) with altered DNA methylation patterns in hippocampal and midbrain tissue, respectively. Such findings

suggest that both early and later experiences can induce epigenetic changes which may result in altered gene expression and subsequent phenotypic change. However, the extent to which epigenetic changes are reversible remains unknown. While such changes have been shown to be lasting (for example, in animal research that has shown that altered methylation patterns in mice that arise as a result of maternal behavior are maintained into adulthood; Weaver et al., 2004), evidence also suggests that the epigenome remains responsive to environmental changes after early life and that DNA methylation induced by environmental circumstances may sometimes be reversible in the event of changes in such circumstances (Fraga et al., 2005; Siuda et al., 2014; Weaver et al., 2004). The extent to which this may occur warrants exploration and would contribute to expansion of current knowledge of how nurture and nature interact in producing phenotypic stability and change.

Cognitive Performance and DNA Methylation

A paucity of work has explored the association between methylation of blood leukocyte DNA and cognitive performance in older individuals who are free of cognitive impairments. Schiepers et al. (2012) assessed the links between performance in individual domains of cognitive functioning (speed, memory, and verbal fluency) and the extent to which blood leukocyte DNA was methylated in 215 older persons free of such impairments. No associations were found between cognitive performance and DNA methylation for any of the cognitive measures. Importantly, however, such findings warrant replication in larger samples and do not preclude the possibility of an association between the methylation at particular loci and cognitive performance (Schiepers et al.,

2012). In support of this possibility, work examining links between cognitive performance and DNA methylation in individuals with cognitive impairments has shown associations between methylation of specific genes and cognitive performance. For example, findings from a recent study showed that DNA methylation at sites within the promotor region of the *APOE* gene in blood leukocytes was associated with elevated incidence of dementia (Karlsson, Ploner, & Wang, 2018). Although specific genotypes for the *APOE* gene have been linked with cognitive impairments, variation in genotype for *APOE* alleles did not moderate the effect of *APOE* methylation on dementia incidence, suggesting that DNA methylation at sites within the promotor region of this gene may contribute to poor cognitive outcomes regardless of *APOE* genotype (Karlsson et al., 2018).

Cognitive impairment in individuals with Down syndrome has also been linked with altered methylation of buccal epithelial DNA at five loci, two of which lie within the Tuberous Sclerosis 2 (*TSC2*) gene, for which an association has been shown with progression of AD (Jones et al., 2013). Moreover, exploration of DNA methylation in individuals with Parkinson disease—most of whom exhibited poor cognition as measured by the Mini-Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) at data collection—has shown altered methylation of frontal cortex tissue in these patients, with concordant methylation differences observed in blood leukocytes for 124 genes (Masliah, Dumaop, Galasko, & Desplats, 2013).

A recent meta-analysis across 11 samples also reported associations between methylation at two CpG sites—one located within a noncoding area on chromosome 12

and one located within the *INPPA* gene—and global cognition and verbal fluency, respectively (Marioni et al., 2018). Such findings suggest that future work on DNA methylation and cognitive performance should aim to assess the association between global DNA methylation patterns and cognition in larger samples and further examine whether and how differential methylation within other specific genes previously linked to cognitive impairments (e.g., *SORL1*, Lambert et al., 2013) or within genes associated with biological pathways believed to potentially be associated with risk of dementia or cognitive decline (e.g., those involved in inflammatory pathways; Elwan et al., 2003; Engelhart et al., 2004; Eriksson et al., 2011; Rafnsson et al., 2007; Komulainen et al., 2007; Ravaglia et al., 2005; Schram et al., 2007; Teunissen et al., 2003) is related to individual differences in cognitive performance and decline.

Purpose and Aims of the Dissertation

Broadly, the aims of this dissertation were (a) to further align empirical investigation of the link between loneliness and cognition with the empirically supported theoretical stance that the effects of loneliness on physiological processes accumulate over time (Caspi et al., 2006; Hawkley & Capitanio, 2015; Shiovitz-Ezra & Ayalon, 2010) by assessing how baseline loneliness and two measures of longitudinal loneliness (i.e., time-varying loneliness, and geometric means for loneliness across waves) each relate to longitudinal cognition in mid and late life, and (b) to assess a potential epigenetic pathway between loneliness and performance and change in multiple domains of cognitive functioning. Specifically, the aims of this dissertation were to (a) assess the associations between baseline and longitudinal loneliness and performance and change in

four specific cognitive abilities, (b) explore the association between longitudinal loneliness and longitudinal methylation of CTRA genes in human blood leukocytes, and (c) evaluate a potential mediational role for DNA methylation at CpG sites within CTRA genes in associations between loneliness and cognitive performance and change in mid and late adulthood. Figure 1.1 illustrates the associations between loneliness, DNA methylation, and cognition explored in this dissertation.

Two studies were conducted to address the aims of this dissertation. In Study 1, the extent to which baseline loneliness, time-varying loneliness, and geometric means for loneliness across waves each predicted performance and change in four domains of cognitive functioning (processing speed, spatial ability, working memory, and verbal comprehension) in mid and late life were assessed across up to 28 years of follow-up in a large representative sample of adults ages 25-102 drawn from eight studies participating in the Consortium on Interplay of Genes and Environment Across Multiple Studies (IGEMS; Pedersen et al., 2013). Baseline loneliness was examined as a predictor of subsequent cognitive performance and change to address that the directionality of the association between loneliness and cognition has yet to be established. Further, as chronic rather than transient loneliness is posited to be associated with negative physiological outcomes, associations between time-varying loneliness and cognitive performance and change were explored, as were associations between geometric means for loneliness across waves and these cognitive outcomes. Geometric means were used as a measure of the propensity to report feelings of loneliness across waves. They were chosen as one way to quantify longitudinal loneliness in this study because they took each loneliness

score into account for each participant, as well as the variability between scores, and are more accurate measures of central tendency when used with non-normal data than the arithmetic mean, as they are not as heavily influenced by single outlying scores (Roenfeldt, 2018). Effects for baseline and time-varying loneliness on cognitive performance and change were compared to assess whether longitudinal loneliness was a stronger predictor of cognitive performance and change than baseline loneliness.

Taken together, findings which indicate a link between perceived and objective social isolation and epigenetic changes suggest that the currently unexplored association between loneliness and DNA methylation warrants examination. In Study 2, this gap in the literature was addressed through exploration of the longitudinal association between loneliness and methylation of genes associated with the CTRA (Cole, 2013; Cole et al., 2007) in blood leukocyte DNA in middle-aged and older adult monozygotic (MZ) and dizygotic (DZ) twin pairs from the Swedish Adoption/Twin Study of Aging (SATSA; Pedersen, Plomin, Nesselroade, & McLearn, 1992) across an 18-year period. While epigenetic changes are often thought of as being induced by contextual influences, it is important that work on DNA methylation take into consideration that additive genetic factors have also been found to contribute to DNA methylation patterns (Gordon et al., 2012; Hannon et al., 2018; van Dongen et al., 2016), although the heritability estimates are small, having been estimated at 5-12% (Gordon et al., 2012) and at an average of 19% for different sites within the genome (van Dongen et al., 2016). This dissertation addressed this through examination of whether DNA methylation patterns varied for individuals in the sample and for MZ and DZ twin pairs discordant for loneliness using

the co-twin control design (Carlin, Gurrin, Sterne, Morley & Dwyer, 2005**;** McGue, Osler, & Christensen, 2010). Use of this design allowed for assessment of the extent to which any observed effects of loneliness on methylation were potentially causal in nature versus partially or completely confounded by genetic factors and/or environmental factors shared within twin pairs (McGue et al., 2010).

Altered DNA methylation in lonely individuals, if found, may set physiological processes in motion which contribute to adverse outcomes with respect to health and cognition. To assess the extent to which altered DNA methylation in lonely individuals may contribute to diminished performance or faster decline in four cognitive domains (processing speed, spatial ability, working memory, and verbal comprehension), DNA methylation at sites associated with loneliness was tested as a potential mediator of associations between loneliness and cognition.

Research Questions

Research question 1.1.

Do baseline loneliness, time-varying loneliness, or geometric means for loneliness across time predict performance or change in specific cognitive abilities?

Research question 1.2.

Is longitudinal loneliness (i.e., time-varying, geometric mean) more strongly associated with cognitive performance and change than baseline loneliness?

Research question 2.1.

Does loneliness predict level or change in methylation of blood leukocyte DNA at CpG sites associated with CTRA genes for (a) individuals in the overall sample (i.e., not

by zygosity group), (b) dizygotic (DZ) twin pairs, and (c) monozygotic (MZ) twin pairs? If observed, do patterns of associations suggest (a) confounding of the loneliness-methylation relationship by genetic or common environmental factors or (b) a potentially causal association?

Research question 2.2.

Is there a potential mediational role for DNA methylation at CpG sites associated with CTRA genes in associations between loneliness and performance or change in four specific cognitive abilities?

Hypotheses

Hypothesis 1.1.

Based on findings that have shown associations between baseline loneliness and cognition (Holwerda et al., 2012; O'Luanaigh et al., 2012; Shankar et al., 2013; Tilvis et al., 2004; Wilson et al., 2007) and loneliness across time and cognition (Wilson et al., 2007; Zhong et al., 2016), it was hypothesized that both baseline and longitudinal loneliness would be associated with poorer performance and/or faster decline in the cognitive domains assessed. Based on prior unpublished cross-sectional work on loneliness and cognition using an overlapping IGEMS sample that showed larger cross-sectional associations for processing speed and spatial ability than for working memory and verbal comprehension (Phillips & Reynolds, 2016) and published work indicating that loneliness may predict both level and change for processing speed and spatial ability, and level only for working memory (Wilson et al., 2007), these associations were expected to be strongest for processing speed and spatial ability.

Hypothesis 1.2.

Based on prior work suggesting that longer periods of loneliness may lead to worse cognitive outcomes than shorter periods of loneliness (Zhong et al., 2016) and theoretical assertions that longer-term rather than shorter-term loneliness is likely to lead to lasting physiological changes (Hawkley & Capitanio, 2015) that may detract from cognitive aging, it was hypothesized that associations between loneliness and cognitive performance and change would be stronger for longitudinal loneliness than for baseline loneliness. This pattern of results was expected to be most prominent for tasks expected to have the largest associations with loneliness (i.e., processing speed and spatial ability).

Hypothesis 2.1.

Analyses assessing the longitudinal association between feelings of loneliness and methylation of CTRA genes were exploratory; prior findings showing altered expression of these genes in lonely persons (Cole et al., 2007) suggest that DNA methylation and/or change in methylation across time may also vary systematically with loneliness for some of these genes, however, this currently remains unknown.

Hypothesis 2.2.

If loneliness is associated with level or change in methylation at CpG sites associated with CTRA genes, methylation at these sites may play a mediational role in the links between loneliness and cognitive performance and change; these analyses were exploratory. As stated in hypothesis 1.1, it was predicted that loneliness would be associated with cognition, and that associations would be strongest for processing speed and spatial ability. Results from initial work showing associations between methylation at

specific CpG sites or within specific genes and cognition (Karlsson et al., 2018; Marioni et al., 2018) suggest that CpG or gene specific methylation may contribute to cognitive outcomes in mid to late adulthood, however, whether methylation at specific CpG sites within CTRA genes is related to cognition remains unknown.

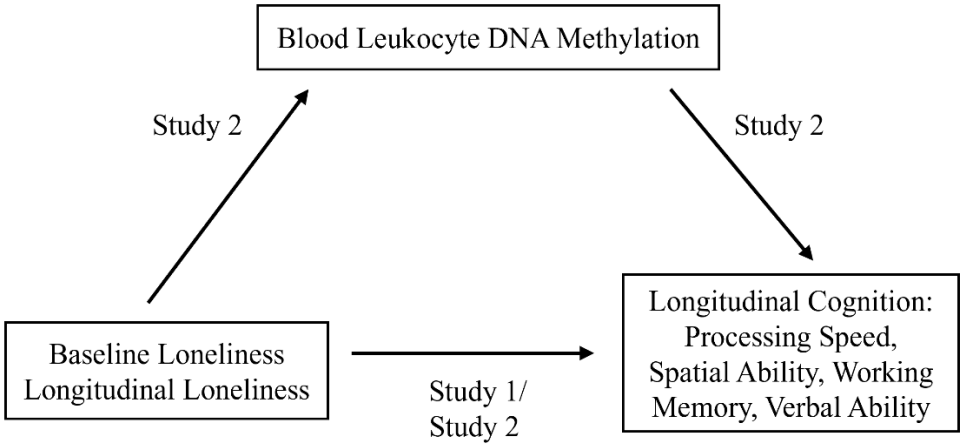

Figure 1.1. Conceptual model showing examined associations between loneliness, DNA methylation, and cognitive performance and change

Study 1

Baseline and Longitudinal Loneliness as Predictors of Cognitive Performance and Change in Mid and Late Life

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INTRODUCTION

Development unfolds in a social context, and social interaction is an essential component of normative cognitive development (Hughes, Waite, Hawkley, & Cacioppo, 2004), especially during critical or sensitive periods earlier in the lifespan. The importance of social interaction to cognition has also been shown to persist throughout adulthood. For example, measures of *quantity* of social interaction such as the size of one's social network (Bennett, Schneider, Tang, Arnold, & Wilson, 2006) and social activity engagement (James, Wilson, Barnes, & Bennett, 2011) have been shown to predict cognitive outcomes in later life, with smaller social networks and lower levels of social activity engagement predicting faster decline in global cognition (Bennett et al., 2006; James et al., 2011) and specific domains of cognitive function (James et al., 2011). Studies of perceived relationship *quality* and cognition suggest that variability in late-life cognitive outcomes is also independently linked with whether individuals feel that their social relationships fulfill their expectations or needs (Amieva et al., 2010). Feelings of loneliness, which arise when one perceives that their social relationships do not meet their current needs or expectations, particularly in terms of quality, but also in terms of quantity (Hawkley & Caccioppo, 2010), have been linked with poorer cognitive performance (O'Luanaigh et al., 2012; Wilson et al., 2007), faster cognitive decline (Holwerda et al., 2014), and elevated dementia risk (Rafnsson, Orrell, d'Orsi, Hogervorst, & Steptoe, 2017; Wilson et al., 2007).

Cognition Across Adulthood

Broadly speaking, age-associated decline in cognitive performance has been shown to occur throughout most of adulthood (Johnson, McGue, & Deary, 2014). Evidence suggests, however, that such decline is not consistent across cognitive domains, with function in different domains peaking on average at different ages and showing different patterns of change across age (Johnson et al., 2014; Schaie & Willis, 2010). For example, the classic fluid crystallized (Gf-Gc) theory (Cattell, 1963) asserts that there are two types of general intelligence—fluid intelligence (Gf) which is assessed using tasks that tap into one's ability to reason in an abstract manner, and crystallized intelligence (Gc) which is assessed using tasks that evaluate reasoning based on information learned throughout one's life (e.g., Horn & Cattell, 1967). Empirical evidence from studies based on this theory indicates that fluid intelligence peaks earlier in adulthood while crystallized intelligence continues to rise with age into later life (Horn & Cattell, 1967), although sex and cohort differences in patterns of change with age have been observed (Schaie & Willis, 2010). Moreover, although crystallized intelligence has been shown to decline in later adulthood, steeper decline has been observed for this broad domain after age 75 (Schaie & Willis, 2010).

Other studies have assessed patterns of cognitive function across age in more specific domains such as processing speed, spatial ability, verbal ability, working memory, and executive function (e.g., Salthouse, 2009; Schaie & Willis, 2010). Although decline has been observed with age for each of these domains, the average pattern across age has been shown to vary among them (Johnson et al., 2014; Schaie & Willis, 2010),

with cross-sectional studies of domain-specific performance suggesting earlier and steeper decline than longitudinal studies due to cohort differences in cross-sectional studies and the potential for practice effects in longitudinal studies (Salthouse, 2009; Schaie & Willis, 2010).

Findings from longitudinal work modeling trajectories of cognitive task performance across age in the Swedish Adoption Twin Study of Aging (SATSA) suggest reduction in performance with age after age 65 for measures of verbal ability, fluid/spatial ability, speed, and working and episodic memory with accelerating non-linear change across age for all tasks assessed except for Digit Span, a measure of working memory (Reynolds et al., 2005). For all tasks with accelerating change, significant individual differences were observed in the rate of acceleration, indicating that patterns of change across age vary among individuals (Reynolds et al., 2005). Linear change with age after age 65 was steepest for Block Design and Card Rotations (two measures of fluid/spatial ability), followed by Symbol Digit and Figure Identification (two measures of perceptual speed). Less steep change with age was observed for Figure Logic (fluid/spatial ability) and Analogies (verbal ability), and measures of episodic and working memory (Thurstone Picture and Digit Span, respectively), with the least change with age observed for Information and Synonyms (two measures of verbal ability). Cross sectional work with twin samples from the Consortium on Interplay of Genes and Environment Across Multiple Studies (IGEMS; Pedersen et al., 2013) has also shown steeper reductions in performance across 10-year age bands between ages >50 and 70+ for Block Design (fluid/spatial) and Symbol Digit (speed) than for Digits Backward

(working memory) (Pahlen et al., 2018). Reduction in performance on a Synonyms task was not observed in the IGEMS samples prior to age 60, with a fairly steep reduction in performance observed between ages 60 and 69, and less pronounced reduction in performance for those 70+ (Pahlen et al., 2018).

Importantly, despite general patterns, substantial inter-individual variability exists in cognitive functioning and in change across age, with some individuals maintaining performance longer than others (Hertzog, Kramer, Wilson, & Lindenberger, 2009; McArdle Ferrer-Caja, Hamagami, & Woodcock, 2002; Salthouse, 2009; Salthouse, 2019; Stern, 2012). The extent of and etiology of individual differences in task performance observed at older ages also shifts for many domains, with greater individual differences in task performance observed at older ages (McArdle & Plassman, 2009; McArdle, Prescott, Hamagami, & Horn, 1998; Reynolds et al., 2005; Tucker-Drob & Briley, 2014). Biometric analyses using twin data suggest that this change in variability is accompanied by a concurrent increase in variability attributed to non-shared environmental influences (i.e., environmental exposures experienced by one twin but not the other within each twin pair) for verbal, memory, and fluid/spatial tasks with age, with patterns of such etiological shifts varying among tasks within this broad pattern (Reynolds et al., 2005). This pattern has also been observed for general cognitive ability (Tucker-Drob & Briley, 2014). These findings suggest increasing importance of environmental exposures with age with respect to variability in cognitive performance and speak to the importance of understanding how environmental exposures contribute to these individual differences.

Although different cognitive domains show different patterns of age-related performance and change, these parameters have been shown to be interrelated across multiple domains. Notably, performance and change in performance on processing speed tasks have been implicated as mediators of performance and change in other cognitive domains in both cross-sectional and longitudinal work (Finkel, Reynolds, McArdle, & Pedersen, 2005; Finkel & Pedersen, 2004; Ghisletta & Lindenberger, 2004; Ghisletta & De Ribaupierre, 2005; Verhaeghen & Salthouse, 1997), as has working memory performance in cross-sectional analyses (Verhaeghen & Salthouse, 1997). Such findings further signify the importance of identification of environmental factors which contribute to declines in performance for these domains.

Loneliness and Cognition

Studies of loneliness and global cognition have shown that feeling lonely is associated with both poorer global cognitive performance (O'Luanaigh et al., 2012; Wilson et al., 2007; Zhong, Chen, Tu, & Conwell, 2017) and faster decline in global cognition (Holwerda et al., 2014; Tilvis et al., 2004; Wilson et al., 2007). Feelings of loneliness have also been associated with elevated risk of developing dementia (Rafnsson et al., 2017; Wilson et al., 2007). For example, Wilson et al. (2007) reported risk of Alzheimer's disease for very lonely persons to be more than two-fold that of persons low in loneliness—an effect that was modestly reduced when controlling for depression (Wilson et al., 2007). Similar results were reported by Rafnsson et al. (2017) and Holwerda et al. (2014) who also found an elevated risk of dementia for lonely persons after adjusting for depressive symptoms.

A small but growing literature on associations between loneliness and various domains of cognitive performance suggests that feelings of loneliness are also associated with domain-specific cognitive function and change, although inconsistency has been observed in such associations across cognitive domains. Prior work has shown negative associations between loneliness and performance on executive function (Shankar et al., 2013), working memory (Wilson et al., 2007) episodic memory (Shankar et al., 2013; Wilson et al., 2007), semantic memory (Wilson et al., 2007), visual memory (O'Luanaigh et al., 2012), visuospatial ability (Wilson et al., 2007), and processing speed (O'Luanaigh et al., 2012; Wilson et al., 2007) tasks. Longitudinal studies of loneliness and domain-specific cognitive performance and change also suggest that loneliness predicts accelerated decline in visuospatial ability (Wilson et al., 2007), episodic (Donovan et al., 2016) and semantic memory, and processing speed (Wilson et al., 2007). Observed associations between loneliness and working memory performance and loneliness and rate of change in processing speed may be of especially great interest in the context of findings that suggest that losses in these cognitive domains may mediate performance and change in other domains (Finkel et al., 2005; Finkel & Pedersen, 2004; Ghisletta & Lindenberger, 2004; Ghisletta & De Ribaupierre, 2005; Verhaeghen & Salthouse, 1997).

It is also important to consider that although longitudinal work has shown associations between earlier loneliness and later cognitive performance and change (Donovan et al., 2016; Holwerda et al., 2014; Tilvis et al., 2004; Wilson et al., 2007), reductions in cognitive performance may also make the experience of loneliness more likely, and this may, in part, explain observed associations between loneliness and

cognition. Findings from recent work that assessed longitudinal associations between baseline loneliness and subsequent cognition and baseline cognition and subsequent loneliness across a 12-year period suggest that the association between loneliness and cognition operates in the direction from loneliness to cognition rather than vice versa (Donovan et al., 2016), however, follow-up work is warranted to further explore potential reciprocal effects within the loneliness-cognition association.

Loneliness theorists have proposed that short-term periods of loneliness are unlikely to lead to lasting physiological changes associated with declining health, and that such changes associated with longer periods of loneliness may diminish or even reverse once loneliness is overcome (Hawkley & Capitanio, 2015). Despite that such theoretical assertions highlight the importance of considering how different patterns of feelings of loneliness across time relate to cognitive outcomes, few studies have assessed associations between loneliness across time and cognition. In one recent study, Zhong, Chen and Conwell (2016) assessed associations between different types of loneliness experienced across a 3-year period and global cognition at a six-year follow-up. Individuals were categorized as transiently lonely if they reported loneliness at either one of two assessment waves, as chronically lonely if they reported loneliness at both waves, and not lonely if they did not report loneliness at either wave. Both loneliness categories were associated with poorer global cognitive function, with a stronger effect observed for chronic than transient loneliness. Interestingly, the effect of chronic loneliness was only observed for individuals with higher cognitive function at follow-up, while the effect of transient loneliness was significant across all participants regardless of cognitive

function. The finding that transient loneliness was associated with global cognition suggests that lasting effects on cognition may result from transient exposures to loneliness, however, more work is needed to determine whether this is the case. As loneliness was assessed in this study twice across three years and individuals who reported loneliness at the second wave (who may have continued to be lonely following assessment of loneliness and prior to assessment of cognition) were categorized as being transiently lonely, further work is needed to determine how transient loneliness relates to cognitive outcomes. In another study that considered loneliness across time, Wilson et al. (2007) found an association between average loneliness scores across up to five assessments across a four-year period and rate of global cognitive decline and risk of developing Alzheimer disease.

Loneliness Trends by Age and Sex

Loneliness has been shown to be most prevalent in adolescence/early adulthood and in late life (Luhmann & Hawkley, 2016; Nicolaisen & Thorsen, 2014; Qualter et al., 2015) with the peak in prevalence observed in adolescence attributed to concurrent social and physical changes that normatively occur at this stage of the lifespan (Qualter et al., 2015). Concurrent physical changes and changes with respect to social roles also become more probabilistic with age in late adulthood (e.g., increasing likelihood of experiencing social loss and decline in cognitive function and physical ability with age) which may increase risk of experiencing loneliness with age in late life (D'Augostino & Canli, 2018; Nicolaisen & Thorsen, 2014; Qualter et al., 2015). Moreover, studies of social relationships in late life suggest that older adults tend to have a declining number of

persons in their social network with age, resulting from the maintenance of only those relationships with the greatest emotional significance (Carstensen, Fung, & Charles, 2003; Lang & Carstensen, 1994). Although such focus on meaningful social interactions and trimming of one's social network in later life appears deliberate and is conceived as adaptive (Lang & Carstensen, 1994), for those whose social networks consist primarily or solely of older individuals, loneliness may become more likely with age if increasing losses to one's social network are experienced.

Prevalence of loneliness has also been shown to be higher in women than in men (Pinquart & Sörensen, 2001). Explanations proposed for this observed sex difference include differences in socialization of males and females (i.e., greater focus on nurturing social relationships for females than males), and differences in likelihood of experiencing the loss of a spouse or declining functional ability in older men and women, with elderly women more likely to experience these or to live alone or suffer from long-term illness than elderly men (Pinquart & Sörensen, 2001). Importantly, it has been shown that this sex difference in prevalence of loneliness is larger in magnitude when single items are used to assess loneliness compared to when loneliness scales are used, an observation thought to result from underreporting of loneliness by males when single items are used due to social desirability (Luhmann & Hawkley, 2016; Nicolaisen & Thorsen, 2014; Pinquart & Sörensen, 2001). A more recent review likewise points to mixed findings with respect to gender differences in loneliness (Qualter et al., 2015).

The aims of the present investigation were to assess associations between loneliness and performance and change in four specific cognitive abilities (processing

speed, spatial ability, working memory, and verbal comprehension) in a large sample with longitudinal data collected across up to 28 years of follow-up and to compare effects of baseline loneliness and two measures of longitudinal loneliness (time-varying loneliness and geometric means for loneliness across waves) on level and change across age for each of these domains. Comparison of effects for baseline and longitudinal loneliness on domain-specific performance and change in cognition allowed for examination of how fluctuations in loneliness across waves and the trait-like propensity to experience loneliness each related to cognition and the extent to which associations for longitudinal measures of loneliness differ from relations for baseline loneliness. The extent to which such patterns of effects were consistent across tasks assessing different cognitive domains was also explored. To reduce the likelihood that any observed associations are due to reverse causation (with declining cognitive function leading to loneliness rather than vice versa), data collected at or after the onset of dementia or low cognitive performance were excluded from analyses.

It was predicted that both baseline and longitudinal loneliness would be associated with poorer cognitive performance and/or faster cognitive decline. Prior work supports this prediction, with associations reported between both baseline (Holwerda et al., 2012; O'Luanaigh et al., 2012; Shankar et al., 2013; Tilvis et al., 2004; Wilson et al., 2007) and longitudinal loneliness (Wilson et al., 2007; Zhong et al., 2016) and cognition. It was also predicted that associations would be largest for processing speed and spatial ability. Limited prior longitudinal work on loneliness and domain-specific cognition suggests that loneliness may predict both level and change for processing speed and

spatial ability, and level only for working memory (Wilson et al., 2007). Moreover, prior unpublished cross-sectional work assessing associations between baseline loneliness and cognitive performance in an overlapping IGEMS sample showed higher correlations between loneliness and scores on tasks assessing processing speed and spatial ability after adjusting for age and sex than were observed for working memory or verbal comprehension (Phillips & Reynolds, 2016).

It was hypothesized that longitudinal measures of loneliness would more strongly predict cognitive performance and/or change than baseline loneliness. Initial findings from recent work on longitudinal loneliness and global cognition suggests that the length of time across which loneliness is experienced may be an important predictor of cognitive outcomes, with longer periods of loneliness associated with worse outcomes than shorter periods of loneliness (Zhong et al., 2016). As associations were expected to be strongest for tasks assessing processing speed and spatial ability, it was also expected that this pattern of results would be more prominent for these tasks.

Method

Samples

Overall IGEMS sample.

The longitudinal association between loneliness and cognition was assessed using cross-sectional and longitudinal data from eight studies from the Consortium on Interplay of Genes and Environment across Multiple Studies (IGEMS; Pedersen et al., 2013). The IGEMS Consortium was formed to pool twin studies to enable large-scale analyses of the interplay between contextual and genetic factors on physical and mental health and

cognition in mid and late life (Pedersen et al., 2013). Eight of the IGEMS samples—those for which overlapping or harmonizable measures of loneliness and cognition were collected—were selected for the current study, allowing for analysis of associations between loneliness and four specific cognitive abilities in a large sample across a follow-up period of up to 28 years. The number of waves of data collection at which both loneliness and cognitive performance were assessed varied by study and ranged from 1 to 10 (see Table 2.1). The median number of follow-ups for the overall sample was 2.

The overall IGEMS sample consisted of 15,302 twins, *n* = 5,703 MZ (2,407 complete pairs, 889 incomplete pairs), *n* = 7,124 same-sex dizygotic (SSDZ; 2,684 complete pairs, 1,756 incomplete pairs), *n* = 2,170 opposite-sex dizygotic (OSDZ; 887 complete pairs, 396 incomplete pairs). Thirty-six complete pairs and 233 incomplete pairs had unknown zygosity (*n* = 305). For the overall sample, *n* = 7,929 were females (51.82%) and *n* = 7,373 (48.18%) were males. At baseline, the age range was 25-102 (*M* = 64.33, *SD* = 13.39).

Analysis sample.

The analysis sample consisted of *n* = 13,114 twins, *n* = 4,952 MZ (2,052 complete pairs, 848 incomplete pairs), *n* = 5,979 SSDZ (2,172 complete pairs, 1,635 incomplete pairs), *n* = 1,968 OSDZ (796 complete pairs, 376 incomplete pairs), and *n* = 215 with unknown zygosity (25 complete pairs, 165 incomplete pairs) who (a) had both loneliness and cognitive data, (b) had data for all relevant covariates, and (c) were not flagged for low cognitive performance or dementia diagnosis at one or more waves of data collection. *N* = 1,424 from the overall IGEMS sample were missing data for one or more

analysis variables; the sample size with data for all analysis variables was *n* = 13,878. Of the participants with at least one complete wave of data, *n* = 764 were flagged for having consistently low cognitive performance below cutoffs or were prevalent dementia cases and were excluded from the analysis sample. At baseline, the age range for the analysis sample was 25 to 101 and average age was 62.69 years (*SD* = 13.03). The sample was 50.5% female (*n* = 6,621) and 49.5% male (*n* = 6,493). The overall and analysis samples are each described below for each IGEMS study. Demographic information for the complete IGEMS sample and the analysis sample are reported in Table 2.2 for the pooled sample and by study.

Swedish studies.

Four of the eight IGEMS studies were conducted in Sweden using Swedish samples; the Swedish Adoption/Twin Study of Aging (SATSA; Pedersen et al., 1992), Origins of Variance in the Oldest-Old (OCTO-Twin; McClearn et al., 1997), the Sex Differences in Health and Aging Study (GENDER; Gold, Malmberg, McClearn, Pedersen, & Berg, 2002), and the Twin and Offspring Study in Sweden (TOSS; Neiderheiser & Lichtenstein, 2008).

SATSA is a longitudinal twin and adoption study of cognitive performance in middle-aged and older adults (Pedersen et al., 1992; Finkel & Pedersen, 2004). The SATSA twins were recruited from the Swedish Twin Registry beginning in 1984 (see Berglund et al., 2016) and include twins raised both together and apart (Finkel & Pedersen, 2004; Pedersen et al., 1992). Following an initial questionnaire wave of data collection, participants ages 50 and older were invited to participate in in-person testing

which occurred approximately every three years, with 10 waves of in-person data collected by 2014 (Berglund et al., 2016). Questionnaires and tests of cognitive and physical function were administered at each IPT wave, and blood samples were collected (Berglund et al., 2016; Finkel & Pedersen, 2004). Six additional questionnaire waves were also administered throughout this 30-year period, for a total of 17 waves. Data collected at the 10 in-person testing waves (with up to 28 years of follow-up) were used for the current study, as cognition was only assessed at these waves. The overall SATSA sample consisted of a subsample of SATSA twins who participated in-person testing (n = 859). N = 340 were MZ twins (163 complete pairs, 14 incomplete pairs), n = 516 were SSDZ twins (246 complete pairs, 24 incomplete pairs), and n = 3 had unknown zygosity (1 complete pair, 1 incomplete pair). The sample was 59.6% female (n = 512) and 40.4% male (n = 347). At baseline, the age range for the SATSA sample was 39 to 87 years (M = 63.56, SD = 8.82). The SATSA analysis sample included 768 SATSA twins (n = 68 were missing on key study variables; n = 23 were excluded for having low cognitive performance or dementia diagnosis across waves at which they had complete data). N = 302 were MZ twins (141 complete pairs, 20 incomplete pairs), n = 465 were SSDZ twins (214 complete pairs, 37 incomplete pairs), and n = 1 had unknown zygosity. The SATSA analysis sample was 59.8% female (n = 459) and 40.2% male (n = 309). The age range at baseline was 44-89 years (M = 64.46, SD = 8.79).

OCTO-Twin is a longitudinal study of twins 80 years of age or older that was established to gain a better understanding of how heritable and contextual influences contribute to interindividual variability in complex phenotypes common in aging

individuals (McClearn et al., 1997). Complete, same sex twin pairs were recruited between 1991 and 1993 from the Swedish Twin Registry (McClearn et al., 1997) to participate in five waves of in-person data collection that took place from 1991 to 2002. Data from all five waves of OCTO-Twin were used for the current study. The OCTO-Twin sample included *n* = 702 twins (351 complete twin pairs; 149 MZ pairs and 202 SSDZ pairs) and was 66.67% female (*n* = 468) and 33.33% male (*n* = 234). The age range at baseline was 79 to 97 years (*M* = 83.58, *SD* = 3.17). The OCTO-Twin analysis sample included 469 twins (*n* = 148 were missing on key study variables; *n* = 85 were dropped for having low cognitive performance or dementia diagnosis across waves at which they had complete data). *N* = 210 were MZ twins (84 complete pairs, 42 incomplete pairs) and *n* = 259 were SSDZ twins (89 complete pairs, 81 incomplete pairs). The age range for the OCTO-Twin analysis sample was 79-97 at baseline (*M* = 83.16, *SD* = 2.81). The sample was 65.0% female (*n* = 305) and 35.0% male (*n* = 164).

GENDER, also known as the Sex Differences in Health and Aging Study, is a longitudinal study of opposite-sex twins recruited from the Swedish Twin Registry created to explore potential sex differences in perceived and objective health (Gold et al., 2002). GENDER participants were 70 to 80 years old at baseline and completed three in-person testing waves and two questionnaire waves of data collection between 1994 and 2007 (Pedersen et al., 2013). As cognitive performance was only assessed at the in-person testing waves, data from these three waves were used for the current study. The GENDER sample (*n* = 498) consisted of 249 complete OSDZ twin pairs. Consequently, the sample was 50% female (*n* = 249) and 50% male (*n* = 249). The sample age range at

baseline was 69 to 80 years (*M* = 74.52, *SD* = 2.64). The GENDER analysis sample consisted of *n* = 440 twins. *N* = 28 were missing on key study variables; *n* = 30 were dropped for having low cognitive performance or dementia diagnosis across waves at which they had complete data. All participants were OSDZ twins (196 complete pairs, 48 incomplete pairs). The analysis sample was 50.2% female (*n* = 221) and 49.8% male (*n* = 219). The age range at baseline was 69-80 years (*M* = 74.46, *SD* = 2.63).

TOSS is a study of twins, their spouses, and their children designed to assess gene-environment interplay related to parenting and familial relations in adulthood (Neiderheiser & Lichtenstein, 2008). Same-sex female twin pairs who had children were initially recruited from the Swedish Twin Registry, along with their spouses and children. Three years later a second sample of male and female same-sex twin pairs was also drawn from the registry. Twin data from the first cohort were used for the current study. The TOSS sample (*n* = 1,602) consisted of *n* = 694 MZ twins (314 complete pairs, 66 incomplete pairs), 904 SSDZ twins (416 complete pairs, 72 incomplete pairs), and 2 complete pairs with unknown zygosity. The sample was 62.91% female (*n* = 1,004) and 37.09% male (*n* = 592). The age range for the TOSS sample was 32 to 59 years (*M* = 44.84, *SD* = 4.86). The TOSS analysis sample included 1,587 twins (*n* = 15 were missing on key study variables). *N* = 690 were MZ twins (312 complete pairs, 66 incomplete pairs) and *n* = 893 were SSDZ twins (407 complete pairs, 79 incomplete pairs). Two complete pairs had unknown zygosity (*n* = 4). The TOSS analysis sample was 62.9% female (*n* = 998) and 37.1% male (*n* = 589) with an age range of 32-59 years (*M* = 44.82, *SD* = 4.86).

American studies.

Two of the IGEMS studies selected for the current study were conducted in the United States with American samples; the Vietnam Era Twin Study of Aging (VETSA; Kremen, Franz, & Lyons, 2013), and the Minnesota Twin Study of Adult Development and Aging (MTSADA; Finkel & McGue, 1993; Finkel, Pedersen, & McGue, 1995).

The VETSA was designed to assess factors which contribute to cognitive aging using a sample of male twins who were enlisted in military service during the Vietnam era (between 1965 and 1975; Kremen et al., 2013; Pedersen et al., 2013). The VETSA is ongoing, with two waves of data currently available. Baseline data were collected from 2003 to 2007; the first follow- up wave began in 2008 and ended in 2012. Data from both completed waves of the VETSA study were used for the current analyses. The VETSA sample (n = 1,237) consisted of 348 complete MZ twin pairs and 266 complete DZ twin pairs, and 3 incomplete MZ twin pairs and 6 incomplete DZ twin pairs. As mentioned above, the sample was 100% male. The sample age range at baseline was 51 to 60 years (M = 55.88, SD = 2.48). The VETSA analysis sample included 1,218 twins (n = 19 were missing on key study variables). N = 687 were MZ twins (339 complete pairs, 9 incomplete pairs) and 531 were SSDZ twins (259 complete pairs, 13 incomplete pairs). The age range for the VETSA analysis sample was 51-64 at baseline (M = 55.91, SD = 2.51).

The MTSADA is a longitudinal twin study that was designed to assess how genetic and environmental factors each contribute to interindividual variability in outcomes related to aging (Minnesota Center for Twin & Family Research, 2007). The

MTSADA twins were drawn from the Minnesota Twin Registry (Minnesota Center for Twin & Family Research, 2007). Baseline data were collected beginning in 1986. Two follow-up waves of data were collected between 1986 and 1996. Baseline data were used for the current study. Although loneliness was assessed at two waves (baseline and the second follow-up wave), it was not assessed more than once for each participant and cognitive data were only collected at the baseline wave. The MTSADA sample (n = 1,359) consisted of n = 724 MZ twins (333 complete pairs, 58 incomplete pairs), n = 633 SSDZ twins (288 complete pairs, 57 incomplete pairs), and 1 complete pair with unknown zygosity. The sample was 57.98% female (n = 788) and 42.02% male (n = 571). The age range at baseline was 25 to 92 years (M = 58.68, SD = 10.73). The MTSADA analysis sample included n = 777 twins (n = 568 were missing on key study variables; n = 14 were dropped for having low cognitive performance). N = 461 were MZ twins (204 complete pairs, 53 incomplete pairs) and n = 316 were SSDZ twins (142 complete pairs, 32 incomplete pairs). The MTSADA analysis sample was 60.9% female (n = 473) and 39.1% male (n = 304). The age range was 25-86 years (M = 55.05, SD = 12.56).

Danish studies.

Two of the IGEMS studies were conducted in Denmark with Danish samples; the Longitudinal Study of Aging Danish Twins (LSADT; Skytthe et al., 2006; Skytthe et al., 2013) and the Middle Age Danish Twin Study (MADT; Skytthe et al., 2013).

The LSADT is a longitudinal study of twins ages 70 and above that began in 1995 and had five follow-up assessments, the last of which took place in 2005 (Skytthe et al.,

2006). The LSADT was implemented to explore the interplay between genetic and contextual influences with respect to a variety of outcomes associated with aging (Skytthe et al., 2006). Data from all six LSADT assessments were used for the current analyses. The LSADT sample (n = 4,731) consisted of n = 1,489 MZ twins (436 complete pairs, 617 incomplete pairs), n = 2,728 SSDZ twins (666 complete pairs, 1,396 incomplete pairs), n = 224 OSDZ twins (21 complete pairs, 182 incomplete pairs), and 29 complete pairs and 232 incomplete pairs with unknown zygosity. The sample was 58.93% female (n = 2,788) and 41.07% male (n = 1,943). The baseline age range was 70 to 102 years (M = 77.74, SD = 5.66). The LSADT analysis sample consisted of n = 3,628 twins (n = 491 were missing on key study variables across waves; n = 612 were dropped for having low cognitive performance or dementia diagnosis across waves at which they had complete data). N = 1,168 were MZ twins (326 complete pairs, 516 incomplete pairs), n = 2,147 were SSDZ twins (486 complete pairs, 1,175 incomplete pairs), n = 109 were OSDZ twins (5 complete pairs, 99 incomplete pairs), and n = 204 had unknown zygosity (20 complete pairs, 164 incomplete pairs). The LSADT analysis sample was 57.6% female (n = 2,090) and 42.4% male (n = 1,538). The age range at baseline was 75-101 years (M = 76.77, SD = 5.04).

The MADT is a longitudinal twin study implemented to assess how physical and cognitive health and mortality in late life relate to midlife functioning and behaviors (Skytthe et al., 2013). Approximately 10 years after the intake wave of data collection, which took place in 1998, a second wave of data was collected beginning in 2008 and ending in 2011 (Skytthe et al., 2013). Both waves of data were used for the current study.

The MADT sample (n = 4,314) consisted of n = 1,459 MZ twins (664 complete pairs, 131 incomplete pairs), n = 1,401 SSDZ twins (600 complete pairs, 201 incomplete pairs), n = 1,448 OSDZ twins (617 complete pairs, 214 incomplete pairs), and 3 complete pairs with unknown zygosity. The sample was 49.05% female (n = 2,116) and 50.95% male (n = 2,198). The baseline age range was 46 to 68 years (M = 56.88, SD = 6.34). The MADT analysis sample included n = 4,227 twins (n = 87 were missing on key study variables across waves). N = 1,434 were MZ twins (646 complete pairs, 142 incomplete pairs), n = 1,368 were SSDZ twins (575 complete pairs, 218 incomplete pairs), n = 1,419 were OSDZ twins (595 complete pairs, 229 incomplete pairs), and n = 6 had unknown zygosity (3 complete pairs). The MADT analysis sample was 49.1% female (n = 2,075) and 50.9% male (n = 2,152). The age range at baseline was 46-68 years (M = 56.86, SD = 6.33).

Measures

Loneliness.

Rasch analysis-based loneliness person measures computed using all available IGEMS loneliness data.

Loneliness was assessed in 12 of 15 IGEMS studies with questionnaire items that varied across studies both with respect to the questions asked and the number of items given. The number of loneliness items asked in each study ranged from one (TOSS, LSADT, VETSA) to seven (GENDER). The loneliness items and response options administered in each IGEMS study are shown in appendix Table A1. To construct a harmonized loneliness measure across the IGEMS studies, the longitudinal IGEMS

loneliness data were pooled with data from a 'crosswalk' sample who filled out a questionnaire (either in-person or online via Mechanical Turk) that included all loneliness items given to each of the IGEMS samples, and a 10-item version of the UCLA Loneliness Scale (ULS; Russell, Peplau, & Cutrano, 1980). Rasch measurement analysis, which uses responses on multiple items measuring a single latent trait to estimate where each item and each participant falls along the trait (Boone, Staver, & Yale, 2014), was conducted using Winsteps v. 3.92.1. The Rasch analysis yielded "person measures" of loneliness for each participant at each wave which represent, in logit units, where each individual fell on the latent construct of loneliness given their responses on the loneliness items they responded to. Rasch analyses used to compute loneliness person measure scores are described in more detail in Appendix 1.

Cognitive performance.

Processing speed (Symbol Digit task).

Processing speed was assessed in six of the nine studies using either a Symbol Digit task (MADT, LSADT, SATSA, OCTO-Twin, and GENDER) or a Digit Symbol task (MTSADA; Wechsler, 1981). In the former task, participants were asked to use a visual display of nine paired shapes and numbers (1-9) to translate subsequently presented shapes into their corresponding numbers (which they reported verbally). In the latter task, participants were asked to use a similar visual display to translate subsequently presented numbers into their corresponding shapes (which they were asked to draw). For the studies in which Symbol Digit was used, 100 trials were given, with one symbol to be translated presented in each trial. For MTSADA, 90 trials were given, with

one number presented per trial. Maximum possible total scores were 100 for the Symbol Digit task and 90 for the Digit Symbol task. Scores were converted to % correct for the Digit Symbol task so that scores on both tasks were out of 100.

Spatial ability (Block Design task).

Spatial ability was assessed in four studies with either the Kohs Block Design test (Stone, 1985) (SATSA, OCTO-Twin, and GENDER) or the Wechsler Adult Intelligence Scale-Revisited (WAIS-R) Block Design subtest (Wechsler, 1981) (MTSADA). In both tasks, participants were asked to use a set of provided blocks to construct a series of pictorially presented shapes. One shape was presented per trial, and either 7 (SATSA, OCTO-Twin, GENDER) or 9 (MTSADA) trials were administered. Participants' performance was scored according to how quickly they were able to construct the shape and how closely the shape they produced matched that originally presented. Scores were converted to percent correct so that possible scores on these tasks ranged from 0 to 100.

Working memory (Digits Backward task).

Working memory was assessed in VETSA, MADT, LSADT, SATSA, and OCTO-Twin using similar Digits Backward tasks in which participants were asked to verbally reproduce a series of 2-8 digits (MADT, LSADT, SATSA, OCTO-Twin) or 2-10 digits (VETSA) read aloud to them by an experimenter in the opposite order from which they heard them (i.e., from last to first). Across studies, shorter series of digits were tested first and the task progressively increased in difficulty (the digit series increased by a single digit after two trials if at least one was successful) until participants failed to reproduce both digit series of a given length or the maximum number of digits for the test

was reached. Within the SATSA sample, one participant had a raw score of 2 at IPT3 for this task. This score was set to missing, as it was outside the range of possible scores for this task and may have resulted from an error in coding. Scores were converted to percent correct so that maximum scores across studies for the task were 100.

Verbal comprehension (Synonyms task).

Verbal comprehension was assessed in SATSA, GENDER, OCTO-Twin, and TOSS using a Synonyms task in which each trial consisted of the presentation of a single word along with response options from which participants were asked to determine which word was closest to the target word in meaning. Scores were converted to percent correct so that scores for this task ranged from 0 to 100.

The measure(s) of cognitive performance assessed in each IGEMS study and the waves at which each was administered are shown in Table 2.1.

Harmonization of cognitive scores across IGEMS studies.

After converting scores on each cognitive task to percent correct so that scores from all studies had a highest possible score of 100, cognitive data from the IGEMS studies were pooled for studies that administered the same cognitive tasks so that the cognitive data could be normalized. The four Swedish studies (SATSA, OCTO-Twin, GENDER, and TOSS) were combined, as were the two Danish studies (LSADT and MADT); each of the two American studies (VETSA and MTSADA) were normalized separately. Scores on each task were normalized for each study/group of studies based on means and standard deviations from referent groups which included participants who were age 65 to 69.99 at baseline and who were not flagged for having a dementia

diagnosis or low cognitive performance (see detailed description of criteria below). For the Swedish studies, the referent means and standard deviations for norming were computed using a referent group from SATSA that was age 65-69.99 at baseline. For VETSA, the baseline age range was 51 to 60. Since no participants fell into the age range for the referent group for this study at wave 1, the means and standard deviations for normalizing the Digits Backward task for VETSA were computed using data from attrition replacements who had their first wave of data collection at wave 2 or wave 3 who were age 65 to 69.99 at their first wave of assessment. For each sample, *z*-scores were computed for each cognitive task based on these means and standard deviations, and scores were converted to a *T*-score scale ($M = 50$, $SD = 10$). The 65 to 69.99 age range was selected based on the necessity of selecting an age range that had sufficient coverage within each study or group of studies to form the referent group for normalizing.

For the two Danish studies, visual inspection of longitudinal plots of Symbol Digit scores revealed that some individuals within both the MADT and LSADT samples had outlying scores that were aberrations to within-person patterns of responding over time for this cognitive task or were uncharacteristically high or low in comparison to scoring patterns across the rest of LSADT and MADT.

To address these issues, Symbol Digit data from the MADT and LSADT samples were pooled with data from the MIddle Age Danish Twin Study (MIDT; Skytthe et al., 2013). Scores of 0 in the MADT sample and scores above 85 in the MADT and LSADT samples were first set to missing. Eighty-five was chosen as the cutoff value for the upper end of the distribution as this was the highest score obtained in the MIDT study and the

MIDT sample was similar in age to the MADT sample and younger than the LSADT sample. Fifty-five scores ranging from 88 to 100 were removed (35 from MADT and 20 from LSADT). For LSADT, scores were also dropped if there was an increase or decrease in score of more than three standard deviations (standard deviation was computed for baseline symbol digit scores across the three Danish samples after dropping aforementioned scores and individual scores corresponding to waves at which a participant scored less than 24 on the MMSE) across adjacent waves (i.e., within an approximately two-year period). The standard deviation was 13.45 and the cutoff for change across adjacent waves was +/-40.35. Thirty-five individuals were flagged for having scores that changed more than 40.35 points across adjacent waves. These scores were examined, case by case, within the context of scores across waves for each individual. For cases with three or more waves of data, the score that did not align with the individual's other responses was removed. For cases with only two waves of data, both scores were dropped from the analysis.

Following removal of these scores, the mean and standard deviation was again computed for the baseline MADT, MIDT, LSADT sample and scores were winsorized to +/- 3 standard deviations around the mean (M = 46.62, SD = 13.45) to pull in remaining extreme values. No scores were winsorized on the high end of the distribution, as the upper boundary for winsorizing was 86.97, and scores above 85 were previously dropped. On the low end, 1,034 low scores were pulled in to the lower boundary of 6.27. Scores were subsequently normalized according to the procedure described above.

Indicators of low cognitive performance.

Participants with low cognitive performance were excluded both from the referent group for norming cognitive scores across studies and from analyses for the current study. For MTSADA and LSADT, participants were excluded if they had a score of 23 or lower on the Mini-Mental State Examination (MMSE). Dementia diagnoses were also available for SATSA, OCTO-Twin, and GENDER. Participants from these studies were excluded if they had a score of 23 or below on the MMSE or if they had been diagnosed with dementia where their age of onset was prior to the in-person testing occasion. For participants who became demented or whose MMSE score dropped below 24 after their first wave of data collection, data were included in analyses for all waves prior to dementia diagnosis or having a low MMSE score. For VETSA, TOSS, and MADT, MMSE data and dementia diagnoses were not available; consequently, screening for low cognitive performance for these studies was not possible. These three samples had the youngest participants of the eight studies—the upper ends of the age ranges for VETSA at baseline and the TOSS study were 60 years; for MADT the upper end of the age range was 68 years at baseline.

Covariates.

Baseline objective social isolation and depression, age, sex, educational attainment, and country of residence were adjusted for in model-fitting analyses.

Objective social isolation.

Objective social isolation is moderately associated with feelings of loneliness (Wilson et al., 2007), and has been linked with diminished cognitive performance

(Stoykova, Matharan, Dartigues, & Amieva, 2011) and greater risk of dementia (Fratiglioni, Wang, Ericsson, Maytan, & Winblad, 2000; Kuiper et al., 2015). Objective social isolation was measured in all eight IGEMS studies with measures of marital status and living arrangement. Marital status was evaluated across studies by asking participants about their current marital status at the time of measurement. Response options for the harmonized marital item were '*unmarried, not cohabitating, or single*', '*married/separated*', '*cohabitating*', '*divorced*', and '*widowed*'. Living arrangement was assessed across the IGEMS studies using various items which asked about the number and identity of others living in the participant's home. For the present analyses, marital status and living arrangement were each coded dichotomously (i.e., married or cohabitating = 0, not married or cohabitating = 1 for the marital item and live with one or more others = 0, live alone = 1 for the living arrangement item).

Depression.

Depressive symptoms are highly correlated with feelings of loneliness and have been shown to significantly predict accelerated rate of cognitive change in older adults (e.g., Donovan et al., 2016; Wilson et al., 2002) and dementia risk (Wilson et al., 2002). Depression was assessed in six of the eight IGEMS studies (SATSA, GENDER, OCTO-Twin, TOSS, VETSA, and MTSADA) with a 20-item version of the CES-D scale (Radloff, 1977). Each item asked about the previous week. Sample items include '*during the last week…I didn't feel like eating, I had a bad appetite*' and '*during the last week…I slept poorly*'. Response options were consistent across all items and included '*rarely or none of the time*', '*some or a little of the time*', '*occasionally or a moderate amount of*

time', and '*most or all of the time*'. Depression was assessed in the remaining two studies (LSADT and MADT) using the CAMDEX (Roth et al., 1986). The CAMDEX consisted of 17 items and asked participants to report on how they felt at the time of the interview compared to how they felt 6-12 months prior. Sample items include '*do you feel sad, depressed, or miserable?*' and '*have you lost pleasure or interest in things you usually cared about or enjoyed?*' Response options were '*yes—most of the time*', '*yes, sometimes*', and '*no*' for all but two items, for which response options were '*yes*' and '*no*'.

To compute harmonized depression scores across the IGEMS studies, data from a crosswalk sample previously recruited for data harmonization across these samples (n = 1,061, see Gatz et al., 2015) who took both the CES-D and the CAMDEX were used to conduct Rasch measurement analyses in Winsteps v. 3.92.1 to create conversion tables for converting total scores on the CES-D to CAMDEX units and vice versa. To achieve this, separate Rasch analyses were conducted for the CES-D and the CAMDEX and person measure (Θ) scores were estimated for each possible total score on each of the two scales. To convert CES-D scores to CAMDEX units, the CAMDEX was rescaled so that the *M* and *SD* of the Θ values were the same as those for the CES-D scale. The test characteristic curve from this analysis was then used to link Θ values for each CES-D score with corresponding values on the CAMDEX. To convert CAMDEX scores to CES-D units, the same procedure was carried out—the CES-D was rescaled so that the *M* and *SD* aligned with those for the CAMDEX, and scores on the CAMDEX were linked with corresponding CES-D values using Θ values and the test characteristic curve. As both

scales included items asking about feelings of loneliness, these items were excluded for computation of total scores and for harmonization of total scores on the depression scales. The CAMDEX also included an item which asked whether participants preferred to be on their own recently which was also excluded for harmonization. For the current analysis, total scores on the CAMDEX were converted to CES-D units. Refer to Appendix 2 for conversion tables for converting total scores on the CAMDEX to CES-D units and vice versa (Tables A2 and A3, respectively).

Educational attainment.

Higher levels of educational attainment have been associated with reduced dementia risk and maintenance of cognitive performance in individuals with pathological brain changes associated with Alzheimer's disease (AD; Evans et al., 1997; Stern, 2012; Stern et al., 1994). Thus, educational attainment was adjusted for in analyses assessing associations between loneliness and cognition. Educational attainment was assessed in all IGEMS studies. For MTSADA and VETSA, participants were asked to report the number of years of education they had completed. Other IGEMS studies asked participants to report the highest level of education they completed, (e.g., '*high school*', or '*master's degree*'). For these studies, educational attainment was recoded to years of education by representatives from the individual studies, such that *some grade school* = 6, *8th grade* = 8, *some high school* = 11, *GED* = 12, *high school graduate* = 12, *1-2 years of college* = 13.5, *associate degree or vocational school* = 14, *3 or more years of college* = 15, *college degree* = 16, *some grad school* = 17, *master's degree* = 18, *professional or Ph.D. degree* = 20.

For the present analyses, educational attainment data were not available for MADT. To address this, model-fitting was carried out initially without adjusting for educational attainment, and follow-up sensitivity analyses adjusting for education were run for all models from which effects of loneliness emerged, which thus excluded the MADT sample.

Country of residence.

Country of residence was added as a covariate in model-fitting analyses. Country was effects coded such that Denmark was coded -.5, the United States was coded 0, and Sweden was coded .5. Coding was based on average loneliness scores for each country. The Danish samples reported the lowest levels of loneliness on average at baseline (M = -2.92, SD = 1.67), followed by the U.S. samples (M = -2.39, SD = 2.00). The Swedish samples reported the highest levels of loneliness on average at baseline (M = -2.31, SD = 2.06). A similar pattern was observed across waves with the lowest average loneliness reported by the Danish samples (M = -2.79, SD = 1.81), followed by the U.S. samples (M = -2.35, SD = 1.99), with the Swedish samples reporting the highest levels of loneliness on average (M = -2.25, SD = 2.18). Of note, other research has shown differences in traits related to loneliness in Sweden and Denmark consistent with this pattern (Christensen, Herskind, & Vaupel, 2006).

Statistical Analyses

Baseline loneliness, time-varying loneliness and geometric means for longitudinal loneliness as predictors of cognitive performance and change.

The multilevel model for change (Singer & Willett, 2003) was fitted to the longitudinal data for each cognitive task to assess the extent to which baseline loneliness, time-varying loneliness, and geometric means for loneliness across waves each predicted performance at age 65 (intercept) or change over time (slope) in processing speed, spatial ability, and verbal ability using SAS software version 9.4 (SAS Inc., Cary, NC). Baseline loneliness was operationalized as the loneliness person measure score from the first wave at which each participant had scores for loneliness, depression, and social isolation. Baseline depression and social isolation were operationalized as (a) each participant's harmonized depression score from this wave (without the loneliness items), and (b) each participant's score for the marital status and living arrangement items from this wave, respectively. Time-varying loneliness referred to each participant's loneliness person measure scores at each wave for which they had loneliness data, and geometric means for loneliness were computed using SAS version 9.4 (SAS Inc., Cary, NC) by first adding 6 to each person's loneliness person measure scores at each wave (which ranged from -5.81 to 6.89 across waves) so that all scores were positive, then using these scores to compute geometric means for each participant, and finally, subtracting 6 from computed geometric means to convert scores back to their original scale. The geometric mean is computed similarly to the arithmetic mean with the exception that multiplication is used in place of addition (i.e., all values are multiplied rather than summed) and the nth root is taken of

the product (where *n* = the number of values) in place of dividing by *n* (Roenfeldt, 2018). For model-fitting analyses, age was centered at 65 years. This was done to improve interpretability of intercepts and increase model stability by centering at an age at which data were abundant and which best accommodated patterns of change in the longitudinal cognitive data across the cognitive tasks. All models accounted for nesting of individuals within twin pairs and nesting within individuals.

Unconditional models.

For each cognitive task, a series of unconditional models was fitted to determine whether an unconditional means model (Model A), an unconditional linear model (Model B), an unconditional quadratic model (Model C1) or an unconditional spline model with two slopes (prior to centering age and at or after centering age; Model C2) provided the best fit to the data. Each unconditional model was fitted both including and excluding a fixed-effect term accounting for practice effects (coded such that each participant had a score of 0 at their first wave of data collection and a score of 1 at each subsequent wave) to assess whether adding a term for practice effects (a) decreased the model's residual variance, suggesting that practice effects may be adjusted for in the model or (b) increased the model's residual variance, indicating potential overfitting when the practice term was included. Chi-square difference tests were conducted to choose the best-fitting unconditional model for each cognitive task.

Conditional models.

For each cognitive task, the unconditional quadratic model or unconditional spline model provided better fit than the unconditional linear model. Both quadratic and spline

conditional models were fitted for each task. Conditional models were fitted with (a) level-2 covariates (Model D), and (b) level-2 covariates and loneliness (baseline, Model E1; time-varying, Model E2; or geometric mean, Model E3). For models for which effects of loneliness emerged, sensitivity analyses were conducted adjusting for educational attainment. Given that four IGEMS studies had 2 or fewer waves of data and four studies had more than 2 waves of data, we evaluated whether models supported inclusion of a term adjusting for practice effects. For models with effects of loneliness for which the unconditional models indicated that practice effects could be added to the model without increasing the residual variance, sensitivity analyses were also conducted adjusting for practice effects. Each conditional model is briefly described below.

Conditional model with covariates added (model D).

For each task, fixed effects for all covariates except educational attainment (i.e., sex, baseline depressive symptoms, marital status, living arrangement, and country of residence) and their interactions with age were added to the unconditional quadratic and unconditional spline models. Sex was effects coded such that males = -.5 and females = .5, as females reported higher levels of loneliness in the overall sample. Country of residence was effects coded such that Denmark = -.5, the United States = 0, and Sweden = .5, as Danish participants reported the lowest levels of loneliness and Swedish participants reported the highest levels of loneliness. Educational attainment was centered at 12 years, as this value was close to the average years of education for the analysis sample (M = 11.02, SD = 3.70) and corresponds with a high-school level education in the United States. Depression scores were not centered, as 0 was a possible score on the

harmonized depression scale, and scores of 0 were observed within the analysis sample. Chi-square difference tests were used to test for significant improvements in model fit for model D in comparison to the corresponding unconditional model.

Conditional models with covariates and loneliness added (models E1, E2, E3).

Fixed-effects terms for baseline loneliness, time-varying loneliness, or loneliness geometric means and interaction terms for loneliness with age were next added to model D to (a) test whether adding loneliness to the model significantly improved model fit, (b) assess effects of loneliness on performance and change across age for each cognitive task, and (c) to compare observed effects of baseline loneliness, time-varying loneliness, and loneliness geometric means on cognition. Participants with a single wave of data were included in Model E3 (with geometric means for loneliness equal to their baseline loneliness person measure score) so that Model E3 could be directly compared to other models within tasks.

Effect sizes similar to Cohen's *d* were computed for baseline loneliness, time-varying loneliness, and loneliness geometric means for the quadratic and spline models to quantify the difference in change in cognitive performance between ages 65 and 80 associated with loneliness for each model for each cognitive task. The formula used to compute each effect size was: model-based predicted differences in cognition between ages 65 and 80 ($\Delta_{80\text{-}65}$) for high lonely – predicted differences in cognition between ages 65 and 80 ($\Delta_{80\text{-}65}$) for low lonely divided by the standard deviation of the outcomes (*SD*) (Feingold, 2009), which was equal to 10 as cognitive scores were on a *T*-scale. For computation of effect sizes, high loneliness was defined as a person measure score of

5.295, while low loneliness was defined as a score of -3.315. These scores were selected by averaging person measure scores corresponding to the highest levels of loneliness for the CESD (5.77) and CAMDEX (4.82) loneliness items and the lowest levels of loneliness on the CESD (-3.14) and CAMDEX (-3.49) items, as each IGEMS study administered one of these measures.

Results

Descriptive Statistics

Loneliness.

At baseline, loneliness person measure scores ranged from -5.81 to 6.04 (M = -2.68, SD = 1.85) for the analysis sample; most participants reported low levels of loneliness (see Table 2.3). Across waves, loneliness person measure scores ranged from -5.81 to 6.89. The average score across waves was similar to that at baseline, with a slightly higher standard deviation (M = -2.59, SD = 1.95). Geometric means for longitudinal loneliness ranged from -5.81 to 5.78 (M = -2.71, SD = 1.52).

Loneliness by age and sex.

Examining average loneliness person measure scores at baseline separately for those >50 years, 50-59 years, 60-69 years, 70-79 years, 80-89 years, and 90+ years within the analysis sample revealed a pattern of decreasing average loneliness with age from 25-49 years through ages 60-69 and increasing loneliness thereafter (See Table 2.3).

At baseline, females (M = -2.57, SD = 1.97) reported significantly greater loneliness than males (M = -2.80, SD = 1.70, $t(13,112) = -7.182$, $p < .001$) on average. This was also observed across waves (M = -2.43, SD = 2.09 for females; M = -2.76, SD =

1.78 for males, $t(13,112) = -8.516$, $p < .001$). Geometric means for loneliness showed a similar pattern with females having significantly higher geometric means for loneliness on average ($M = -2.56$, $SD = 1.72$) than males ($M = -2.80$, $SD = 1.50$), $t(13,112) = -8.516$, $p < .001$.

Cognition.

Processing speed (Symbol Digit task).

Symbol Digit scores showed a general trend of decline with age, both within and between persons. The longitudinal trajectory plot for Symbol Digit *T*-scores across age for the analysis sample (see Figure 2.1a) illustrates this trend along with individual differences in change over time for this task. Examination of average Symbol Digit *T*-scores for six age groups (<50, 50-59, 60-69, 70-79, 80-89, and 90+) at baseline revealed that, for the analysis sample, reduced performance was also associated with higher age cross-sectionally (see Table 2.4).

Spatial ability (Block Design task).

Block Design scores also showed both cross-sectional and longitudinal trends of age-associated decrease and decline. The longitudinal trajectory plot for Block Design (see Figure 2.2a) shows a general trend of within-person decline with age, especially after age 65. Cross-sectionally, Block Design scores at baseline also showed a pattern of decrease with age (see Table 2.4).

Working memory (Digits Backward task).

Individual differences in within-person change in Digits Backward scores with age are shown in Figure 2.3 for the analysis sample. For this task, scores showed a

different pattern of change across age than was observed for the Symbol Digit and Block Design tasks, with more stable performance observed before age 75 and more decline observed after age 75. Cross-sectionally, a similar pattern was observed for the analysis sample at baseline with scores showing a pattern of decrease with age after age 59 and stability prior to age 60 (see Table 2.4).

Verbal comprehension (Synonyms task).

In general, scores on the Synonyms task showed stability across age, with increasing variability with age after age 70 and a higher frequency of lower scores observed after age 70 than prior to age 70. This pattern is illustrated in the longitudinal trajectory plot for Synonyms scores in Figure 2.4a. Scores on this task showed more cross-sectional stability across age than scores on the other three cognitive tasks (see Table 2.4).

Covariates.

Baseline social isolation.

Marital status.

At baseline, n = 9,457 (72.11% of the analysis sample) reported that they were married or cohabitating and n = 3,657 (27.89%) reported that they were not married or cohabitating.

Living arrangement.

At baseline, n = 9,889 (75.41% of the analysis sample) reported that they lived with at least one other person and 3,225 (24.59%) reported that they lived alone.

Baseline depressive symptoms.

Baseline harmonized depression scores ranged from 0 to 46.92 (M = 7.05, SD = 7.23). Most participants reported low levels of depression.

Educational attainment.

Educational attainment data were available for n = 5,682 participants from the Swedish and American studies and LSADT. For this subsample, the range of years of education attained was 0 (n = 1) to 25. The average number of years of education attained was 11.02 (SD = 3.70). Descriptive statistics for covariates are summarized in Table 2.3.

Correlations.

Table 2.5 lists correlations between study variables at baseline. Partial correlations were computed adjusting for age, sex, and country of residence (except where one of these variables was being correlated). Loneliness was not associated with age (r = -.01, p = .2172) or sex (ρ = .01, p = .2761). The strongest association for loneliness and cognition was observed for Block Design, which was weakly negatively correlated with loneliness at baseline (r = -.14, p < .0001). Associations were smaller in magnitude for Symbol Digit and Synonyms (r = -.06, p < .0001 and r = -.06, p = .0003 respectively) and even smaller for Digits Backward (r = -.03, p = .0071). The association between loneliness and depressive symptoms was strong and positive (r = .48, p < .0001); there was no association between loneliness and educational attainment (r = -.01, p = .4088). A positive moderate to strong correlation was observed between loneliness and country of residence (ρ = .40, p < .0001).

Scores on each of the Symbol Digit, Block Design, and Digits Backward tasks were weakly positively correlated with country of residence (range$_\rho$ = .06 to .10, p < .0001). All Synonyms data were from Swedish studies. Scores on each of the four cognitive tasks were moderately positively associated with educational attainment (range$_r$ = .20 to .35, p < .0001). Symbol Digit and Synonyms scores were weakly negatively associated with marital status (ρ = -.06 and ρ = -.08, p < .0001, respectively), as were Block Design scores (ρ = -.06, p = .0061). A weaker negative association was found between Digits Backward scores and marital status (ρ = -.02, p = .0246). Scores on the Symbol Digit and Synonyms tasks were weakly negatively associated with living arrangement (ρ = -.06 and ρ = -.07, p < .0001, respectively), while scores on the Block Design and Digits Backward tasks were not associated with living arrangement (ρ = -.03, p = .1106 and ρ = -.02, p = .1130, respectively). Depressive symptoms were weakly to moderately associated with Symbol Digit and Block Design scores (r = -.15 and r = -.17, p < .0001, respectively), and weakly negatively associated with Digits Backward scores (r = -.08, p < .0001). A weak negative association at trend significance was found between Synonyms scores and depressive symptoms (r = -.03, p = .0758).

Model-Fitting Analyses

Loneliness and processing speed (Symbol Digit).

Figure 2.1b shows expected trajectories for the unconditional quadratic and spline models and observed scores across age for the Symbol Digit task. The unconditional spline model ($\Delta\chi^2(7)$ = 234.8, p < .0001) initially fit better than the unconditional quadratic model ($\Delta\chi^2(7)$ = 229.2, p < .0001). After dropping covariance parameters from

the unconditional quadratic and spline models that hit a boundary of 0 (i.e., the random effect term for individuals within twin pairs for the quadratic effect in quadratic models and the random effect term for individuals within twin pairs for the linear age term at or after age 65 in spline models), the unconditional quadratic model ($\Delta\chi^2(4) = 227.1$, $p < .0001$) fit better than the unconditional spline model ($\Delta\chi^2(4) = 202.1$, $p < .0001$).

Including practice effects improved fit for both the unconditional quadratic and spline models ($\Delta\chi^2(1) = 236.4$ and 255.7, $p < .0001$, respectively), with a regression weight of $b = 2.03$ ($p < .0001$) for practice effects for the quadratic model and a regression weight of $b = 2.09$ ($p < .0001$) for the spline model. While there appeared to be an overall effect of practice in both models, inclusion of the term increased the residual variance for the unconditional quadratic model (from 27.98 to 28.02) and decreased the residual variance for the spline model (from 28.47 to 28.46) suggesting potential overfitting when the term was included for the quadratic model and that a term for practice may be added to the spline model. Sensitivity analyses adjusting for practice effects were therefore only conducted for spline models for this task. For brevity and consistency across tasks, results are reported here for the quadratic model; see Appendix 3 for spline model results.

Adding covariates to the unconditional quadratic model significantly improved model fit ($\Delta\chi^2(15) = 550.8$, $p < .0001$). Adding baseline loneliness to the model with covariates improved fit at trend significance ($\Delta\chi^2(3) = 7.2$, $p = .0658$). Adding geometric means for loneliness to the model significantly improved fit ($\Delta\chi^2(3) = 16.9$, $p = .0007$). Higher loneliness scores were associated with higher performance on the Symbol Digit

task at age 65. This effect was strongest for geometric means for loneliness (b = .20, p = .0294), followed by baseline loneliness (b = .19, p = .0148) and time-varying loneliness (b = .10, p = .0384). After adjusting for educational attainment, the effect of baseline loneliness on the intercept was attenuated and non-significant (b = .15, p = .2238), and effects of time-varying loneliness (b = .12, p = .0845) and loneliness geometric means (b = .21, p = .1402) were slightly larger in magnitude, but were trend significant and non-significant, respectively. Time-varying loneliness and loneliness geometric means were negatively associated with linear slope (b = -.01, p = .016 and b = -.01, p = .01, respectively), suggesting that higher scores on these loneliness measures were associated with faster linear decline in Symbol Digit scores with age. Effects were similar after adjusting for educational attainment (b = -.009, p = .1148 for time-varying loneliness; b = -.01, p < .0757 for loneliness geometric means) although these effects were non-significant and trend significant, respectively. No effect of baseline loneliness on linear slope was observed. Loneliness geometric means were negatively associated with quadratic slope (b = -.0008, p = .022), suggesting an association between higher geometric means for loneliness and faster acceleration in linear change in Symbol Digit scores with age. This effect was slightly larger in magnitude after adjusting for educational attainment (b = -.001, p = .0061). A negative effect of time-varying loneliness on quadratic slope also emerged after adjusting for education (b = -.0006, p = .0326) suggesting a relation between higher loneliness scores and faster acceleration in linear decline in Symbol Digit scores with age. See Tables 2.6 and 2.7 for modeling results for quadratic models for Symbol Digit.

Effect sizes (*d*) were computed for each of the quadratic models (i.e., Models E1, E2, and E3) to quantify the difference in change between ages 65 and 80 (15 years elapsed) associated with loneliness for the Symbol Digit task. The effect was smaller in magnitude for baseline loneliness (d = -0.14) and time-varying loneliness (d = -0.19); the largest effect (d = -0.34) was observed for loneliness geometric means. These effect sizes suggest small to moderate negative effects of loneliness on change in Symbol Digit scores between ages 65 and 80, with faster decline associated with loneliness across this age range. Figure 2.5 shows predicted change in Symbol Digit scores across age for high, intermediate, and low loneliness.

Loneliness and spatial ability (Block Design).

Figure 2.2b shows expected trajectories for the unconditional quadratic and spline models and observed scores across age for the Block Design task. For this task, the unconditional quadratic model provided the best fit to the data. Compared to the unconditional linear model, the quadratic model had a greater reduction in -2 log likelihood ($\Delta\chi^2(7) = 116.1$, $p < .0001$) than did the spline model ($\Delta\chi^2(7) = 100.3$, $p < .0001$). Adding a term for practice effects improved fit for both the unconditional quadratic ($\Delta\chi^2(1) = 42.6$, $p < .0001$) and spline ($\Delta\chi^2(1) = 41.9$, $p < .0001$) models, with regression weights for practice of 1.22 and 1.18 ($p < .0001$), respectively. Although there appeared to be similar effects of practice for both the quadratic and spline models, adding practice effects to these models slightly increased each model's residual variance, indicating potential overfitting when the term was included. Therefore, practice effects

were excluded from conditional quadratic and spline models. Results are reported here for quadratic models; see Appendix 3 for spline model results.

Adding covariates to the unconditional quadratic model significantly improved model fit ($\Delta\chi^2(15) = 161.5$, $p < .0001$), Subsequently adding loneliness to this model also significantly improved model fit (for baseline loneliness, $\Delta\chi^2(3) = 10.5$, $p = .0148$; for loneliness geometric means, $\Delta\chi^2(3) = 12.5$, $p = .0058$). No effects of loneliness on intercept were found. A trend significant effect of loneliness geometric means on linear slope was observed ($b = -.01$, $p = .0982$), suggesting that this loneliness measure was associated with slightly faster linear decline in Block Design scores with age; no effects were observed on linear slope for baseline or time-varying loneliness. Effects of loneliness on quadratic change across age were observed for baseline ($b = -.0007$, $p = .025$) and time-varying loneliness ($b = -.0007$, $p = .0035$), suggestive of an association between higher levels of loneliness and faster acceleration in decline in Block Design scores across age. No effect of loneliness geometric means on quadratic change was found. For baseline and time-varying loneliness, regression weights remained the same and *p*-values were similar after adjusting for educational attainment. See Tables 2.8 and 2.9 for modeling results for quadratic models.

Effect sizes (*d*) for Block Design quadratic models showed a small negative effect of baseline loneliness on change in Block Design scores between ages 65 and 80 ($d = -0.21$). The effect was smaller in magnitude for time-varying loneliness ($d = -0.10$), and larger in magnitude for loneliness geometric means ($d = -0.25$). These effect sizes suggest small negative effects of loneliness on change in Block Design scores between ages 65

and 80, with faster decline associated with loneliness across this age range. As was seen for Symbol Digit, the effect was largest in magnitude for loneliness geometric means. Predicted change in Block Design scores across age associated with high, intermediate, and low loneliness is illustrated in Figure 2.6.

Loneliness and working memory (Digits Backward).

Descriptive statistics for loneliness and Digits Backward scores revealed that the association between loneliness and performance on this task was very small (r = -.03, p = .0071) at baseline. Heterogeneity in patterns of responding across IGEMS studies was also observed for this task which may have resulted from differences in how the task was administered within individual studies. Therefore, we did not proceed with model-fitting analyses for this task.

Loneliness and verbal comprehension (Synonyms).

Figure 2.4b shows expected trajectories for the unconditional quadratic and spline models and observed scores across age for the Synonyms task. The unconditional quadratic model ($\Delta\chi^2(7) = 112.9$, $p < .0001$) initially fit the data better than the unconditional spline model ($\Delta\chi^2(7) = 52.9$, $p < .0001$). After dropping covariance parameters from the unconditional quadratic and spline models that hit a boundary of 0 in the unconditional models (i.e., random effect terms for centered linear age (AgeC) for both twin pairs and individuals within twin pairs for the quadratic model and random effect terms for linear change across age prior to age 65 (AgeC65A) for twin pairs and linear change at or after age 65 (AgeC65B) for twin pairs for the spline model), the unconditional spline model ($\Delta\chi^2(4) = 49.8$, $p < .0001$) provided the best fit to the data and

the unconditional quadratic model fit significantly worse than the unconditional linear model ($\Delta\chi^2(1) = -22.3$, $p = .0002$).

Adding a term for practice effects to the unconditional quadratic model significantly improved model fit ($\Delta\chi^2(1) = 21.1$, $p < .0001$), and a positive effect of practice was observed ($b = .77$, $p < .0001$). Adding a term for practice effects to the model resulted in a slight increase in the model's residual variance, indicating potential overfitting when the term was included. Practice effects were therefore excluded from quadratic models for this task.

Adding covariates to the unconditional quadratic model resulted in a significant improvement in model fit ($\Delta\chi^2(15) = 43.2$, $p < .0001$); adding loneliness to the model with covariates did not ($\Delta\chi^2(2) = 2.7$, $p = .2592$ for baseline loneliness and $\Delta\chi^2(2) = 2.3$, $p = .3166$ for loneliness geometric means). No effects of loneliness on Synonyms performance at age 65 (i.e., intercept) were observed. After adjusting for education, a trend significant of effect of time-varying loneliness on the intercept emerged ($b = -0.10$, $p = .0841$). A negative effect of loneliness on quadratic change was found for time-varying loneliness ($b = -0.0004$, $p = .0376$), with higher loneliness scores associated with faster acceleration in decline in Synonyms scores. This effect was attenuated and non-significant after adjusting for educational attainment, ($b = -0.00028$, $p = .1838$), and was attenuated after adjusting for practice effects ($b = -0.00035$, $p = .0779$). See Tables 2.10 and 2.11 for modeling results for quadratic models for Synonyms.

Effect sizes (*d*) for Synonyms quadratic models indicated that effects of loneliness on change in Synonyms performance across ages 65 to 80 were small. For baseline

loneliness, the effect was positive (d = 0.01), while for time-varying loneliness and loneliness geometric means effects were negative (d = -0.08 and d = -0.03, respectively). These effect sizes suggest that effects of loneliness on change in Synonyms performance between age 65 and age 80 are minimal. Effect sizes (d) for loneliness on change in cognitive scores between ages 65 and 80 for each cognitive task are shown in Table 2.12 for quadratic models. Figure 2.7 shows predicted change in Synonyms scores across age for high, intermediate, and low loneliness.

Discussion

This study explored longitudinal relations between feelings of loneliness and four specific cognitive abilities (processing speed, spatial ability, working memory, and verbal comprehension) in mid to late adulthood in a large multinational sample with up to 28 years of follow-up from eight studies participating in the IGEMS Consortium. Prior work on loneliness and cognition suggests that loneliness is associated with poorer performance and faster decline in both global cognition (Holwerda et al., 2014; O'Luanaigh et al., 2012; Tilvis et al., 2004; Wilson et al., 2007; Zhong, Chen, Tu, & Conwell, 2017) and specific domains of cognitive functioning (Donovan et al., 2017; O'Luanaigh et al., 2012; Shankar et al., 2013; Wilson et al., 2007), and elevated dementia risk (Rafnsson et al., 2017; Wilson et al., 2007). Our efforts sought to confirm findings and further elucidate how loneliness relates to performance and change within specific cognitive domains.

A primary objective of this study was to compare effects of baseline versus longitudinal loneliness on cognitive performance and change. Longitudinal loneliness

was operationalized as time-varying loneliness scores across waves and as geometric means for loneliness across waves which captured the relative endurance of loneliness and was less influenced by single fluctuations than the arithmetic average. Assessment of how these different loneliness measures relate to late-life cognition within a single study has the potential to aid in elucidation of how different measures of loneliness (i.e., baseline scores versus individual scores across waves or average scores across waves) relate to cognitive outcomes, and whether patterns of associations vary among different cognitive domains. In the present study, baseline loneliness, time-varying loneliness, and geometric means for loneliness were each added to separate longitudinal growth models for each cognitive domain. Based on prior findings linking baseline loneliness (Holwerda et al., 2012; O'Luanaigh et al., 2012; Shankar et al., 2013; Tilvis et al., 2004; Wilson et al., 2007) and measures of longitudinal loneliness (Wilson et al., 2007; Zhong et al., 2016) to adverse cognitive outcomes, it was hypothesized that both baseline and longitudinal loneliness would be associated with poorer cognitive performance and/or faster cognitive decline for the domains assessed. It was predicted that associations would be strongest for processing speed and spatial ability, as prior unpublished cross-sectional work on loneliness and cognition using an overlapping IGEMS sample showed higher cross-sectional correlations for these domains than for working memory and verbal comprehension (Phillips & Reynolds, 2016), and limited longitudinal work suggests an association between loneliness and both poorer performance and faster decline for processing speed and spatial ability, with effects on performance only for other domains, including working memory (Wilson et al., 2007). Based on recent work suggesting that

longer periods of loneliness may be associated with worse global cognitive functioning than shorter periods of loneliness (Zhong et al., 2016), it was hypothesized that associations would be larger in magnitude for longitudinal measures of loneliness than for baseline loneliness. As associations were expected to be strongest for processing speed and spatial ability, it was hypothesized that this pattern of results would be most prominent for tasks assessing these domains.

Overall, results showed small effects of loneliness on cognition that varied across cognitive domains, with faster processing speed at age 65 and faster decline in processing speed, spatial ability, and verbal comprehension (prior to adjusting for education) associated with loneliness. Effects of loneliness tended to be on change rather than level (with the exception of the positive effects on performance at 65 observed for processing speed), and greater loneliness was associated with somewhat faster acceleration in linear decline with age for all three domains, although this effect was attenuated for verbal comprehension in sensitivity analyses. Effects of loneliness on cognition were observed adjusting for two indices of objective social isolation, suggesting that feeling lonely contributed to worse cognitive outcomes independently of objective isolation. This finding aligns with prior work indicating that lower perceived relationship quality and feelings of loneliness are uniquely associated with poorer cognition (Amieva et al., 2010; Holwerda et al., 2012; Wilson et al., 2007). Effects of loneliness also largely endured adjusting for education. Patterns of effects for the different loneliness measures on cognition varied across cognitive domains, suggesting that the loneliness measure used for analysis of associations between loneliness and cognitive performance and change has

important implications for study results. Further work is needed to understand how different patterns of loneliness across age relate to performance and change in specific domains of cognition. Additional work assessing such relations between loneliness and performance and change in other cognitive domains is also essential (e.g., episodic memory, executive functioning).

The prediction that baseline and longitudinal loneliness would each predict poorer cognition or faster cognitive decline was supported by results for spatial ability, but not by those for processing speed, verbal comprehension, or working memory. For processing speed, baseline loneliness was associated with better cognitive performance at age 65, while longitudinal measures of loneliness were associated with faster cognitive decline. For verbal comprehension, effects of loneliness on cognition were only observed for time-varying loneliness, and for working memory, task performance was not associated with loneliness in preliminary analyses.

As no association was found between loneliness and working memory and verbal comprehension was minimally associated with loneliness in this study, results aligned with the prediction that effects of loneliness on cognition would be strongest for processing speed and spatial ability. Standardized effects of loneliness on change in performance between ages 65 and 80 were also strongest for these domains. The prediction that effects of longitudinal loneliness on cognition would be stronger than those for baseline loneliness was supported by results for verbal comprehension, with small negative effects on level and change observed only for time-varying loneliness. This prediction was not fully supported, however, by results for processing speed and

spatial ability. Effects of baseline loneliness and geometric means for loneliness on processing speed performance at age 65 were similar, while the effect of time-varying loneliness was smaller. Effects on slope for this domain followed the predicted pattern, with effects on change observed only for longitudinal loneliness. For spatial ability, effects of loneliness on linear change followed the expected pattern, with an effect only observed for loneliness geometric means, while effects of loneliness on quadratic change did not, with similar effects observed for baseline and time-varying loneliness and no association for loneliness geometric means. Standardized effects of loneliness on cognitive change between ages 65 and 80 revealed larger effects for longitudinal loneliness for processing speed and verbal comprehension than for baseline loneliness, however for spatial ability, a deviation from this pattern was noted, with larger effects for baseline loneliness and loneliness geometric means than for time-varying loneliness.

The finding that higher loneliness was associated with faster age-related decline in processing speed and spatial ability was consistent with prior work showing accelerated decline across time associated with loneliness for these domains (Wilson et al., 2007). Positive effects of loneliness on processing speed performance at age 65, however, were inconsistent with prior results showing reduced performance on tasks tapping processing speed in both cross-sectional and time-based longitudinal analyses (O'Luanaigh et al., 2012; Wilson et al., 2007). This discrepancy in findings may stem from differences in how these associations were assessed—the present study used age-based analyses, with intercepts representing performance at age 65, while prior work estimated effects of loneliness on processing speed performance at baseline (Wilson et

al., 2007) or cross-sectionally (O'Luanaigh et al., 2012). The lack of an association between loneliness and scores on the working memory task did not align with prior work suggesting a link between higher loneliness and reduced working memory performance (Wilson et al., 2007).

The present results suggested that associations between loneliness and domain-specific cognitive performance and change were small, and most often observed for change rather than level at age 65. Consistent with our results, small effects of loneliness on performance (O'Luanaigh et al., 2012; Wilson et al., 2007) and change (Wilson et al., 2007) have been previously reported for processing speed (O'Luanaigh et al., 2012; Wilson et al., 2012) and spatial ability (Wilson et al., 2007). Limited prior work has consistently suggested, however, that loneliness is associated with reduced performance for these domains. As noted above, these discrepancies in findings may result from the use of age-based analyses in the present study. Prior work assessing associations between subtypes of longitudinal loneliness (across 2 time points) and global cognitive function suggests that associations may be stronger for longitudinal measures of loneliness than for baseline loneliness. The results of the present study suggest that patterns of effects for baseline and longitudinal measures of loneliness may vary for different specific cognitive domains and for different measures of longitudinal loneliness.

Strengths of this study included the use of a large, multinational sample with up to 28 years of follow-up to explore relations between loneliness and domain-specific cognitive performance and change, assessment of effects of both baseline and longitudinal loneliness on cognition within a single study, and comparison of effects for

baseline and longitudinal loneliness for multiple domains of cognitive functioning. One limitation of this study involved heterogeneity in the number of waves of data collected across IGEMS studies, which ranged from 1 to 10 and likely adversely impacted model stability. Another limitation was the use of a single cognitive task from each cognitive domain for analyses. Although additional measures assessing these domains are available for individual IGEMS studies, lack of overlap across studies selected for analysis resulted in use of single tasks for each domain. The number and nature of loneliness items asked also varied among studies—some studies asked a single, direct item assessing loneliness, while others asked multiple items which varied in terms of how directly they asked participants about loneliness. Loneliness person measure scores were likely more accurate for studies with more loneliness items, not only because they were based on responses to multiple items, but also because items that asked more directly about loneliness may have been more likely to yield responses susceptible to social desirability than less direct items (Victor, Grenade, & Boldy, 2005). Moreover, such items may be interpreted differently from individuals from different cultures (Victor et al., 2005). Finally, heterogeneity across studies in the number of waves and time between waves limited how longitudinal loneliness could be characterized, and long periods of time between waves limited our ability to clearly differentiate transient loneliness from other types of loneliness.

Future studies can build on these and other findings on effects of longitudinal loneliness and cognition (Wilson et al., 2007; Zhong et al., 2016) by assessing how different patterns of loneliness across time relate to cognitive outcomes. To truly

distinguish between effects of transient loneliness from those of longer-term loneliness, or to examine the duration of loneliness, loneliness should be assessed frequently across shorter periods of time as well as across longer periods of time (e.g., measurement burst design; Nesselroade, 1991; Sliwinski, 2008). Such work can further understanding of whether loneliness that is truly short-term is associated with adverse cognitive outcomes, how duration of loneliness relates to cognition, whether effects of loneliness on cognition might lessen or subside once loneliness is overcome, and how different patterns of loneliness (e.g., intermittent loneliness, chronic intense loneliness, chronic moderate loneliness) relate to cognitive performance, cognitive change, and dementia risk.

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Table 2.1

Measures of Cognitive Performance Given at Each Wave in Each IGEMS Study

	Study/Wave	1	2	3	4	5	6	7	8	9	10
Processing Speed	SATSA	●	●	●	●	●	●	●	●	●	●
	OCTO-Twin	●	●	●	●	●					
	GENDER	●	●	●							
	MTSADA	♦									
	LSADT	--	--	●	●	●	●				
	MADT	●	●								
Spatial Ability	SATSA	●	●	●	●	●	●	●	●	●	●
	OCTO-Twin	●	●	●	●	●					
	GENDER	●	●	●							
	MTSADA	♦									
Working Memory	SATSA	●	●	●	●	●	●	●	●	●	●
	OCTO-Twin	●	●	●	●	●					
	VETSA	●	●								
	LSADT	●	●	●	●	●	●				
	MADT	●	●								
Verbal Comprehension	SATSA	●	●	●	●	●	●	●	●	●	●
	OCTO-Twin	●	●	●	●	●					
	GENDER	●	●	●							
	TOSS	●									
	VETSA	●	●								

Note. Processing speed: Symbol Digit task = •, Digit Symbol task = ♦; spatial ability: Koh's Block Design = •, WAIS-R Block Design = ♦; working memory: Digits Backward task = •; verbal comprehension: Synonyms task = •

Table 2.2.

Demographic Information for the IGEMS Sample and Each IGEMS Study

	IGEMS	SATSA	OCTO-Twin	GENDER	TOSS	LSADT	MADT	VETSA	MTSADA
Overall Sample									
N	15,302	859	702	498	1,602	4,731	4,314	1,237	1,359
N_{MZ}	5,703	340	298	---	694	1,489	1,459	699	724
($N_{Complete\ Pairs}$)	(2,407)	(163)	(149)	---	(314)	(436)	(664)	(348)	(333)
N_{SSDZ}/N_{OSDZ}	7,124/2,170	516/0	404/0	0/498	904/0	2,728/224	1,401/1,448	538/0	633/0
($N_{Complete\ SSDZ/OSDZ\ Pairs}$)	(2,684/887)	(246/0)	(202/0)	(0/249)	(416/0)	(666/21)	(600/617)	(266/0)	(288/0)
N_{UZ}	305	3	---	---	4	290	6	---	2
($N_{Complete\ Pairs}$)	(36)	(1)	---	---	(2)	(29)	(3)	---	(1)
Age Range	25-102	39-87	79-97	69-80	32-59	70-102	46-68	51-60	25-92
Age *M*	64.33	63.56	83.58	74.52	44.84	77.74	56.88	55.88	58.68
(*SD*)	(13.39)	(8.82)	(3.17)	(2.64)	(4.86)	(5.66)	(6.34)	(2.48)	(10.73)
%	51.8%	59.6%	66.7%	50.0%	62.9%	58.9%	49.0%	0.0%	58.0%
(*n*) female	(7,373)	(512)	(468)	(249)	(1,004)	(2,788)	(2,116)	(0)	(788)
Analysis Sample									
N	13,114	768	469	440	1,587	3,628	4,227	1,218	777
N_{MZ}	4,952	302	210	---	690	1,168	1,434	687	461
($N_{Complete\ Pairs}$)	(2,052)	(141)	(84)	---	(312)	(325)	(646)	(339)	(204)
$N_{SSDZ/OSDZ}$	5,979/1,968	465/0	259/0	0/440	893/0	2,147/109	1,368/1,419	531/0	316/0
($N_{Complete\ SSDZ/OSDZ\ Pairs}$)	(2,172/796)	(214/0)	(89/0)	(0/196)	(407)/0	(486/5)	(575/595)	(259/0)	(142/0)
N_{UZ}	215	1	---	---	4	204	6	---	---
($N_{Complete\ Pairs}$)	(25)	(0)	---	---	(2)	(20)	(3)	---	---
Age Range	25-101	44-89	79-97	69-80	32.59	75-101	46-68	51-64	25-86
Age *M*	62.69	64.46	83.16	74.46	44.82	76.77	56.86	55.91	55.05
(*SD*)	(13.03)	(8.79)	(2.81)	(2.63)	(4.86)	(5.04)	(6.33)	(2.51)	(12.56)
%	50.5%	59.8%	65.0%	50.2%	62.9%	57.6%	49.1%	0.0%	60.9 %
(*n*) female	(6,621)	(459)	(305)	(221)	(998)	(2,090)	(2,075)	(0)	(473)

Note. Age statistics reflect baseline. Analysis sample consists of all who will be included in analyses (i.e., have data for study variables and at least one cognitive measure at $\geq$ one waves; observations where low MMSE or dementia status observed have been dropped. MZ = monozygotic, SSDZ = same-sex dizygotic, OSDZ = opposite-sex dizygotic, UZ = unknown zygosity

Table 2.3

Descriptive Measures of Baseline Covariates

Baseline	*N*	*M*	*SD*	*Min*	*Max*
Age	13114	62.69	13.03	25	101
Sex	13114	.005	.500	-.5	.5
Marital Status	13114	.25	.431	0	1
Living Arrangement	13114	.28	.448	0	1
Depressive Symptoms	13114	7.05	7.23	0	46.92
Educational Attainment	5682	11.02	3.70	0	25
Baseline Loneliness	13114	-2.68	1.85	-5.81	6.04
Loneliness Geometric Mean	13114	-2.71	1.52	-5.81	5.78

Table 2.4

Descriptive Measures of Loneliness and Cognitive Measures by Age Group

Baseline	***<50 years***		***50-59 years***		***60-69 years***		***70-79 years***		***80-89 years***		***90+ years***	
	N = 237 to 2,257		*N* = 384 to 3,840		*N* = 275 to 2,210		*N* = 616 to 3,426		*N* = 414 to 1,299		*N* = 7 to 82	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Loneliness	-2.40	1.80	-2.66	1.76	-2.94	1.55	-2.82	1.90	-2.51	2.31	-2.00	2.66
Loneliness GM	-2.37	1.73	-2.62	1.53	-2.70	1.38	-2.84	1.44	-2.75	1.61	-2.59	1.67
Symbol Digit	62.75	11.27	57.13	9.70	52.11	10.19	45.09	10.75	38.44	10.85	26.73	7.47
Block Design	61.64	10.36	57.50	10.03	52.75	10.75	49.60	9.193	42.65	10.21	37.92	7.02
Digits Backward	52.44	9.99	52.97	11.04	50.48	9.75	48.86	9.53	46.51	9.54	42.09	10.12
Synonyms	55.20	8.44	56.57	8.78	51.81	10.09	51.98	10.07	46.62	11.96	51.32	10.28

Note. GM = geometric mean.

Table 2.5.

Correlations Between Key Study Variables at Baseline

	Symbol Digit *r*(*n*)	**Block Design** *r*(*n*)	**Digits Backward** *r*(*n*)	**Synonyms** *r*(*n*)	**Baseline Loneliness** *r*(*n*)	**Time-Varying Loneliness** *r*(*n*)	**Loneliness Geometric Mean** *r*(*n*)
Baseline Loneliness	-.06*** (7,853)	-.14*** (2,245)	-.03** (10,302)	-.06** (3,171)	1.00 (13,114)	.98*** (13,109)	.86*** (13,114)
Time-Varying Loneliness	-.06*** (7,849)	-.14*** (2,245)	-.03** (10,297)	-.06** (3,171)	.98*** (13,109)	1.00 (13,109)	.86*** (13,109)
Loneliness Geometric Mean	-.06*** (7,853)	-.15*** (2,245)	-.02* (10,302)	-.05** (3,171)	.86*** (13,114)	.86*** (13,109)	1.00 (13,114)
Age	-.58*** (7,853)	-.47*** (2,245)	-.22*** (10,302)	-.25** (3,171)	-.01 (13,114)	-.01 (13,109)	-.04*** (13,114)
Sex	.07*** (7,853)	-.08** (2,245)	-.03** (10,302)	-.01 (3,171)	.01 (13,114)	.01 (13,109)	.04*** (13,114)
Country of Residence	.10*** (7,853)	.10*** (2,245)	.06*** (10,302)	--- ---	.40*** (13,114)	.40*** (13,109)	.36*** (13,114)
Baseline Marital Status	-.06*** (7,853)	-.06** (2,245)	-.02* (10,302)	-.08*** (3,171)	.21*** (13,114)	.21*** (13,109)	.20*** (13,114)
Baseline Living Arrangement	-.06*** (7,853)	-.03 (2,245)	-.02 (10,302)	-.07*** (3,171)	.22*** (13,114)	.21*** (13,109)	.20*** (13,114)
Baseline Depression	-.150*** (7,853)	-.17*** (2,245)	-.08*** (10,302)	-.03^{t} (3,171)	.48*** (13,114)	.47*** (13,109)	.48*** (13,114)
Years of Education	.30*** (2,791)	.28*** (2,192)	.20*** (3,522)	.35*** (2,575)	-.01 (5,682)	-.01 (5,681)	-.006 (5,682)

*$p < .05$, **$p < .01$, ***$p < .0001$, $^{t}p < .10$. *Note*. Correlations are partial correlations adjusting for age and sex. Pearson and Spearman correlations were computed for continuous and categorical variables, respectively.

Table 2.6.

Model Fit Statistics for Symbol Digit Quadratic Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***p***
Unconditional						
A. Intercept Only	9,042	134484.8	134492.8	---	---	---
B. Age	9,042	129419.4	129437.4	5,065.4	5	< .0001
C1. Age + Age^2	9,042	129190.2	129220.2	229.2	7	< .0001
C1: Age + Age^2 ♦	9,042	129192.3	129218.3	227.1	4	< .0001
Model C1♦ + Practice	9,042	128955.9	128983.9	236.4	1	< .0001
Conditional						
D: Model C1♦ + Covariates	9,042	128641.5	128697.5	550.8	15	< .0001
E1: Model D + Baseline Loneliness	9,042	128634.3	128696.3	7.2	3	.0658
E2: Model D + Time-Varying Loneliness	9,032	127863.6	127925.6	---	---	---
E3: Model D + Loneliness Geomeans	9,042	128624.6	128686.6	16.9	3	.0007
Sensitivity (Education)						
Model E1 + Education	2,897	46841.8	46909.8	---	---	---
Model E2 + Education	2,897	46402.4	46470.4	---	---	---
Model E3 + Education	2,897	46830.9	46898.9	---	---	---

Note. Model C1♦ = Unconditional model with the covariance parameter estimate for individuals within twin pairs for the quadratic effect removed. This parameter hit a boundary of 0 in the unconditional quadratic model and was removed from subsequent quadratic models.

Table 2.7

Unstandardized Parameters (b) for Symbol Digit Quadratic Models

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**
Level								
Performance (age 65)	51.62**	54.49**	55.19**	54.75**	55.14**	56.04**	55.86**	56.18**
Sex	---	1.76**	1.76**	1.74**	1.74**	3.11**	3.08**	3.06**
Country	---	3.25**	3.22**	3.10**	3.15**	5.58**	5.43**	5.48**
Marital Status	---	-0.12	-0.24	-0.22	-0.22	0.04	-0.01	0.05
Living Arrangement	---	-1.00^{t}	-1.03^{t}	-0.95^{t}	-1.00^{t}	-1.14	-1.00	-1.10
Depression	---	-0.25**	-0.27**	-0.26*	-0.27**	-0.20**	-0.20**	-0.21**
Baseline Loneliness	---	---	**0.19***	---	---	0.15	---	---
Time-Varying Loneliness	---	---	---	**0.10***	---	---	0.12^{t}	---
Loneliness Geomeans	---	---	---	---	**0.20***	---	---	0.21
Education	---	---	---	---	---	1.05**	1.05**	1.05**
Linear Change								
Linear slope	-0.56**	-0.58**	-0.61**	-0.61**	-0.63**	-0.58**	-0.57**	-0.60**
Sex	---	-0.01	-0.01	-0.01	-0.01	-0.10**	-0.10**	-0.09**
Country	---	-0.11**	-0.11**	-0.11**	-0.11**	0.25**	-0.05	0.25**
Marital status	---	-0.11**	-0.10**	-0.10**	-0.10**	-0.05	-0.05	-0.06
Living Arrangement	---	0.07*	0.07*	0.07*	0.08*	0.02	0.02	0.03
Depression	---	-0.001	-0.00067	-0.00051	-0.00009	0.00219	0.00207	0.00272
Baseline Loneliness	---	---	-0.007	---	---	-0.008	---	---
Time-Varying Loneliness	---	---	---	**-0.010***	---	---	-0.009	---
Loneliness Geomeans	---	---	---	---	**-0.015***	---	---	**-0.015^{t}**
Education		---	---	---	---	0.02**	0.02**	0.02**
Quadratic Change								
Quadratic slope	-0.0060**	-0.0042**	-0.0052**	-0.0049**	-0.0068**	0.00029	0.00007	-0.00176
Sex	---	0.003**	0.003**	0.003**	0.003**	0.003*	0.004*	0.003*
Country	---	0.008**	0.008**	0.009**	0.008**	-0.016**	-0.014**	-0.016**

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**
Marital Status	---	-0.001	-0.001	-0.001	-0.001	-.003	-0.003	-0.003
Living Arrangement	---	-0.00004	0.00008	0.00001	0.00009	0.00226	-0.00185	0.00220
Depression	---	0.00009	0.00011	0.00012^{t}	0.00016*	0.00002	0.00003	0.00007
Baseline Loneliness	---	---	-0.0003	---	---	-0.0005	---	---
Time-Varying Loneliness	---	---	---	-0.0003	---	---	**-0.0006***	---
Loneliness Geomeans	---	---	---	---	**-0.0008***	---	---	**-0.0012****
Education	---	---	---	---	---	-0.0004	-0.0004	-0.0004

**$p < .01$, * $p < .05$, ^{t} = $p < .10$. *Note*. Educ. = years of education. Significant effects of loneliness are in bold. Model C1♦ = Unconditional model with the covariance parameter estimate for individuals within twin pairs for the quadratic effect removed. This parameter hit a boundary of 0 in the unconditional quadratic model and was removed from subsequent quadratic models.

Table 2.8

Model Fit Statistics for Block Design Quadratic Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***P***
Unconditional						
A. Intercept Only	2,263	41853.3	41861.3	---	---	---
B. Age	2,263	40669.5	40687.5	1,183.8	5	< .0001
C1. Age + Age^2	2,263	40553.4	40585.4	116.1	7	< .0001
Model C1 + Practice	2,263	40510.8	40544.8	42.6	1	< .0001
Conditional						
D. Model C1 + Covariates	2,263	40391.9	40453.9	161.5	15	< .0001
E1. Model D + Baseline Loneliness	2,263	40381.4	40449.4	10.5	3	.0148
E2. Model D + Time-Varying Loneliness	2,263	39889.4	39957.4	---	---	---
E3. Model D + Loneliness Geomeans	2,263	40379.4	40447.4	12.5	3	.0058
Sensitivity (Education)						
E1 + Education	2,210	39819.1	39893.1	---	---	---
E2 + Education	2,210	39326.6	39400.6	---	---	---
E3 + Education	2,210	39816.9	39890.9	---	---	---

Table 2.9

Unstandardized Parameters (b) for Block Design Quadratic Models

Fixed Effects	**Model C1**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**
Level								
Performance (age 65)	53.11**	52.61**	52.63**	52.74**	52.36**	51.45**	51.47**	51.28**
Sex	---	-1.22*	-1.22*	-1.19*	-1.22*	-0.70	-0.68	-0.71
Country	---	6.50**	6.40**	6.37**	6.19**	18.42**	18.29**	18.25**
Marital Status	---	-1.30	-1.29	-1.33	-1.26	-0.93	-0.95	-0.92
Living Arrangement	---	0.32	0.34	0.39	0.37	0.11	0.15	0.14
Depression	---	-0.24**	-0.24**	-0.24**	-0.23**	-0.23**	-0.23**	-0.22**
Baseline Loneliness	---	---	0.003	---	---	0.027	---	---
Time-Varying Loneliness	---	---	---	0.043	---	---	0.039	---
Loneliness Geomeans	---	---	---	---	-0.080	---	---	-0.025
Education	---	---	---	---	---	1.22**	1.21**	1.22**
Linear Change								
Linear slope	-0.35**	-0.57**	-0.59**	-0.56**	-0.60**	-0.50**	-0.47**	-0.52**
Sex	---	0.12**	0.13**	0.13**	0.13**	0.12**	0.12**	0.12**
Country	---	0.59**	0.57**	0.57**	0.57**	0.40*	0.41*	0.41*
Marital status	---	-0.06^{t}	-0.07^{t}	-0.07^{t}	-0.07^{t}	-0.05	-0.05	-0.05
Living Arrangement	---	0.06	0.06	0.05	0.06	0.05	0.04	0.05
Depression	---	-0.0007	0.0002	-0.0009	0.0006	0.0001	-0.0010	0.0006
Baseline Loneliness	---	---	-0.006	---	---	-0.006	---	---
Time-Varying Loneliness	---	---	---	0.003	---	---	0.003	---
Loneliness Geomeans	---	---	---	---	**-0.011^{t}**	---	---	**-0.013^{t}**
Education	---	---	---	---	---	-0.0009	-0.0006	-0.0004
Quadratic Change								
Quadratic slope	-0.004**	-0.004	-0.006^{t}	-0.006^{t}	-0.006	-0.004	-0.003	-0.003
Sex	---	0.00017	0.00002	-0.00021	-0.00022	-0.00027	-0.00049	-0.00005
Country	---	-0.007	-0.009	-0.008	-0.009	-0.020**	-0.018*	-0.020**

Fixed Effects	**Model C1**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**
Marital Status	---	-0.004*	-0.004^{t}	-0.004^{t}	-0.004*	-0.004*	-0.004*	-0.005*
Living Arrangement	---	0.001	0.002	0.001	0.001	0.002	0.002	0.002
Depression	---	0.0002^{t}	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*	0.0002*
Baseline Loneliness	---	---	**-0.0007***	---	---	**-0.0007***	---	---
Time-Varying Loneliness	---	---	---	**-0.0007****	---	---	**-0.0007****	---
Loneliness Geomeans	---	---	---	---	-0.0006	---	---	-0.0006
Education	---	---	---	---	---	-0.0008**	-0.0008**	-0.0008**

**$p < .01$, * $p < .05$, ^{t} = $p < .10$. *Note*. Educ. = years of education. Significant effects of loneliness are in bold.

Table 2.10

Model Fit Statistics for Synonyms Quadratic Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***P***
Unconditional						
A. Intercept Only	3,204	45904.3	45912.3	---	---	---
B. Age	3,204	45586.2	45602.2	318.1	5	< .0001
C1. Age + Age^2	3,204	45473.3	45501.3	112.9	7	< .0001
C1. Age + Age^2 ♦	3,204	45608.5	45628.5	-22.3	1	< .0001
Model C1♦ + Practice	3,204	45587.4	45609.4	21.1	1	< .0001
Conditional						
D: Model C1♦ + Covariates	3,204	45565.3	45601.3	43.2	15	< .0001
E1: Model D + Baseline Loneliness	3,204	45562.6	45602.6	2.7	2	.2592
E2: Model D + Time-Varying Loneliness	3,204	45162.0	45202	---	---	---
E3: Model D + Loneliness Geomeans	3,204	45563.0	45603	2.3	2	.3166
Sensitivity (Education)						
Model E2 + Education	2,608	40664.4	40708.4	---	---	---
Sensitivity (Practice)						
Model E2 + Practice	3,204	45145.8	45187.8	16.2	1	< .0001

Note. Model C1♦ = Unconditional quadratic model with the covariance parameter estimates for (a) individuals within twin pairs, and (b) twin pairs for the linear age effect removed. Both effects hit a boundary of 0 in the unconditional quadratic model and were excluded from subsequent quadratic models. Since no random effects were modeled on the linear age term, no interactions with this term were included in quadratic models.

Table 2.11

Unstandardized Parameters (b) for Synonyms Quadratic Models

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E2 + Educ.**	**Model E2 + Practice**
Level							
Performance (age 65)	53.61**	54.20**	53.58**	53.91**	53.91**	57.08**	53.73**
Sex	---	-0.14	-0.14	-0.21	-0.14	0.17	-0.24
Country	---	---	---	---	---	---	---
Marital Status	---	-1.74	-1.68	-1.76	-1.72	0.07	-1.60
Living Arrangement	---	-0.75	-0.71	-0.65	-0.72	-1.02	-0.52
Depression	---	0.017	0.039	0.026	0.026	-0.004	0.026
Baseline Loneliness	---	---	-0.15	---	---	---	---
Time-Varying Loneliness	---	---	---	-0.04	---	**-0.10^{t}**	-0.06
Loneliness Geomeans	---	---	---	---	-0.08	---	---
Education	---	---	---	---	---	1.29**	---
Practice	---	---	---	---	---	---	0.69**
Linear Change							
Linear slope	-0.12**	-0.10**	-0.10**	-0.10**	-0.10**	-0.01	-0.12**
Sex	---	---	---	---	---	---	---
Country	---	---	---	---	---	---	---
Marital status	---	---	---	---	---	---	---
Living Arrangement	---	---	---	---	---	---	---
Depression	---	---	---	---	---	---	---
Baseline Loneliness	---	---	---	---	---	---	---
Time-Varying Loneliness	---	---	---	---	---	---	---
Loneliness Geomeans	---	---	---	---	---	---	---
Education	---	---	---	---	---	---	---
Quadratic Change							
Quadratic slope	-0.003**	-0.003**	-0.003*	-0.004**	-0.004*	-0.008**	-0.005**
Sex	---	0.004**	0.004**	0.004**	0.004**	0.004**	0.004**

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E2 + Educ.**	**Model E2 + Practice**
Country	---	---	---	---	---	---	---
Marital Status	---	-0.002	-0.002	-0.002	-0.002	-0.002	-0.002
Living Arrangement	---	0.003	0.003	0.003	0.003	0.003	0.003
Depression	---	-0.00012^{t}	-0.00013	-0.00008	-0.0001	-0.00012	-0.00008
Baseline Loneliness	---	---	0.00004	---	---	---	---
Time-Varying Loneliness	---	---	---	**-0.00041***	---	-0.00028	**-0.00035^{t}**
Loneliness Geomeans	---	---	---	---	-0.00017	---	---
Education	---	---	---	---	---	-0.0005*	---

**$p < .01$, * $p < .05$, ^{t} = $p < .10$. *Note*. Educ. = years of education. Significant and trend significant effects of loneliness are in bold. Model C1♦ = Unconditional quadratic model with the covariance parameter estimates for (a) individuals within twin pairs, and (b) twin pairs for the linear age effect removed. Both effects hit a boundary of 0 in the unconditional quadratic model and were excluded from subsequent quadratic models. Since no random effects were modeled on the linear age term, no interactions with this term were included in quadratic models.

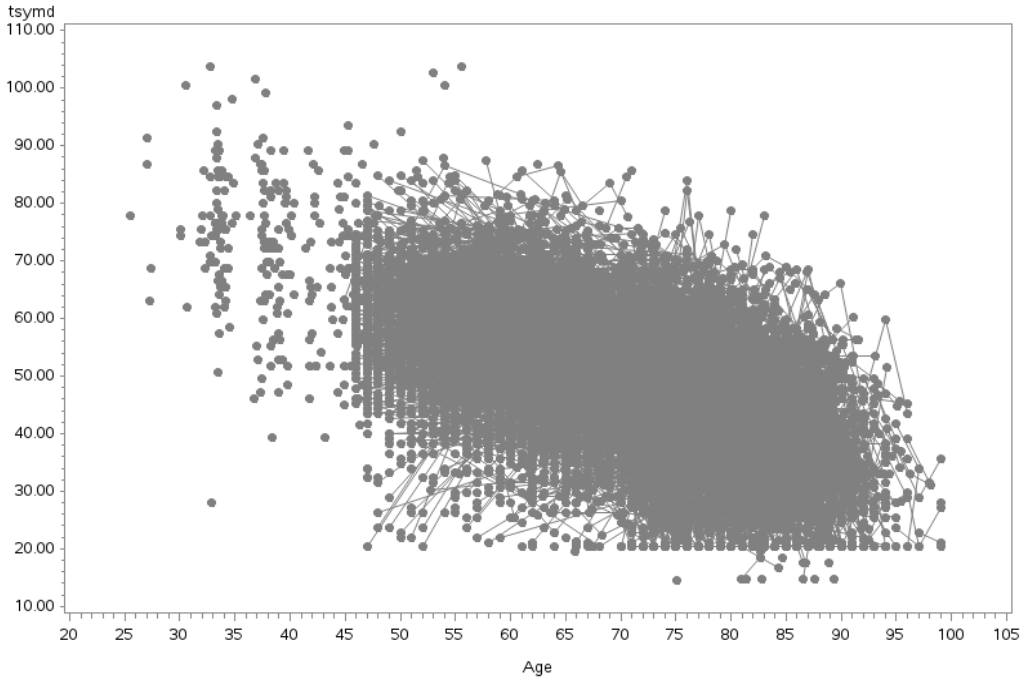
Table 2.12

Effect Sizes (d) for Loneliness on Change in Cognitive Performance Between Ages 65 and 80 for Quadratic Models

	Baseline Loneliness (*d*)	Time-Varying Loneliness (*d*)	Loneliness Geometric Means (*d*)
Symbol Digit	-0.14	-0.19	-0.34
Block Design	-0.21	-0.10	-0.25
Synonyms	0.01	-0.08	-0.03

Note. Effect sizes (*d*) quantify the difference in change in cognitive performance between ages 65 and 80 associated with high vs. low loneliness.

(a)



(b)

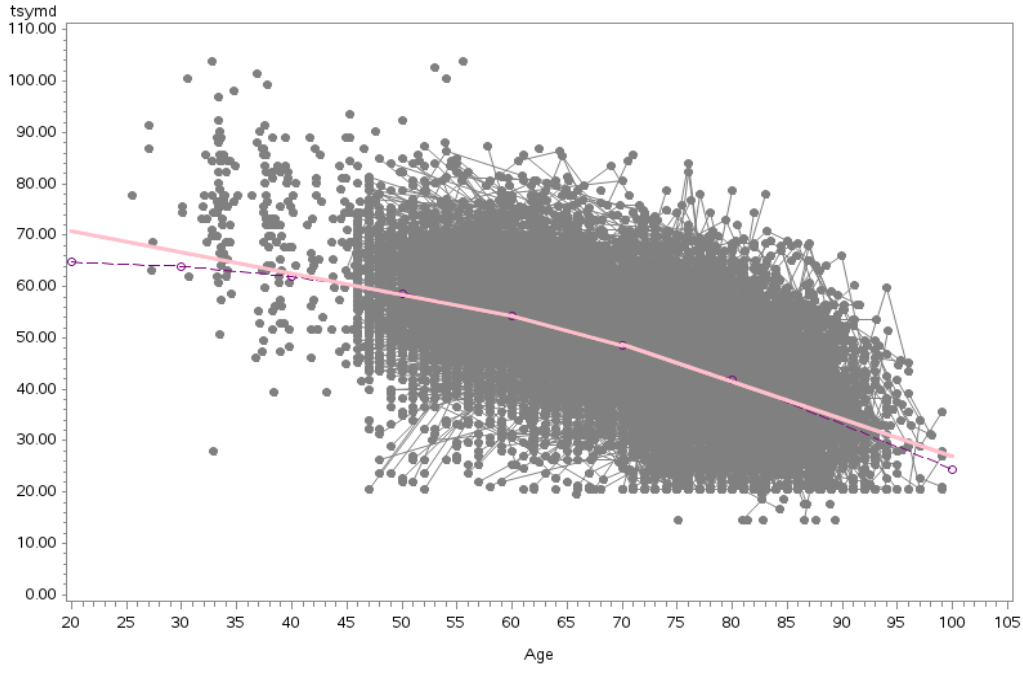
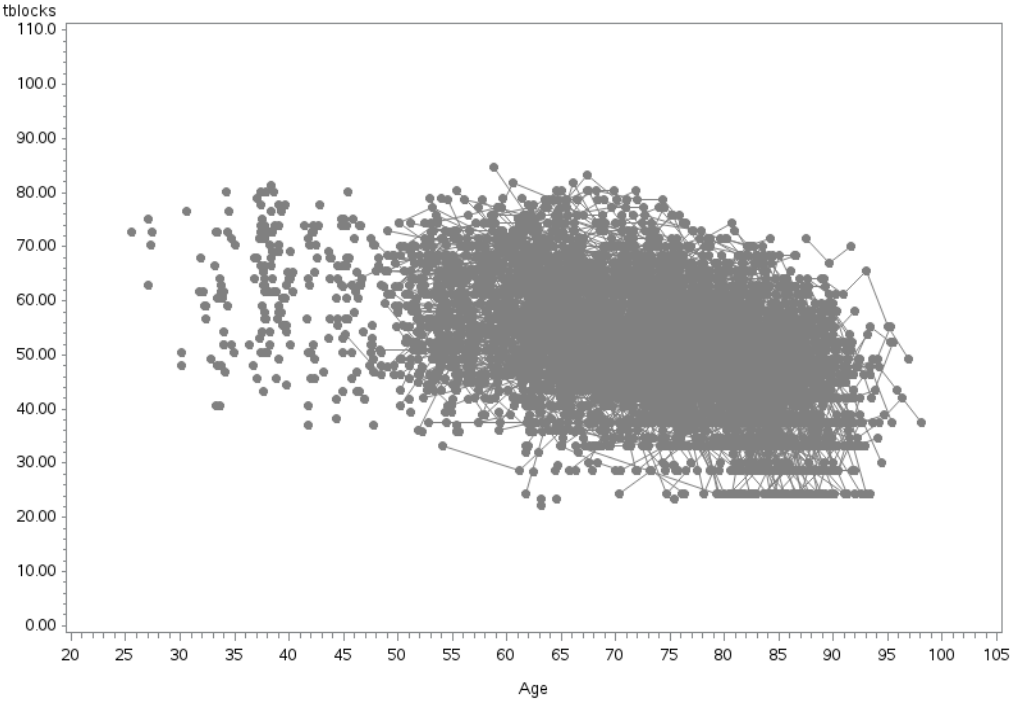


Figure 2.1. (a) Longitudinal trajectory plot for *T*-scores on the Symbol Digit task (y-axis) by age for the analysis sample. (b) Observed Symbol Digit *T*-scores (y-axis) across age and expected trajectories for the unconditional quadratic (purple, dash) and spline (pink, solid) models.

(a)



(b)

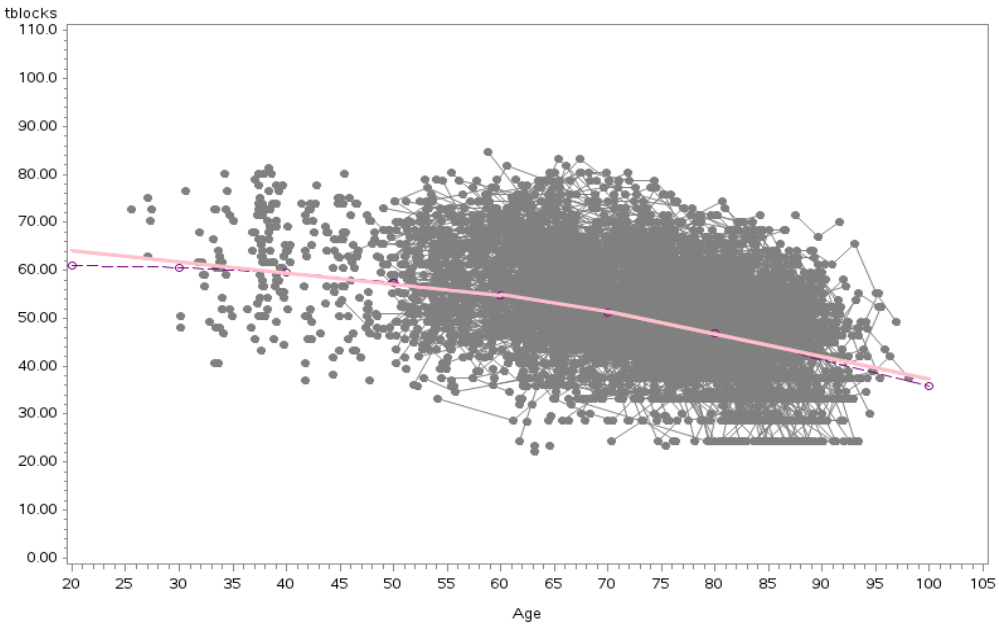


Figure 2.2. (a) Longitudinal trajectory plot for *T*-scores on the Block Design task (y-axis) by age for the analysis sample. (b) Observed Block Design *T*-scores (y-axis) across age and expected trajectories for the unconditional quadratic (purple, dash) and spline (pink, solid) models.

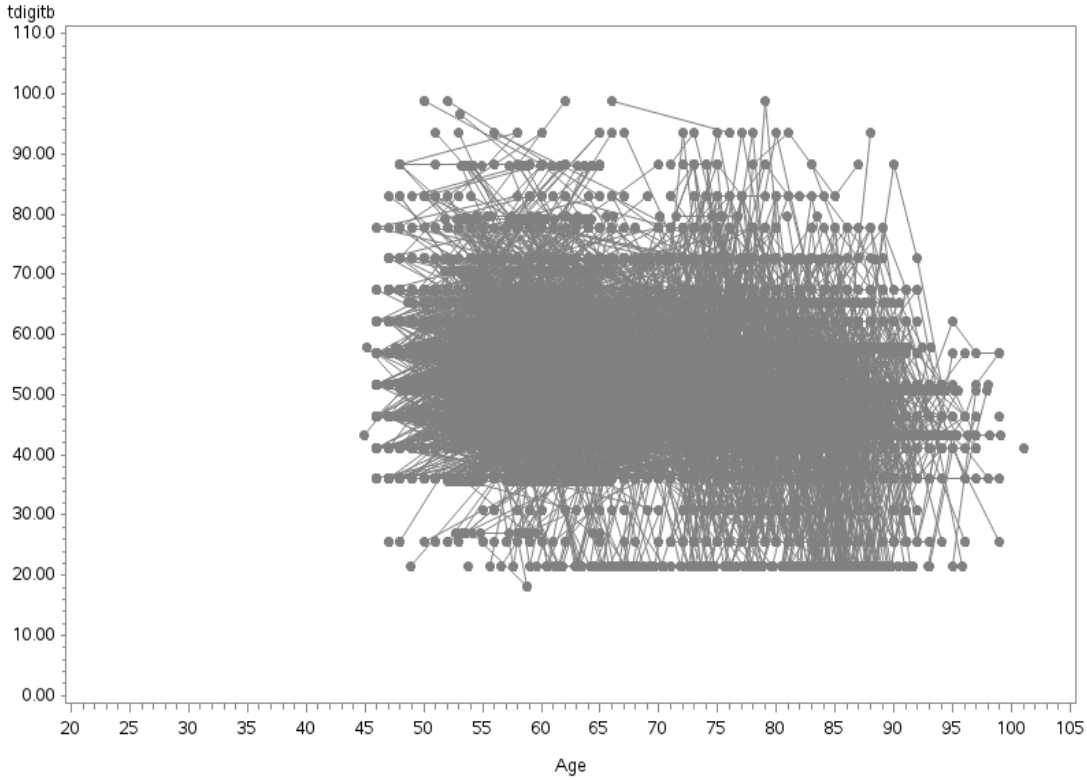
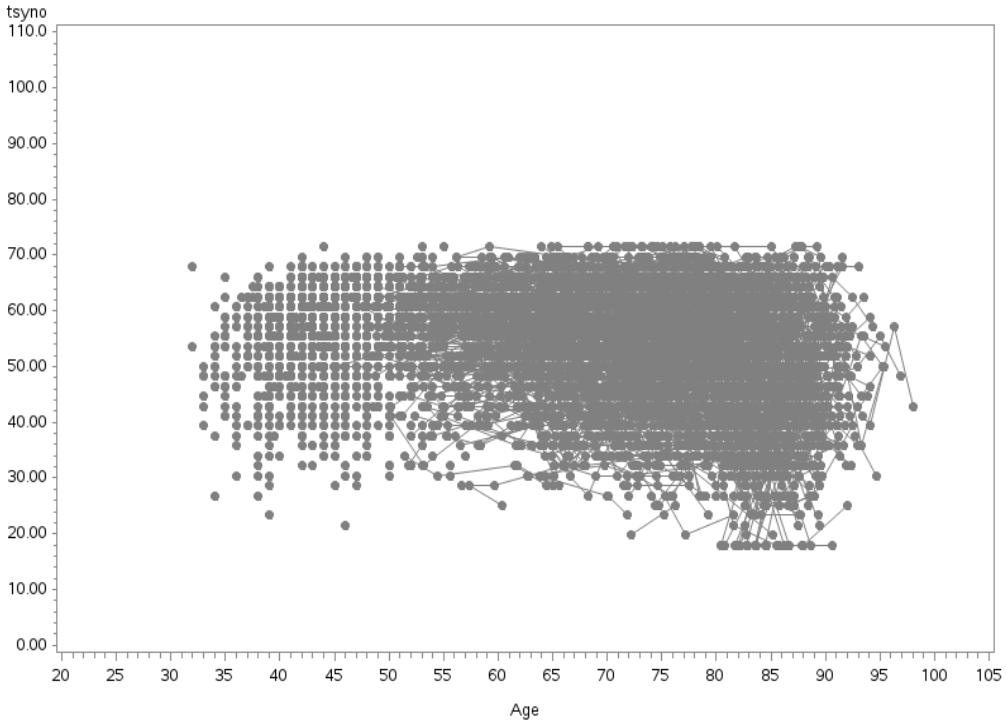


Figure 2.3. Longitudinal trajectory plot for the Digits Backward task by age for the analysis sample. Digits Backward scores are shown on a *T*-score scale ($M = 50$, $SD = 10$).

(a)



(b)

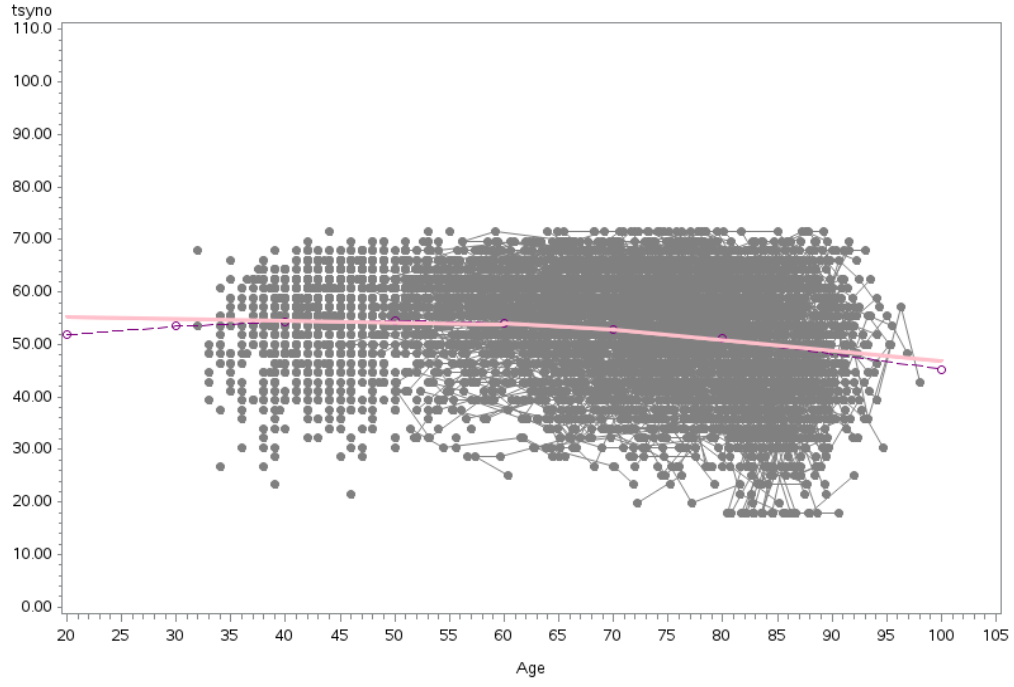


Figure 2.4. (a) Longitudinal trajectory plot for *T*-scores on the Synonyms task (y-axis) by age for the analysis sample. (b) Observed Synonyms *T*-scores (y-axis) across age and expected trajectories for the unconditional quadratic (purple, dash) and spline (pink, solid) models

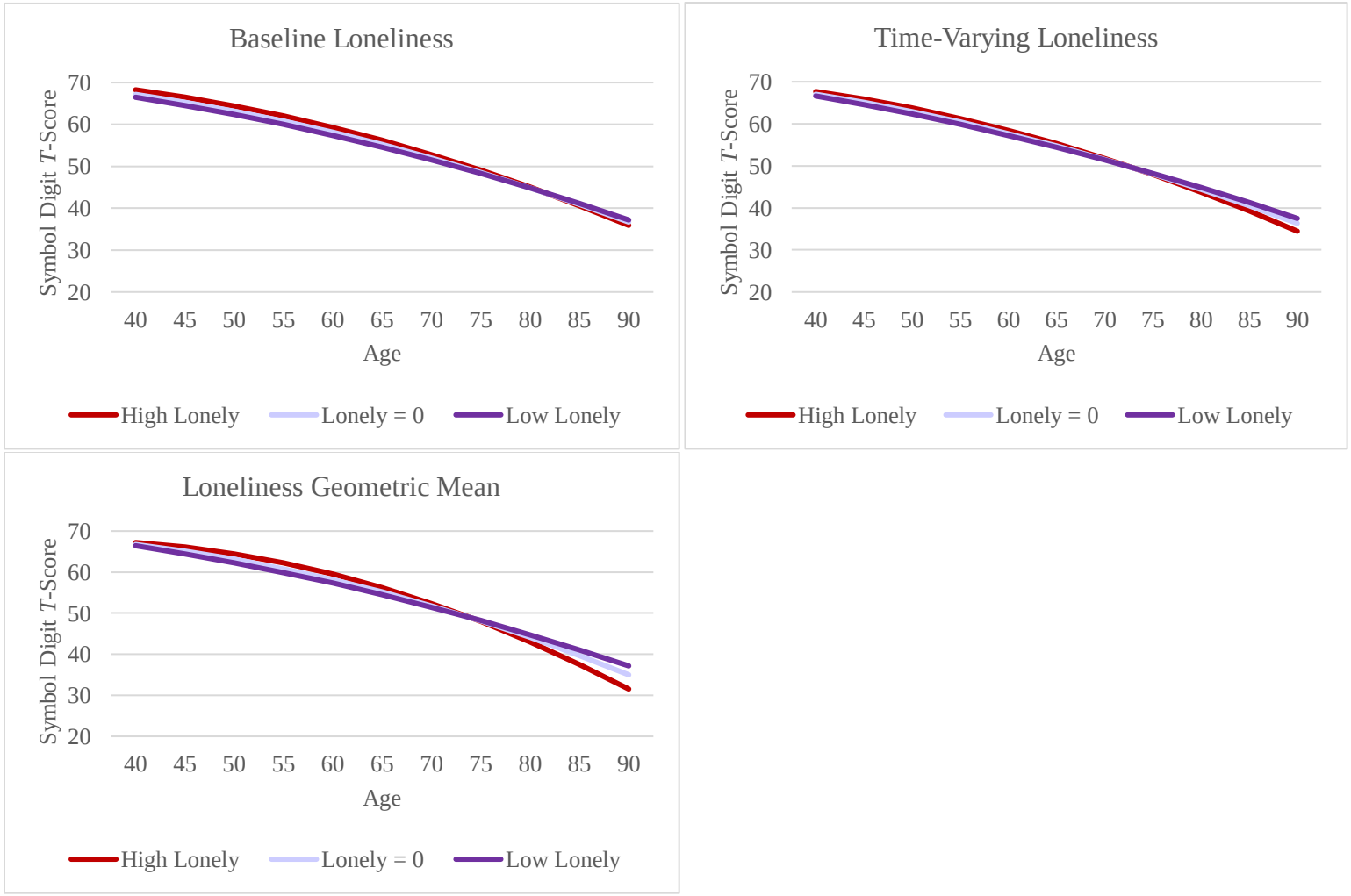


Figure 2.5. Predicted trajectory curves by loneliness for Symbol Digit *T*-scores estimated from quadratic models

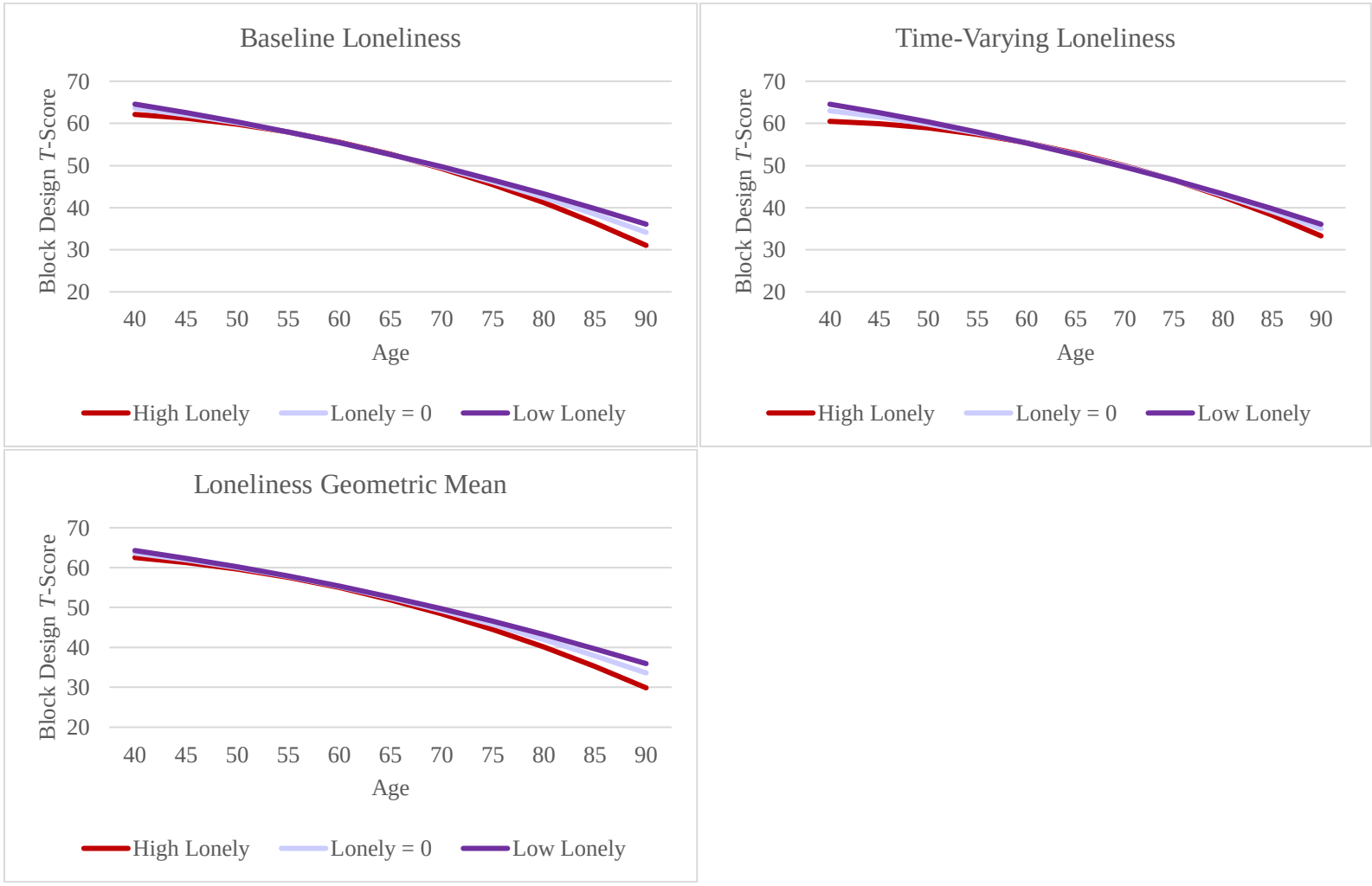


Figure 2.6. Predicted trajectory curves by loneliness for Block Design *T*-scores estimated from quadratic models.

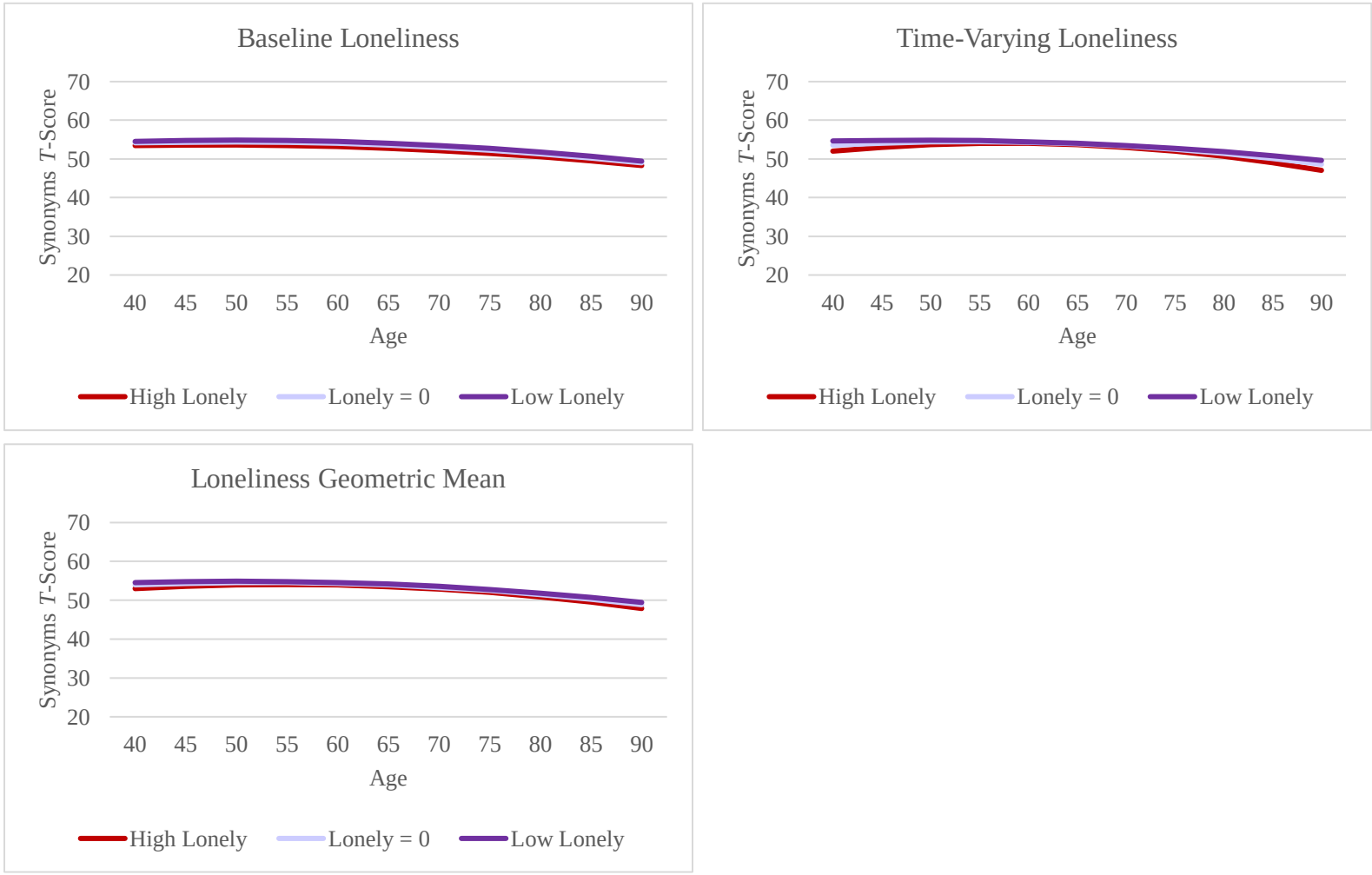


Figure 2.7. Predicted trajectory curves by loneliness for Synonyms *T*-scores estimated from quadratic models

Study 2

Methylation of Blood Leukocyte DNA in Lonely and Non-Lonely Individuals and a Potential Role for this Epigenetic Biomarker in the Association between Loneliness and Cognition

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INTRODUCTION

Feeling lonely is an intense adverse emotional experience that accompanies the perception that the quality or quantity of one's social relationships does not meet their current needs or expectations (Spithoven, Cacioppo, Goossens, & Cacioppo, 2019). Prior work suggests that feelings of loneliness detract from healthy cognitive aging, with associations reported between loneliness and poorer cognitive performance (O'Luaniagh et al., 2012; Wilson et al., 2007), faster cognitive decline (Donovan et al., 2016; Tilvis et al., 2004; Wilson et al., 2007) and an increased risk of dementia (Amieva et al., 2010; Holwerda et al., 2012; Rafnsson et al., 2017; Wilson et al., 2007). Although mechanisms of the associations between loneliness and these cognitive outcomes remain to be determined, a growing body of literature linking loneliness and altered expression patterns for genes in blood leukocytes (Cole et al., 2007; Cresswell et al., 2012) and brain tissue (Canli et al., 2016; Canli et al., 2018) suggests that one pathway by which loneliness may undermine healthy cognitive aging is by inducing epigenetic changes (i.e., changes to DNA that are sensitive to contextual influence and alter the function of DNA without changing the DNA sequence; Feinberg, 2013) that lead to physiological changes that have a deleterious impact on cognitive functioning over time.

DNA methylation, which involves attachment of a methyl (CH_3) group to a cytosine nucleotide within a DNA strand (Meloni, 2014; Moore, Le, & Fan, 2012), is one type of epigenetic change that can affect gene expression (Meloni, 2014). Methylation most often occurs at sites where guanine nucleotides follow cytosine nucleotides (i.e., CpG sites), although it can occur elsewhere along DNA strands (Moore et al., 2012).

Methylation within promotor regions of genes (i.e., locations where transcription factors bind to DNA to initiate gene expression) can prevent transcription factors from binding at transcription sites resulting in gene silencing (Meloni, 2014; Moore et al., 2012). Despite the growing body of work on loneliness and gene expression, the association between loneliness and DNA methylation remains unexplored. Such work has the potential to further understanding of the interplay between genes and social context and to shed light on a potential pathway by which social context which may lead to physiological changes that detract from healthy cognitive aging.

Feelings of loneliness have been associated with poorer performance and faster decline in global cognitive functioning (O'Luanaigh et al., 2012; Wilson et al., 2007; Zhong, Chen & Conwell, 2016). They have also been linked with poorer performance on tasks assessing processing speed, semantic memory, episodic memory, working memory, and spatial ability, and faster decline in processing speed, semantic memory (Wilson et al., 2007), episodic memory (Donovan et al., 2016), and spatial ability (Wilson et al., 2007). Longitudinal studies of loneliness and dementia suggest a greatly increased risk of developing dementia for individuals who report feelings of loneliness compared to those who do not. The increased risk for lonely persons has been reported to be as high as 1.64 (Holwerda et al., 2012) to more than 2 times (Wilson et al., 2007) that for non-lonely persons.

Findings from studies of loneliness and gene expression in blood leukocytes indicate that genes associated with inflammation and fighting viral infections are differentially expressed in individuals who experience high levels of loneliness across

time compared to those who are not lonely, such that genes associated with inflammatory processes are over-expressed in lonely individuals and genes associated with fighting viral infections are under-expressed in lonely individuals compared to non-lonely persons (Cole et al., 2007; Cole et al., 2015; Creswell et al., 2012). This expressional pattern has been referred to as a 'conserved transcriptional response to adversity' (CTRA; Cole, 2013; Cole, 2014), and has been linked with other stress-inducing experiences such as low socioeconomic status, social isolation, receiving a potentially terminal medical diagnosis, and impending loss of a loved one (Cole, 2013). Altered CTRA gene expression has also been linked with hedonic well-being, while the opposite expressional pattern has been observed in individuals who report high levels of eudaimonic well-being (Frederickson et al., 2013; Frederickson et al., 2015).

The evolutionary theory of loneliness (ETL) provides a framework for understanding how observed differential CTRA gene expression in individuals who experience chronic loneliness might have been adaptive in an evolutionary context (Goossens et al., 2015; Spithoven et al., 2019). Findings from studies on loneliness and gene expression suggest that when one chronically experiences strong feelings of loneliness, their immune system shifts away from prioritizing fighting viral infections (i.e., which we encounter when in close proximity with others) toward prioritizing fighting bacterial infections (which we are more likely to encounter than viral infections when isolated from others) (Goossens et al., 2015; Spithoven et al., 2019). Such a response to objective and perceived isolation is likely less adaptive for most individuals

in modern times, especially those who live in urban contexts where contact with others is likely to occur whether one perceives themselves as socially isolated or not.

Loneliness has also been linked with altered expression of genes in nucleus accumbens and dorsolateral prefrontal cortex tissue (Canli et al., 2016; Canli et al., 2018). Canli et al. (2016) found a relation between loneliness scores collected more than two years prior to death and expression of 1,599 genes in the nucleus accumbens, a brain region linked with social processing, after death. These genes have been linked with social behaviors and emotional responses, mental health disorders, and diseases including Alzheimer's disease (AD; 169 genes) and cancer. A relation has also been observed between loneliness scores collected five years earlier and altered expression for sets of genes associated with AD, cancer, inflammation, immune function, and mental health disorders in the dorsolateral prefrontal cortex after death (Canli et al., 2018). The observed relation between loneliness and altered expression of genes linked with these cognitive, physical, and mental health outcomes in brain tissue suggests potential overlap for genes associated with loneliness and these outcomes, however, whether loneliness causes altered expression for these genes remains unknown (Canli et al., 2016).

Altered patterns of gene expression associated with loneliness may contribute to the stability of loneliness by altering behaviors, social perceptions, and inflammatory processes which may negatively influence how lonely individuals are perceived by others. For example, altered gene expression can influence central nervous system function which in turn can lead to behaviors that make remaining lonely more likely (e.g., assuming a sick role; Cole, 2014). Altered CTRA gene expression associated with

loneliness has been found to predict later loneliness (Cole et al., 2015); it has been posited that inflammation associated with altered CTRA expression may also result in shunning by others, as outward signs of inflammation are suggestive of poor health (Spithoven et al., 2019). Collectively, altered behavioral patterns and inflammatory processes may increase the likelihood of remaining lonely and continued altered CTRA expression; such altered expression may set physiological processes in motion which undermine cognitive, physical, and mental health over time (Cole, 2013).

On the other hand, findings suggest that epigenetic changes associated with loneliness may also be reversible. For example, altered expression of genes associated with inflammation in lonely individuals has been found to be reduced following an eight-week training intervention on stress reduction using mindfulness (Creswell et al., 2012). This finding, taken together with the observation that an expressional pattern opposite of the CTRA has been associated with eudaimonic well-being (Cole et al., 2015; Frederickson et al., 2013; Frederickson et al., 2015), suggest that interventions that reduce loneliness and/or promote eudaimonic well-being may have important implications for health (Cole et al., 2015). Changes in gene expression associated with such interventions may also lead to changes in physiological processes and behaviors that may facilitate healthier social interactions (Cole et al., 2015).

Inflammation associated with loneliness may in part explain the link between loneliness and cognition, although this has yet to be investigated. High serum concentrations of inflammatory markers have been linked in some studies with dementia risk, and cognitive performance and change (e.g., Schram et al., 2007; Teunissen et al.,

2003; Trollor et al., 2012; Yaffe et al., 2003). Inflammatory markers that have been associated with cognition include C-reactive protein (CRP; Komulainen et al., 2007; Ravaglia et al., 2005; Schram et al., 2007; Teunissen et al., 2003), interleukin-6 (IL-6; Elwan et al., 2003; Rafnsson et al., 2007; Schram et al., 2007), intercellular adhesion molecule-1 (ICAM-1) (Rafnsson et al., 2007), haptoglobin (Teunissen et al., 2003), and α1-antichymotrypsin (Engelhart et al., 2004). Much of the work on inflammation and cognition has focused on CRP and IL-6. Findings from these studies show a link between high serum concentrations of CRP and poorer concurrent executive (Schram et al., 2007) and global (Ravaglia et al., 2005; Schram et al., 2007) cognitive function, poorer memory function 6-12 years later (Komulainen et al., 2007; Teunissen et al., 2003), decline in performance on memory tasks (Schram et al., 2007), and heightened dementia risk (Engelhart et al., 2004; Schmidt et al., 2002). IL-6 levels have been linked with poorer executive function (Schram et al., 2007), sensory memory, attention (Elwan et al., 2003), and global cognitive performance (Schram et al., 2007), faster decline in performance on measures of memory (Schram et al., 2007), and speed of processing (Rafnsson et al., 2007), elevated risk of dementia (Engelhart et al., 2004), and active dementia (Eriksson et al., 2011). Although much evidence of a relation between inflammatory markers and cognition exists, it is important to consider that several studies of inflammation and cognition have produced null findings (e.g., Alley, Crimmins, Karlamangla, Hu, & Seeman, 2008; Dik et al., 2005; Trollor et al., 2012; Yaffe et al., 2003).

DNA methylation may play a role in altered gene expression observed in lonely individuals (Cole, 2013) and has been linked with both stress in adulthood (Lam et al.,

2012) and cognitive outcomes including dementia in late life (Karlsson, Ploner, & Wang, 2018) and cognitive impairment in individuals with Down syndrome (Jones et al., 2013). Stress in adulthood has been associated with altered variability in methylation across over 27,000 CpG sites (Lam et al., 2012) while methylation at particular sites (e.g., within the *APOE* gene; Karlsson et al., 2018) has been linked with cognition. A recent epigenome-wide study of methylation and domain-specific cognition reported associations between methylation at a single CpG site within a noncoding area within chromosome 12 and global cognition and methylation at a second CpG site within the *INPP5A* gene and verbal fluency (Marioni et al., 2018). However, a single study of the relation between global DNA methylation and performance on speed, memory, and verbal fluency tasks in persons that were non-cognitively impaired found no association between global methylation and performance on these tasks (Schiepers et al., 2012), suggesting that methylation at particular sites may be more strongly associated with cognitive outcomes than global methylation, however, further work is needed to determine how methylation relates to cognition.

Importantly, the role of DNA methylation in gene expression remains unclear (Cole, 2013; Lam et al., 2012). Although methylation has been determined to play a role in altering gene expression (Umov & Wolffe, 2001; Wolffe & Matzke, 1999), findings suggest this association has been found to be moderate on average (i.e., -.29), with higher associations for some genes and expressional patterns that appear largely independent of methylation for others (Lam et al., 2012). Such findings reflect that gene expression

results from the interplay of multiple factors (Lam et al., 2012) and that methylation may affect expression of some genes more than others.

In the present study, longitudinal associations between loneliness and DNA methylation at 1,586 CpG sites within 105 CTRA genes in blood leukocytes were assessed in a twin sample from the Swedish Adoption/Twin Study of Aging (SATSA; Pedersen, Plomin, Nesselroade, & McLearn, 1992). Both phenotypic and discordant twin approaches were used. Phenotypic analyses explored whether methylation level or change in methylation across an 18-year follow up period at CpG sites within these CTRA genes were associated with loneliness. Discordant twin analyses used the co-twin control design (Carlin, Gurrin, Sterne, Morley, & Dwyer, 2005; McGue, Osler, & Christensen, 2010; Vitaro, Brendgen, & Arseneault, 2009) to test for potential causality versus genetic or common environmental confounding in observed associations between loneliness and methylation at individual CpG sites. Importantly, the use of a twin sample addressed that the propensity to experience epigenetic changes in response to environmental circumstances is heritable (Goossens et al., 2015). Heritability for methylation has been found to be site-specific, with the average heritability for CpGs genome-wide reported at .19 (van Dongen et al., 2016). A potential mediational role for altered methylation patterns at these CpG sites in associations between loneliness and performance and change in specific domains of cognition was also explored. Prior findings showing altered expression of CTRA genes in lonely persons (Cole et al., 2007; Creswell et al., 2012) suggest that DNA methylation and/or change in methylation across time may also systematically vary with loneliness for some of these genes, however this

currently remains unknown. Based on prior work linking loneliness with poor outcomes in specific domains of cognitive function (Donovan et al., 2016; Wilson et al., 2007), it was hypothesized that loneliness would significantly predict reduced performance or faster decline in the cognitive domains assessed. Analyses assessing associations between DNA methylation and cognition were considered exploratory. It was predicted that if loneliness was associated with methylation at CpG sites associated with CTRA genes and if methylation was associated with cognition for these CpGs, that methylation at these sites may play a mediational role in the link between loneliness and cognitive performance and change.

Method

Sample

SATSA methylation sample

The sample included 385 twins from the Swedish Adoption Twin Study of Aging (SATSA; Pedersen et al., 1992) who participated in one or more of the study's five in-person testing (IPT) waves for which DNA methylation data are currently available (IPT waves 3, 5, 6, 8, and 9). Across waves, n =1,017 total observations were available for this sample. IPT3 assessments took place between 1992-95 and IPT9 between 2010-12, with the resulting follow-up period spanning 18 years. All twins in the sample were from same-sex twin pairs. The sample consisted of n = 173 MZ twins (76 complete, 21 incomplete twin pairs), n = 211 DZ twins (91 complete, 29 incomplete twin pairs) and n = 1 with unknown zygosity. The sample was 60.00% female (n = 231) and 40.00% male (n = 154). The age range at baseline was 48 to 94 (M = 68.96, SD = 9.66).

Analysis sample

The analysis sample for the loneliness and methylation analyses (i.e., individuals from the SATSA methylation sample who were not missing on loneliness or covariates adjusted for in loneliness and methylation analyses at one or more waves) consisted of n = 357 participants. N = 213 (59.66%) were female and n = 144 (40.34%) were male. The age range for the analysis sample at baseline (i.e., the first wave at which each participant had complete data) was 48 to 99 (M = 68.95, SD = 9.83). For 92 twin pairs (40 MZ and 52 DZ pairs), both twins had complete data at one or more measurement occasions.

The analysis sample for assessing relations between loneliness, methylation, and cognition included individuals from the SATSA methylation sample who had at least one wave of complete data (i.e., those not missing on loneliness or any covariates adjusted for in cognitive analyses and who had at a score for at least one cognitive task, n = 372, n_{obs} = 919) and who were not diagnosed with dementia at the first wave for which they have complete data for study variables (n = 361, n_{obs} = 896). N = 23 observations were dropped across waves due to dementia status (n = 11 participants dropped out of the analyses completely).

Measures

Loneliness.

Loneliness was assessed at each IPT wave of the SATSA with a single loneliness item from the Center for Epidemiologic Studies Depression (CES-D) scale (Radloff, 1977) which asked participants how often they '*felt lonely*' during the previous week.

Response options were '*rarely or none of the time*, '*some or a little of the time*', '*occasionally or a moderate amount of time*', and '*most or all of the time*'.

For the present study, an IRT-based measure of loneliness that was informed by participants' scores on the single CES-D loneliness item was used for data analysis. The loneliness measure was computed using Winsteps v. 3.92.1 for the purpose of creating a harmonized score of loneliness across studies participating in the Consortium on Interplay of Genes and Environment Across Multiple Studies (IGEMS; Pedersen et al., 2013) that used all available loneliness information for each study and was scaled against a 10-item version of the UCLA Loneliness Scale (ULS; Russell, Peplau, & Cutrano, 1980). The computed loneliness scores (referred to here as '*person measures*') are expressed in logit units and reflect where the four possible response choices fell along the latent construct of loneliness (Boone, Staver, & Yale, 2014). Loneliness person measure scores were used to assess associations between loneliness, DNA methylation at CpG sites of interest, and cognition. See Appendix 1 for an in-depth description of the computation of the loneliness person measure scores.

Blood leukocyte DNA methylation.

Blood samples were taken as part of the IPT protocol for a subset of SATSA participants at waves at which methylation data were collected (Berglund et al., 2016). DNA was extracted and extent of DNA methylation at 485,512 CpG sites was assessed using the Infinium 450 K HumanMethylation BeadChip (Illumina, San Diego, CA, USA). For quality control purposes samples were removed from the set if they failed to produce a sufficiently strong signal, if they had correlations less than .7 with genotype

controls, or if probe signals on sex chromosomes incorrectly predicted the sex of the participant. Probes were removed from the set if any sample had a signal detection *p*-value greater than 0.05 for a particular probe, if they corresponded to sites with single-nucleotide polymorphisms, if they corresponded to sites on sex chromosomes, or if they did not correspond with sites within CpGs. After removing these samples and probes, 1.094 samples and 329,341 probes remained. Normalizations and adjustments were made for cell counts and batch effects. These have been previously described in detail, as have other quality control procedures for the SATSA methylation data (see Jylhävä et al., 2019; Karlsson et al., 2018; Wang et al., 2018).

Methylation log ratio (M) values were used as measures of the extent of methylation at each CpG site assessed. The M values were previously computed from beta values obtained from analysis of the BeadChips. Although use of beta values is advantageous in terms of interpretability (i.e., beta values represent an approximate estimation of the methylation percentage for each site examined, ranging from 0 to 1), use of the M values is more advantageous because they are more normally distributed (Du et al., 2010). M values are calculated as the log 2 ratio of the beta values and have been shown to provide more accurate measures of methylation, as beta values are approximate estimates of the extent of methylation at particular sites (Du et al., 2010). M values can be either positive or negative, with positive M values representing greater methylation at a particular CpG site and negative M values representing a more unmethylated state at a CpG site.

Identification of CpGs associated with CTRA genes.

The list of CTRA-associated genes was obtained from supplementary materials published by Cole et al. (2007) that listed genes found to be differentially expressed in lonely vs. non-lonely persons. The gene names from this list were used to search the content descriptor file for the HumanMethylation450K BeadChip for CpGs associated with these genes. 2,324 CpG sites were identified. Of these, 1,586 were measured and passed quality control for the SATSA sample. The 105 genes associated with these CpGs and the n_{CpG} for each are listed in Table A11 in Appendix 4.

Cognition.

Processing speed (Symbol Digit task).

Processing speed was assessed at each IPT wave of the SATSA using the Symbol Digit Modalities task (Smith,1982). Participants were first shown an image of nine shapes, each paired with a number between 1 and 9. Then, on each of 100 trials, they were shown a shape and asked to verbally report the number corresponding with that shape. Scores on this task could range from 0-100 and represent the number of correct responses across trials. Scores were normalized prior to data analysis.

Spatial ability (Block Design task).

Spatial ability was assessed using the Koh's Block Design test (Stone, 1985). On each of seven trials, participants were shown an image of a shape and asked to construct the shape using a set of blocks that was provided for the task. Scores on the task reflect both the time it took to construct the shape and how similar the shape they produced was

to the shape shown in the image. The highest possible score on this task was 42. Scores were converted to percent correct and normalized prior to data analysis.

Working memory (Digits Backward task).

Working memory was assessed at each IPT wave with a Digits Backward task. Participants were read series of digits 3 to 8 digits in length and asked to repeat the numbers in the reverse order to which they heard them. Two trials were given for each series length. The first trials tested participants using the two-digit series. The digit series increased in length by one digit in each pair of subsequent trials. This continued until the participant failed to accurately repeat the digit series in both trials for a given series length or until they completed all 12 trials. Scores on this task corresponded with the highest number series length achieved; possible scores on this task ranged from 3 to 8. Scores were converted to percent correct and normed prior to data analysis.

Verbal comprehension (Synonyms task).

SATSA's cognitive battery included a Synonyms task which assessed participants' verbal comprehension. For this task, participants were asked to respond to two sets of 15 items; for each, a word was presented along with response options from which the participants were asked to choose the word closest in meaning to the presented word. Participants were given 3 and a half minutes to complete each set of 15 items. Possible scores on this task ranged from 0 to 30. Scores were converted to percent correct and normed prior to data analysis.

Dementia screening.

Participants diagnosed with dementia at their first wave of methylation data collection (n = 11) were excluded from analyses exploring associations between loneliness, DNA methylation, and cognition. For participants who became demented after their first wave of methylation data collection, data for all waves prior to dementia diagnosis were included in analyses. Across waves, a total of n = 23 observations were dropped. SATSA participants were screened for dementia using several criteria and a consensus conference was held to classify participants as demented or not demented using criteria outlined by the latest edition of the DSM at the time (either the DSM-III-R or the DSM-IV). Factors considered included (a) whether rapid decline in Mini-Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) scores (i.e., $\geq$ 3 points) was observed across adjacent waves, (b) poor performance on the cognitive battery, (c) medical records indicating demented status, and (d) whether participants were suspected of having dementia by someone close to them or the nurses who administered the cognitive battery.

Cognitive data normalizing.

For each cognitive measure, scores were normalized based on means and standard deviations computed for a referent group of SATSA participants age 65-69.99 at their first wave of data collection who were not flagged for having a diagnosis of dementia at that wave. This referent group was selected for purposes of harmonization of cognitive measures across IGEMS studies, as this was the only five-year age band across studies with sufficient coverage within each study or group of studies (i.e., that administered the

same cognitive measures using the same protocol and were therefore pooled for norming) to form the referent group for norming. The referent group consisted of 156 participants. For this group, Symbol Digit (n = 155), scores ranged from 5 to 58 out of 100 (M = 37.06, SD = 9.99); for Block Design (n = 152), scores ranged from 7.14 to 88.10 out of 100 (M = 42.59, SD = 15.46); for Synonyms (n = 155), scores ranged from 16.67 to 100 out of 100 (M = 61.76, SD = 17.04); for Digits backward, (n = 156), scores ranged from 0 to 87.5 out of 100 (M = 49.36, SD = 16.97). For each task, z-scores were computed for each participant in the SATSA sample at each wave using these means and standard deviations. T-scores were then computed by multiplying the z-scores by 10 and adding 50, such that the mean and standard deviation for each task for the referent group were 50 and 10, respectively. T-scores above 50 represented scores above the referent group mean for each task, while T-scores below 50 represent scores below the referent group mean for each task.

Covariates.

Objective social isolation.

As social isolation has been linked with loneliness, cognition, and expression of CTRA genes (Bennett, Schneider, Tang, Arnold, & Wilson, 2006; Cole, 2013; Wilson et al., 2007), analyses assessing relations between loneliness and methylation at CpG sites within CTRA genes were carried out adjusting for longitudinal social isolation. Four items assessing objective social isolation were asked at each of the five waves of the SATSA for which methylation were available. These items asked participants about their marital status, living arrangement, and how often they interact with their twin partners.

The marital status item asked '*what is your marital status?*' Response options varied slightly across waves. At IPT 3, the option '*married/cohabitating*' was offered; at later waves this category was further broken down the separate categories '*married*' and '*cohabitating*'. Response options '*single*', '*divorced*', and '*widow/widower*', were available at each wave. At IPT 5, 6, 8, and 9, the option '*separated*' was also included. Responses on the marital item were coded dichotomously, so that 0 = '*married or cohabitating*' and 1 = '*not married or cohabitating*'.

Living arrangement was assessed with a single item which asked participants '*who do you live with?*' Response options available at each wave were '*alone*', '*husband/wife, fiancé, significant other*', '*twin partner*', '*brothers/sisters*', '*adult children*', '*grandchildren*', '*other relatives*', and '*friend(s)*'. At IPT 3 '*I have a lodger*' and '*I am a lodger*' were also included as possible responses for this item; at IPT 5, 6, 8, 9, and 10 '*paid home help*' was included as a possible response for this item. These three response options were rarely selected—a total of 5 persons selected one of these options across two of these waves (IPT3, $n = 4$ and IPT5, $n = 1$). Living arrangement was coded dichotomously, with 0 = '*live with one or more others*' and 1 = '*live alone*'.

The twin contact items asked '*how often do you see your twin?'* and '*how often do you have telephone or email contact with your twin?*' Response options for each item ranged from '*daily*' to '*never*'. A single twin contact score was computed based on scores on these two items. Participants who reported having in-person or telephone/email contact with their twin partner once a month or more were given scores of 0, those who had contact with their twin less than once a month but at least once a year were given

scores of .5, and those who were in contact with their twin partner less than annually were given scores of 1.

A composite social isolation score was computed by summing scores on the dichotomous marital status and living arrangement variables and the single twin contact score. Scores ranged from 0 to 3 with higher scores representing greater social isolation.

Educational attainment.

Educational attainment was coded from SATSA and other Swedish Twin Registry sources where year equivalents were assigned to the highest level of education attained. The SATSA item asked about the highest level of education individuals had attained with response options of *elementary school*, *vocational school or folk high school* (0-level), *gymnasium* (A-level), and *university or higher*. The four year-equivalent values most common based on responses to the single SATSA item were 6 or 7, 10, 11, and 14 years. Scores were adjusted for country, cohort, and sex differences in required education to reflect individuals' attained education in relation to the standards in place when they attended school in their country of residence for members of each sex. Assigned years ranged from 6 to 16.

Statistical Analyses

Loneliness and Methylation of CpGs Associated with CTRA genes.

The longitudinal associations between loneliness and methylation at 1,586 CpG sites associated with 105 CTRA genes was assessed using both a phenotypic approach and the co-twin control or discordant twin design (Carlin, Gurrin, Sterne, Morley, & Dwyer, 2005; McGue, Osler, & Christensen, 2010; Vitaro, Brendgen, & Arseneault,

2009), a genetically-sensitive approach that is a valuable tool for assessing potential causal links between behavioral and environmental factors and health (Lichtenstein, Gatz, Pedersen, Berg, & McLearn, 1996; Vitaro, Brendgen, & Arseneault, 2009).

Phenotypic approach.

For the phenotypic approach, two sets of Bayesian mixed-effects linear growth curve models were fitted using the 'blme' package version 1.0-4 (Dorie, 2015) in R v.3.4.3 to the longitudinal methylation data for each of the 1,586 CpGs, with (a) baseline loneliness, and (b) time-varying loneliness as predictors of methylation level at age 70 and change in methylation across age. Sex and time-varying social isolation were adjusted for in all model-fitting analyses, and age-based weights, centered on age 70, were used as slope weights. REML estimation was used. Figure 3.1 depicts the growth model fitted to the longitudinal methylation data for the phenotypic analyses.

The models estimated fixed effects of loneliness, centered age, and loneliness x centered age, with random effects on methylation level for each twin pair and zygosity group (MZ and DZ) and for individuals on change in methylation across age, adjusting for fixed effects of sex and time-varying social isolation. Age was centered at age 70 to improve interpretability of intercepts and to enhance model stability; the average age for the sample was 68.96 years at baseline and 72.67 years across waves and data were abundant at this age. The model equation was:

$$Methyl_{ijt} = B_{1i} + B_{2i}AgeC_{ijt} + B_3Sex_j + B_4Soc_{ijt} + B_5Lon_{ijt} + B_6(Lon_{ijt}*AgeC_{ijt}) \quad [1]$$

Methyl refers to longitudinal methylation (M) values for the i^{th} individual in the j^{th} pair at age *t*. CpG, AgeC, Sex, Soc, and Lon refer to effects of centered age, sex, time-varying

social isolation, and either baseline or time-varying loneliness on methylation level at age 70, respectively, and Lon*AgeC refers to the effect of loneliness on methylation slope. In addition, random effects were denoted in the blme equation as follows: "1|Pairid" refers to the intercept variance between twin pairs for MZ twins, "Zyg-1|Pairid" refers to the intercept variance between pairs for DZ twins, and "AgeC|Twinnr" refers to the slope variance among individuals in the sample, assuming no pair similarity for the slope variance. For model-fitting analyses, zygosity was coded 0 = DZ and 1 = MZ. Bayesian models were used to aid in model convergence by imposing priors on the covariance matrices for each of the modeled effects (Dorie, 2015). Effect sizes (*d*) were computed to quantify effects of loneliness on methylation intercept or slope by dividing regression weights (*b*) by the standard deviation of methylation values at each CpG site (Feingold, 2009).

Discordant twin approach.

For the discordant twin analyses, the "mixed approach" to the cotwin control design was used. This approach uses mixed regression models which allow for both dependence of twin data and independence of non-twin data and permit estimation of both between and within-pair effects (Carlin et al., 2005; McGue et al., 2010; Vitaro et al., 2009). The advantage of these models in comparison to the traditionally used difference score models which permit estimation of within-pair effects only (which allows for the assessment of the effect of non-shared experiences for members of each twin pair), the "mixed method" models also permit estimation of between-pair effects (Vitaro et al., 2009).

For these models, loneliness difference scores were computed for each twin pair by first calculating the average loneliness scores for each twin pair at baseline, then subtracting this average score from each of the twins' scores, such that, for pairs discordant in loneliness, the twin who had a loneliness score above the pair mean had a positive difference score and the twin who had a loneliness score below the mean had a negative difference score (Vitaro et al., 2009). Bayesian mixed-effects linear growth models were then fitted to the longitudinal methylation data for each CpG with these mean and difference scores entered as predictors using the 'blme' package version 1.0-4 (Dorie, 2015) in R v.3.4.3. Pair means for loneliness were used for the computation of between-pair effects, and within-pair difference scores for loneliness were used for computation of within-pair effects. Between-pair effects were assumed not to differ between MZ and DZ twin pairs and were constrained to be equal in the models, while within-pair effects of loneliness were allowed to vary for MZ and DZ twins. All models were adjusted for sex and time-varying social isolation. Age-based weights, centered on age 70, were used as slope weights. REML estimation was used. Figure 3.2 depicts a schematic of the growth model fitted to the longitudinal methylation data for the co-twin control analyses.

Since MZ twin partners share 100% of their genetic material and multiple demographic characteristics (e.g., age and sex), the non-lonely twins in MZ twin pairs discordant for loneliness serve as ideal controls to use when comparing DNA methylation in these twins to that of their lonely co-twins (Lichtenstein et al., 1996), resulting in a more precise effect size than would be obtained from groups of unrelated lonely and non-

lonely persons. These analyses accounted for the observation that although DNA methylation is often conceptualized as being driven by environmental exposures, the extent to which each individual is susceptible to DNA methylation given particular environmental exposures is in part determined by genetic influences (Klengel, Pape, Binder, & Mehta, 2014).

Comparing within-pair effects for baseline loneliness on methylation intercept and slope in MZ and DZ twin pairs also permitted testing for potential confounding of the association between loneliness and DNA methylation by genetic or common environmental factors. Specifically, the comparison of within-pair effects for these groups enabled assessment of whether any observed effects of loneliness on methylation level or change were (a) potentially causal in nature (implied when effects were similar for MZ and DZ twins), (b) partially confounded with genetic and common environmental factors (implied when the within-pair association was smaller for MZ twin pairs than for DZ twin pairs), suggesting potential for partial causality in the link between loneliness and methylation at particular CpG sites, or (c) fully confounded with genetic and common environmental factors (implied when a within-pair association is observed for DZ twins but the within-pair association is non-existent for MZ twins), suggesting no causal association between loneliness and methylation (McGue et al., 2010). For co-twin control analyses, baseline was defined as the first wave at which both members of a twin pair had loneliness data. The equation for the models was:

$$Methyl_{ijt} = B_{1i} + B_{2i}AgeC_{ijt} + B_3Sex_j + B_4Soc_{ijt} + B_5\overline{Lon}_{.j} + B_6(\overline{Lon}_{.j}*AgeC_{jt}) +$$

$$B_7(Lon_{ij} - \overline{Lon}_{.j}) + B_8(Lon_{ij} - \overline{Lon}_{.j})*AgeC_{ijt} + B_9(Lon_{ij} - \overline{Lon}_{.j})*(Zyg_j) +$$

$$B_{10}(Lon_{ij} - \overline{Lon}_{.j})*(Zyg_j)*AgeC_{ijt} \quad [2]$$

Methyl refers to methylation (M) values for each CpG site, AgeC refers to age centered at age 70, Soc refers to the fixed effect of time-varying social isolation on methylation level at age 70 and Lon refers to baseline loneliness. Within pair effects of loneliness on methylation intercept and slope were permitted to vary for MZ (B_9 and B_{10}) and DZ (B_7 and B_8) twin pairs. In addition, random effects were denoted in the blme equation as follows: "1|Pairid" refers to the intercept variance between twin pairs for DZ twins, "Zyg-1|Pairid" refers to the intercept variance for MZ twins, and "AgeC|Twinnr" refers to the slope variance among individuals in the sample. As mentioned above, zygosity was coded such that 0 = DZ and 1 = MZ. Fixed effects for MZ twins estimated the deviation of each effect for MZ twins from that for DZ twins. MZ effects were computed by adding estimated effects for MZ and DZ twins.

For all analyses, parameters with *z*-values $\geq$ |1.96| were considered nominally significant at $p = .05$. The critical *z*-value after correcting for multiple testing was computed using a Bonferroni correction. The significance criterion of .05 was divided by the number of tests (1,586). The resulting value (.0000315) was divided by 2 to obtain the values at each end of the distribution (|.0000158|) associated with significance at .05 correcting for multiple testing. The critical *z*-value corresponding to this *p*-value was computed using R software v.3.4.3 using the qnorm function. The *z*-value required to reach significance at .05 after correcting for multiple testing was |4.16|.

Loneliness, Methylation, and Cognition.

Associations between time-varying loneliness, methylation at sites associated with loneliness (as indicated by results of phenotypic and co-twin control analyses), and cognitive performance and change were assessed using a series of hierarchical regression models to explore the extent to which (a) loneliness predicted cognitive performance or change, (b) methylation at each site associated with loneliness predicted cognitive performance or change, and (c) associations between loneliness and cognition were attenuated when methylation at each of these sites was added to the model. For model-fitting analyses, age was centered at 70 years, education was centered at 12 years, and methylation values mean centered for each CpG site.

Unconditional models (Models A – C).

For each cognitive task, unconditional linear (Model A), quadratic (Model B), and spline (Model C) models were first fitted to the data to determine which best characterized cognitive change across age. Spline models were 2-slope models that estimated effects on change in cognitive performance across age prior to age 70 (slope A) and at/after Age 70 (slope B). Nested models were compared using chi-square difference tests. All models accounted for nesting of individuals within twin pairs, specifically by allowing between pair and within pair random effects for the intercept. Random effects for slope were modeled at the individual level only and did not include between pair effects to increase model stability.

Conditional models (Models D – G).

In Model D, fixed effects for covariates (i.e., sex and centered education) on intercept were added to the model. Fixed effects terms were not added for covariates on

slope, due to issues with model stability when these terms were included. In Model E, fixed effects terms for loneliness on intercept and slope were added to assess associations between loneliness and cognitive performance and change for each domain. To assess associations between methylation and cognition and to explore whether associations between loneliness and cognition were attenuated when methylation was added to the model, in Models F and G, fixed effects terms for methylation values on intercept and slope were added; separate models were run for each CpG of interest. Chi-square difference tests were used to assess whether model fit significantly improved for each successive model. As social isolation scores were associated with loneliness but not with cognition, social isolation was not adjusted for in model-fitting analyses of cognition.

Results

Descriptive Statistics

Loneliness.

For the analysis sample at baseline, loneliness person measure scores ranged from -3.14 to 5.77 (*M* = -1.62, *SD* = 2.34). For context, *N* = 224 (62.75%) reported on the raw item that they felt lonely '*rarely or none of the time*', *n* = 86 (24.09%) reported feeling lonely '*some of the time*', *n* = 38 (10.64%) reported feeling lonely '*occasionally*', and *n* = 9 (2.52%) reported feeling lonely '*most of the time*'. A similar pattern was observed across waves, with person measure scores ranging from -3.14 to 5.77 (*M* = -1.64, *SD* = 2.33). Descriptive statistics for loneliness and covariates are listed in Table 3.1.

Cognition.

Processing speed (Symbol Digit task).

Scores on the Symbol Digit task (n = 343) tended to decline with age both longitudinally and cross-sectionally at baseline. Figure 3.3 shows a longitudinal trajectory plot of Symbol Digit *T*-scores across age that illustrates this within-person trend of change across age. Cross-sectional means by age group at baseline are reported in Table 3.2. The average Symbol Digit *T*-score at baseline was M = 52.08 (SD = 12.16).

Spatial ability (Block Design task).

Scores on the Block Design task (n = 349) also showed a pattern of decline with age, both cross-sectionally at baseline and longitudinally. Figure 3.4 shows a longitudinal trajectory plot of Block Design *T*-scores across age by age that illustrates this trend. Cross-sectional means by age group at baseline are reported in Table 3.2. The average Block Design score at baseline was M = 53.98 (SD = 11.97).

Working memory (Digits Backward task).

Scores on the Digits Backward task (N = 360) showed consistency in range and variability across age and did not clearly show a pattern of decline with age (see Figure 3.5 for a longitudinal trajectory plot of Digits Backward *T*-scores by age that illustrates this trend). At baseline, scores showed a pattern of cross-sectional decline with age that was less drastic than that observed for the previously described tasks (see Table 3.2). The average *T*-score on the Digits Backward task at baseline was M = 51.46 (SD = 10.44). For this task, one participant had a raw score of 2 at IPT3 for this task, which was set to missing as this score was outside the possible range of scores for this task and may have resulted from a coding error.

Verbal comprehension (Synonyms task).

Scores on the Synonyms task (n = 347) showed stability both longitudinally and cross-sectionally at baseline, although individual differences in change with age were observed (see Figure 3.6). Cross-sectional means by age group at baseline are reported in Table 3.2. The average T-score on the Synonyms task at baseline was M = 53.80 (SD = 9.36).

Covariates.

Social isolation composite.

Scores on the social isolation composite measure ranged from 0 (least isolated based on available measures across waves) to 3 (most isolated based on available measures across waves). For the analysis sample at baseline, the average social isolation score was .88 (SD = 1.00). The average score and standard deviation were slightly higher across waves (M = .93, SD = 1.03).

Educational attainment.

Educational attainment was assessed for all participants in the sample. For the analysis sample for cognitive data analyses (n = 361), adjusted scores for years of education ranged from 6 to 16 (M = 8.55 years, SD = 2.55).

Correlations

Table 3.3 lists correlations between study variables at baseline for the analysis sample. Partial correlations were computed adjusting for age and sex (except when one of these variables was being correlated). Loneliness showed small positive correlations with age (r = .16, p = .0014) and sex (ρ = .13, p = .0145), suggesting that greater loneliness was associated with being older and female. Loneliness was moderately positively

associated with social isolation scores (r = .32, p < .0001), which indicated that there was a relation between greater loneliness and greater social isolation. Associations between loneliness and scores on the Symbol Digit (r = -.10, p = .0592), Block Design (r = -.13, p = .0149), and Synonyms tasks (r = -.09, p = .0843) were small and negative, suggesting minor relations between greater loneliness and poorer performance on these tasks, although the correlations between loneliness and Symbol Digit and Synonyms *T*-scores only approached significance. There was no association observed between loneliness and Digits Backward *T*-scores (r = -.06, p = .2187). The association between loneliness and years of education was small and negative (r = -.12, p = .0230), indicating a small relation between having fewer years of education and reporting greater feelings of loneliness. Correlations between years of education and *T*-scores on each of the cognitive tasks were positive and ranged from small to moderate. *T*-scores for each of the cognitive tasks were not associated with social isolation after adjusting for age and sex.

Baseline Loneliness Growth Analysis

Estimates of effects of baseline loneliness on methylation level at age 70 (intercept) or linear change in methylation across age (slope) had nominal significance with z values $\geq$ |1.96| for 88 and 46 of the 1,586 CpGs, respectively (overall N_{CpG} = 130). No effects reached significance after correcting for multiple testing ($range_{z}$ = -3.49 to 3.15, $z_{critical}$ = 4.16). The 130 CpGs with nominal effects of baseline loneliness on methylation level or slope and their associated genes are listed in Table 3.4. Frequency distributions for unstandardized effects of baseline loneliness on methylation intercept and slope with $z \geq$ |1.96| are shown in Figure 3.7.

As an initial exploration of effects of loneliness on methylation, the distributions of unstandardized regression weights (*b*) for intercept and slope were examined. The 5 CpGs with the largest unstandardized regression weights (with *b* between .047 and .067) for effects of baseline loneliness on methylation intercepts were all located within *PF4* (cg02530824, cg05509609, cg06834998, cg16072462, cg21043213), a gene involved in blood clot formation, inflammation, and immune function (National Center for Biotechnology Information, 2019a). All effects for CpGs within this gene were positive, suggesting that higher loneliness was associated with greater methylation at these sites at age 70. Standard deviations for methylation at these sites ranged from 0.96 to 1.38, and standardized effects (*d*) ranged from 0.05 to 0.06 (see Table 3.5 for regression weights, standard deviations, and effect sizes for effects of baseline loneliness on methylation intercept and slope for these CpGs). No effects of baseline loneliness on slope were observed at these sites. Information from the Ensembl database indicated that each of these CpGs was located within a promotor region, or a region within a DNA strand where transcription factors bind to DNA to initiate or inhibit gene expression (Adcock & Caramori, 2009; National Center for Biotechnology Information, 2019b). A single CpG (cg05468843, *IL10RA*) had a regression weight of .045 ($z = 2.14$) for the effect of loneliness on slope ($SD = 1.12$, $d = 0.04$). This effect indicates a Cohen's *d* equivalent increase in methylation at this site of 0.04 per year and hence would equal .18 across five years for each one-unit increase in loneliness. Information from the Ensembl regulatory database indicated that this CpG was also located within a promotor region; this gene has been reported to be involved in inhibition of inflammatory processes (National Center for

Biotechnology Information, 2019c). Note that other CpG sites may have different scaling for methylation values, and *d* values may vary more from the unstandardized estimates; *M* values generally range from -6 to 6 with *SD*s that are generally 1.5 or lower.

For 4 CpG sites (cg26009195 in *CDC25B*, cg22147449 in *DDX17*, cg01085225 in *STAT1*, and cg19472303 in *TOP2B*), effects of baseline loneliness on *both* intercept and slope were observed with $z \geq |1.96|$. For three of these CpGs, effects of loneliness on intercept were negative while effects on slope were positive, suggesting slightly lower methylation at age 70 for lonely persons and slightly faster increase in methylation for lonely persons at these sites. For the fourth CpG, the opposite pattern was observed, with effects of baseline loneliness indicating slightly higher levels of methylation at age 70 and slightly faster decline in methylation at these sites for lonely persons. Information from the Ensembl regulatory database indicated that each of these four CpGs was located within a promotor region. Table 3.6 shows regression weights, standard deviations, and effect sizes (*d*) for effects of baseline loneliness on methylation intercept and slope for these CpGs.

As no effects reached significance after correcting for multiple testing, we explored any effects with *z*-values above |3|. For 5 CpGs, effects of baseline loneliness on methylation level at $z \geq |3|$ were observed. For cg01085225 (*STAT1*) and cg22147449 (*DDX17*), effects of loneliness on the intercept were negative (b_{I70} = -0.01, *SD* = 0.13, *d* = -0.08 and b_{I70} = -0.01, *SD* = 0.20, *d* = -0.05 respectively), suggestive of reduced methylation at these sites at age 70 in lonely persons. Effects of baseline loneliness on intercept were positive for cg16787284 (*NEDD5*; b_{I70} = 0.01, *SD* = 0.09, *d* = 0.11),

cg20357806 (*PPBP*; b_{I70} = 0.03, *SD* = 0.54, *d* = 0.05), and cg26439015 (*SLC12A7*; b_{I70} = 0.01, *SD* = 0.12, *d* = 0.08), indicating that higher methylation at age 70 was associated with loneliness at these sites. An effect of baseline loneliness on methylation slope at $z \geq |3|$ was observed for 1 CpG. For cg26661481 (*IL10RA*), the effect of loneliness on slope was positive (b_S = 0.03, *SD* = 0.47, *d* = 0.06), suggestive of a faster increase in methylation with age in lonely persons at age 70. Table 3.7 shows regression weights and *z*-values for effects of baseline loneliness on methylation intercept and slope for these 6 CpGs. Regression weights and *z*-values for effects of baseline loneliness on methylation intercept and slope for the 130 CpGs with $z \geq |1.96|$ are listed in Table A9 in Appendix 5. Regression weights and *z*-values for all predictors are shown for all 1,586 CpGs in Supplement 3.1 for the baseline phenotypic analysis.

Regulatory feature types for 81 of the 130 CpGs with $z \geq |1.96|$ were extracted from the Ensembl database. Frequencies for each feature type for these CpGs are listed in Table 3.8. The majority of the CpGs with regulatory feature information (N_{CpG} = 68; 52.3%) were located within promotor regions. Others (N_{CpG} = 6, 4.6%) were located within open chromatin (regions that contain several types of regulatory sequences; Song et al., 2011), promotor flanking regions (i.e., regions located next to promotors; Shimoyama et al., 2015; N_{CpG} = 4, 3.1%), a CTCF binding site (a site at which CTCF—a protein that can either facilitate or hinder gene expression—binds to DNA; Holwerda & de Laat, 2013; N_{CpG} = 1, 0.8%), an enhancer region (i.e., a region located near a promotor that facilitates gene transcription; Nature Education, 2014; N_{CpG} = 1; 0.8%), and a

transcription factor (TF) binding site (i.e., a site at which transcription factors bind to DNA to initiate or inhibit gene expression; Adcock & Caramori, 2009; $N_{CpG} = 1$; 0.8%).

Time-Varying Loneliness Growth Analysis

Effects of time-varying loneliness on methylation level and change were examined for the 130 CpGs with nominally significant effects of baseline loneliness on methylation. Effects of time-varying loneliness on intercept or slope at $z \geq |1.96|$ were observed for 32 of these CpGs. Regression weights (*b*) and effect sizes (*d*) for both the baseline and time-varying analyses are shown for these CpGs in Table 3.9. For one CpG (cg00619097, *CPT1B*), the effect of time-varying loneliness on slope ($b = .04$, $z = 4.22$) reached significance after correcting for multiple testing ($SD = 0.59$, $d = 0.07$). This effect indicates a Cohen's *d* equivalent increase in methylation at this site of 0.07 per year and 0.35 across five years for each one-unit increase in loneliness. This is illustrated in the longitudinal plot of *M* values by loneliness for this CpG in Figure 3.8. The *CPT1B* gene encodes a protein involved in oxidation of long chain fatty acids in mitochondria of cells and may protect against cellular damage in response to particular (cellular) environmental exposures (Henique et al., 2010; National Center for Biotechnology Information, 2019d).

Half of the 32 CpGs had effects for both baseline and time-varying loneliness on methylation level at age 70 at $z \geq |1.96|$ and no effects of loneliness on slope. For these CpGs, unstandardized regression weights were approximately equal across the two analyses; and otherwise deviations tended to be slightly smaller effects for time-varying loneliness. One exception where the effect was slightly larger for time-varying loneliness

was observed (see cg11235297 in *SLC12A7* in Table 3.9). Four CpGs had effects for both baseline and time-varying loneliness on slope at $z \geq |1.96|$ and no effects of loneliness on intercept. For 3 of these CpGs, effects did not vary between the two analyses. For the fourth CpG (cg00733150 in *C22orf8*), the effect was smaller for time-varying loneliness (b = .01) than for baseline loneliness (b = .03). For 6 CpGs, the effect for one analysis (i.e., baseline or time-varying loneliness) was on the intercept while the effect for the other analysis was on the slope. For 5 CpGs, 3 of the 4 effects of loneliness on intercept and slope for the baseline and time-varying analyses were nominally significant at $z \geq |1.96|$, (see cg00619097 in *CPT1B*, cg12262427 in *PTPN12*, cg01085225 in *STAT1*, cg22147449 in *DDX17*, and cg10435849 in *COL6A2* in Table 3.9), and for 1 CpG (cg19472303 in *TOP2B*) all 4 effects were nominally significant. For this CpG, effects of baseline and time-varying loneliness on the intercept were negative (b = -.01) and effects on slope were positive (b = .01). See Supplement 3.2 for regression weights and *z*-values for all predictors for each of the 1,586 CpGs from the time-varying phenotypic analysis. Table 3.10 summarizes results for baseline and time-varying growth analyses.

Co-Twin Control Analyses

Growth modeling results for between and within-pair effects of loneliness on methylation intercept and slope were examined for the 130 CpGs with effects of baseline loneliness at $z \geq |1.96|$ in phenotypic analyses. Among these, co-twin control results were interpreted for CpGs that met certain criteria. These included (a) a nominally significant within-pair effect for DZ twins, (b) within-pair effects for MZ and DZ twins that were in the same direction (i.e., both were either positive or negative), and (c) a nominally

significant between-pair effect. Co-twin control results were examined for CpGs for which these criteria were met *either* for the effect of loneliness on methylation intercept or for the effect of loneliness on methylation slope. Potential confounding of observed associations was examined by computing the percent reduction in nominally significant effects for MZ twins compared to DZ twins. The formula used to compute percent reduction was: % reduction = $1 - (MZ_{effect}/DZ_{effect})$. A percent reduction value of 0 signifies the absence of genetic or common environmental confounding, while a value of 1 indicates complete confounding. Values in between 0 and 1 indicate varying levels of partial confounding by genetic or common environmental factors.

Within-pair DZ effects of baseline loneliness on methylation intercept or slope with *z*-values above |1.96| were observed for 25 of these CpGs. Of these, 8 also had nominally significant between-pair effects for effects of baseline loneliness on methylation intercept or slope (see bolded rows in Supplement 3.3). For 2 CpGs (cg00403457 in *PTPN12* and cg26661481 in *IL10RA*) all three criteria were met for the effect of loneliness on methylation intercept and the effect of loneliness on methylation slope, respectively. For cg00403457 in *PTPN12*, both between (B) and within-pair (W) DZ effects of loneliness on methylation level at age 70 were nominally significant and within-pair effects of loneliness on intercept were negative for both MZ and DZ twins (see Table 3.11 for summary of co-twin control results). The observed effects of loneliness on intercept suggest an association between loneliness and reduced methylation at this site at age 70. A reduction in effect size for MZ compared to DZ twins of 87.4% was observed, suggesting near complete confounding of this association by

genetic and common environmental factors (see Table 3.11). These results were consistent with phenotypic results showing small negative effects of baseline and time-varying loneliness on intercept with $z \geq |1.96|$ and no effects of baseline or time-varying loneliness on slope (see Table 3.9). The gene *PTPN12* is a tumor suppressor gene involved in cellular development and reproduction, and conversion of normally functioning cells into cancer cells (Luo et al., 2014; National Center for Biotechnology Information, 2019e; Xunyi, Xhentao, Dandan, & Funian, 2012).

For cg26661481 in *IL10RA*, both between and within-pair effects of loneliness on age-related change in methylation were nominally significant, and within-pair effects of loneliness on slope were positive for both MZ and DZ twins (see Table 3.11). Effects of loneliness on slope were indicative of faster age-associated increase in methylation associated with loneliness at this site. A reduction in effect size for MZ compared to DZ twins of 36.8% was observed, suggesting partial confounding of this association by genetic and environmental factors. These results were consistent with phenotypic results showing very small nominally significant positive effects of baseline and time-varying loneliness on slope. A very small negative effect at nominal significance was also observed for time-varying loneliness on methylation intercept in the phenotypic analyses, which was consistent with non-significant estimates for effects of loneliness on intercept in co-twin control analyses (see Table 3.9). See Figures 3.9 and 3.10 for longitudinal plots of methylation values for cg00403457 in *PTPN12* and cg26661481 in *IL10RA*. Supplement 3.3 lists effect sizes and *z*-values for each of the 1,586 CpGs from the co-twin control analysis.

Loneliness, Methylation, and Cognition

The extent to which DNA methylation at the 2 CpG sites for which associations between loneliness and methylation were examined in co-twin control analyses (cg00403457 in *PTPN12* and cg26661481 in *IL10RA*) were associated with cognition and/or attenuated associations between loneliness and performance and change in processing speed (Symbol Digit task), spatial ability (Block Design task), and verbal comprehension (Synonyms task) was explored. Models were not fitted for working memory (Digits Backward), as there was no correlation between loneliness and scores on this task (see Table 3.3).

Comparison of fit for unconditional models showed that the quadratic model fit better for the Block Design and Synonyms tasks ($\Delta\chi^2(4) = 29.8$, $p < .0001$, and $\Delta\chi^2(4) = 22.7$, $p < .0001$, respectively) than the spline model ($\Delta\chi^2(4) = 26.9$, $p < .0001$, and $\Delta\chi^2(4) = 20.0$, $p < .0001$, respectively). For Symbol Digit, the spline model ($\Delta\chi^2(4) = 29.3$, $p < .0001$) fit slightly better than the quadratic model ($\Delta\chi^2(4) = 27.5$, $p < .0001$). For this task, the covariance parameter for linear change hit a boundary of 0 in the unconditional quadratic model and the covariance parameter for linear change prior to age 70 hit 0 in the unconditional spline model. Parameters that hit 0 in unconditional models were removed from successive models. After dropping these parameters, model fit remained slightly better for the spline model ($\Delta\chi^2(1) = 27.0$, $p < .0001$) than the quadratic model ($\Delta\chi^2(1) = 22.8$, $p < .0001$). As the difference in fit for these Symbol Digit models was small and the quadratic model fit best for the other tasks, the quadratic model was selected for model fitting analyses.

Processing speed (Symbol Digit).

As the random effect for linear slope hit 0 in the unconditional quadratic model, interactions of covariates with linear age were not modeled for this task. Adding covariates (fixed effects on level for sex and years of education) to the reduced unconditional quadratic model significantly improved model fit ($\Delta\chi^2(2) = 34.6$, $p < .0001$), whereas subsequently adding loneliness to the model with covariates added did not ($\Delta\chi^2(2) = 1.7$, $p = .4274$). No effects of loneliness on processing speed level at 70 or quadratic change with age were observed. Adding methylation at cg00403457 (*PTPN12*) to the model significantly improved model fit ($\Delta\chi^2(2) = 8.5$, $p = .0143$). Significant effects for methylation at this site on Symbol Digit performance at age 70 ($b = -1.44$, $p = .0040$) and quadratic change ($b = 0.0079$, $p = .0207$) were found, suggesting that greater methylation at this site was associated with reduced performance at age 70 on the Symbol Digit task and dampened the quadratic trend. Sensitivity analyses retaining the linear random effect and interaction terms in the model were run to exclude the possibility that these effects were artifacts of restructuring the model's random effects. Effects of methylation at cg00403457 (*PTPN12*) on both intercept ($b = -1.39$, $p = .0054$) and quadratic slope ($b = 0.007$, $p = .0262$) remained, indicating that these effects were observed regardless of whether these linear terms were included in the model. As the spline model fit better than the quadratic model for Symbol Digit, spline models were also fitted for cg00403457 in *PTPN12* (the random effect for the linear trend prior to 70 years (slope A) hit a boundary of 0 and was excluded from this model). Contrary to what was observed for quadratic models, adding methylation at cg00403457 (*PTPN12*) to the

spline model did not significantly improve model fit ($\Delta\chi^2(2) = 4.4$, $p = .1108$). Despite this, the spline model indicated a significant negative effect of methylation at this site on the intercept ($b = -1.04$, $p = .0361$) and no effect on linear change after age 70 (slope B; $b = 0.08$, $p = .2091$). Adding cg26661481 (*IL10RA*) to the model did not significantly improve model fit ($\Delta\chi^2(2) = 0.9$, $p = .6376$). Overall, adding methylation to the model did not attenuate non-significant effects of loneliness. Fit statistics and unstandardized estimates of fixed effects are displayed in Tables 3.12 and 3.13 for Symbol Digit models.

Spatial ability (Block Design).

As was seen for Symbol Digit, adding covariates to the unconditional quadratic model for the Block Design task significantly improved fit ($\Delta\chi^2(2) = 32.6$, $p < .0001$), while subsequently adding loneliness to the model with covariates did not ($\Delta\chi^2(3) = 5.2$, $p = .1577$). No significant effects of loneliness on level at 70, linear change, or quadratic change were found. Model fit did not significantly improve after adding methylation values for cg00403457 (*PTPN12*) or cg26661481 (*IL10RA*) to the model (see Table 3.14). No significant effects of methylation at these sites were observed on Block Design scores at age 70, linear change at 70 years or quadratic change in scores across age. No attenuation of non-significant effects of loneliness were observed after adding methylation values for each CpG to the model. See Tables 3.14 and 3.15 for fit statistics and unstandardized estimates of fixed effects for Block Design models.

Verbal comprehension (Synonyms).

As was observed for the other tasks, adding covariates to the unconditional quadratic model significantly improved fit ($\Delta\chi^2(2) = 41.9$, $p < .0001$), and adding

loneliness to the model with covariates did not ($\Delta\chi^2(3) = 1.0$, $p = .8012$). No significant effects of loneliness on Synonyms performance at age 70, linear slope, or quadratic slope were observed. Model fit did not significantly improve after adding methylation values for cg00403457 (*PTPN12*), or cg26661481 (*IL10RA*) to the model ($\Delta\chi^2(3) = 3.9$, $p = .2725$, and $\Delta\chi^2(3) = 6.1$, $p = .1068$, respectively). For cg26661481 in *IL10RA*, a significant effect of methylation on linear slope was found ($b = 0.09$, $p = .0147$), and a trend significant attenuated effect on linear slope showing less change at age 70 was observed for cg00403457 in *PTPN12*. No significant effects were observed for this CpG on level at 70 or quadratic change with age. Fit statistics and unstandardized estimates of fixed effects are displayed in Tables 3.16 and 3.17 for Synonyms models.

Discussion

This study assessed associations between feelings of loneliness and DNA methylation at 1,586 CpG sites within 105 CTRA genes in blood leukocytes using both phenotypic and co-twin control approaches and explored whether a potential mediational role may exist for methylation at sites with loneliness-methylation associations in the relation between loneliness and domain-specific cognitive performance and change. Although prior work has linked enduring feelings of loneliness and altered expression of CTRA genes (Cole et al., 2007; Creswell et al., 2012), no published work to date has examined relations between loneliness and DNA methylation. Evidence for methylation and cognitive and dementia outcomes exists (i.e., APOE and dementia, Karlsson et al., 2018; Marioni et al, 2018; but see Schiepers et al., 2012), however, links between DNA

methylation and cognitive change trajectories have not been evaluated considering pathways vis-à-vis loneliness.

In the first part of this study, associations between loneliness and methylation at CpG sites within CTRA genes were assessed in a subsample from the SATSA study for whom methylation data were collected. Potential confounding of associations between loneliness and methylation by genetic or common environmental factors was assessed by comparing within-pair effects of loneliness on methylation for MZ and DZ twins. As the relation between loneliness and DNA methylation was previously unknown, these analyses were considered exploratory. Although feelings of loneliness have been linked to altered expression of CTRA genes (Cole et al., 2007; Creswell et al., 2012), much remains to be understood about the interplay of epigenetic factors in regulating gene expression (Lam et al., 2012) and whether DNA methylation plays a role in such altered expression remains unknown. Observed effects of loneliness on methylation level at age 70 or change in methylation with age were small and most did not survive multiple testing. Phenotypic modeling analyses revealed one significant effect of time-varying loneliness on methylation slope for cg00619097 in *CPT1B* that surpassed the multiple testing, with faster increase in methylation with age associated with greater loneliness. Non-significant negative effects of loneliness on intercept agreed with prior work indicating a link between loneliness and increased expression of the *CPT1B* gene (Cole et al., 2007), although significant age-related increases in methylation associated with loneliness were also observed at this site. The *CPT1B* gene is involved in cellular metabolism, specifically in oxidation of long chain fatty acids, and its expression has

been linked with potential protective effects against cellular damage in response to particular exposures and accumulation of cellular toxins in mitochondria (Henique et al., 2010; Karlic et al., 2003; National Center for Biotechnology Information, 2019d). Age-associated decreases in expression have been observed for this gene, and it has been proposed that such decreased expression may play a role in aging processes in healthy adults (Karlic et al., 2003). The observed increase in methylation with age associated with loneliness at this site suggests a possible link between loneliness and decreased expression of this gene with age, which could further reduce its protective effects over time for lonely persons. Examination of effects of loneliness on methylation slope for 17 other sites within *CPT1B,* however, showed that associations between loneliness and methylation were negative for 14 of these sites (unstandardized regression weights ranged from -0.013 to 0.006 for these sites; none of these effects reached nominal significance), suggesting that the positive association observed between loneliness and methylation slope at this site may not represent patterns of change in methylation with age associated with loneliness at other sites within this gene.

Co-twin control analyses indicated nominally significant associations between baseline loneliness and methylation level for cg00403457 in *PTPN12* and age-associated change in methylation for cg26661481 in *IL10RA*, with reduced methylation at age 70 associated with loneliness for cg00403457 (*PTPN12*), and faster age-related increase in methylation associated with loneliness for cg26661481 (*IL10RA*). Comparison of within-pair effects for MZ and DZ twin pairs suggested near complete confounding of effects of loneliness on methylation level at age 70 for cg00403457 in *PTPN12* and partial

confounding of effects of loneliness on change in methylation with age for cg26661481 in *IL10RA*. Results for cg00403457 in *PTPN12* did not align with previous results indicating that loneliness is associated with reduced expression of this gene (Cole et al., 2007). Interestingly, *PTPN12* is a tumor-suppressor gene and increased expression of this gene has been shown to be protective against tumor formation (e.g., Luo et al., 2014; Xunyi, Xhentao, Dandan, & Funian, 2012). It has also been shown that methylation at sites within this gene results in silencing of its expression (Luo et al., 2014; Xunyi et al., 2012). Additional exploration of results for 23 additional sites within this gene from the baseline phenotypic analysis revealed that effects of loneliness on methylation intercept were also negative for 17 of these sites (unstandardized regression weights ranged from -0.021 to 0.007; only one effect reached nominal significance). Collectively, our results are indicative of a potential protective effect of loneliness with respect to changes in expression of this gene with age, although the extent to which expression of this gene is linked with methylation at these sites is currently unknown.

For cg26661481 in *IL10RA*, non-significant negative effects of loneliness on intercept were in line with prior work indicating a relation between increased expression of the *IL10RA* gene and loneliness (Cole et al., 2007), although the observation that loneliness predicted faster increase in methylation with age at this site at nominal significance was not. The *IL10RA* gene has been shown to be involved in inhibition of inflammatory processes and inhibition of the development of inflammatory intestinal disorders including inflammatory bowel disease and Chron's disease (National Center for Biotechnology Information, 2019c; Shoval et al., 2014). Our result showing greater age-

associated increases in methylation at this site is suggestive of a potential reduction in expression of this protective factor with age associated with loneliness, although further work using a gene-wide approach is warranted and, as mentioned above, the extent to which methylation at these sites is linked with altered expression of this gene remains unknown. Exploration of effects of loneliness on slope for 6 other CpG sites within this gene from the baseline phenotypic analysis revealed that effects of loneliness on slope were positive for 5 of these CpGs (unstandardized regression weights ranged from -0.004 to 0.04; only one effect reached nominal significance), further supporting a potential link between loneliness and reduced expression of this protective factor with age that warrants further exploration.

In the second part of this study, the extent to which loneliness and DNA methylation (at CpG sites for which associations between loneliness and methylation were observed) each predicted scores on tasks measuring processing speed, spatial ability, and verbal comprehension was explored. It was hypothesized that loneliness would be associated with cognitive performance and/or change; analyses assessing associations between methylation and cognition were considered exploratory. Follow-up analyses assessed whether methylation levels at these sites may play a mediational role in associations between loneliness and domain-specific cognition. We reasoned that if methylation at CpG sites within genes linked with inflammatory processes was linked with loneliness, that methylation at these sites may play a mediational role in the loneliness-cognition association.

Associations between loneliness, cognition, and methylation at sites associated with loneliness identified in the first stage (i.e., cg00403457 in *PTPN12* and cg26661481 in *IL10RA*) were explored by fitting a series of hierarchical regression models to the longitudinal cognitive data with time-varying loneliness and time-varying methylation as predictors of performance on tasks tapping processing speed, spatial ability, and verbal comprehension. The hypothesis that loneliness would be associated with cognitive performance or change for the domains assessed was not supported—no associations between loneliness and cognitive performance or change were observed for processing speed, spatial ability, or verbal comprehension. Importantly, however, non-significant effects of loneliness on processing speed, spatial ability, and verbal comprehension performance at age 70 were in the expected direction (i.e., were negative), as were effects of loneliness on quadratic change in spatial ability and of loneliness on linear change with age in verbal comprehension. The finding that loneliness is not significantly associated with cognition does not align with prior work showing associations between higher levels of loneliness and reduced processing speed performance (O'Luanaigh et al., 2012; Wilson et al., 2007), faster decline in processing speed (Wilson et al., 2007), poorer performance and faster decline in spatial ability (Wilson et al., 2007), and reduced working memory performance in older adults. This discrepancy may stem from the limited power to detect (potentially small) effects in the present study due to small sample size.

Effects of methylation at each of the CpG sites on cognitive performance and change varied both among CpGs and within CpG for different domains of cognition. For

spatial ability (Block Design), no effects of methylation on performance at age 70 or change with age were observed. For processing speed (Symbol Digit), methylation at cg00403457 in *PTPN12* predicted significantly reduced performance at age 70 and dampened the quadratic trend, while no associations were observed for cg26661481 in *IL10RA* for this domain. For verbal comprehension (Synonyms), methylation at cg26661481 in *IL10RA* predicted significantly predicted faster linear increase in verbal comprehension with age, while methylation at cg00403457 in *PTPN12* predicted an attenuated effect on linear slope showing less change at age 70 at trend significance, although likelihood ratio tests indicated that model fit did not significantly improve when methylation at these sites was added to each model. These results build upon previous findings indicating a link between methylation at CpG sites within the *APOE* gene (Karlsson et al., 2018) and provide further support that CpG-specific methylation is important to domain-specific cognitive function. However, none of the three prioritized CpGs achieved significance in a recent epigenome-wide study of cognitive abilities across 11 samples (Marioni et al., 2018). Our results hint that methylation at particular CpG sites may differentially relate to different domains of cognition, and that methylation at different sites may be differentially important to different domains of cognitive functioning warranting future work. Although direct effects of loneliness on cognition were not observed, the results were suggestive of a potential role in the loneliness—processing speed association for methylation at cg00403457 in *PTPN12*. Although it has been suggested that mediation should not be assessed for factors not significantly directly associated, it has also been argued that a significant direct

association is not required for mediation to occur, and that factors may be linked indirectly even when a direct effect is not observed (MacKinnon & Fairchild, 2009).

Overall, results from this study suggest hints of altered methylation associated with loneliness, indicating possible sensitivity of methylation levels at particular CpG sites within CTRA genes to experiences of perceived loneliness. Comparison of the current results and prior work on loneliness and CTRA gene expression was not strongly suggestive of a potential role for DNA methylation in altered expression of CTRA genes observed by Cole et al. (2007), as results did not align in terms of directionality for cg00403457 in *PTPN12* (with reduced methylation at age 70 associated with loneliness in the current study and reduced expression of the *PTPN12* gene associated with loneliness reported by Cole et al. (2007)), and weakly aligned for cg00619097 in *CPT1B* and cg26661481 in *IL10RA*—only non-significant negative effects of loneliness on methylation level aligned with prior findings of elevated expression of these genes linked with loneliness (Cole et al., 2007), while nominally significant increases in methylation with age associated with loneliness were also noted, which suggest potential reductions in expression of this gene with age associated with loneliness. The current results indicated no association between loneliness and processing speed, spatial ability, working memory, or verbal comprehension, but were suggestive of a relation between methylation at a CpG site within the *PTPN12* gene and performance and change in processing speed.

One strength of this study was the use of five waves of twin data collected across 18 years, which enabled assessment of longitudinal associations between loneliness, DNA methylation of CTRA genes, and domain-specific cognitive performance and

change accounting for differences in genetic relatedness among twins in the sample. Another strength of this study involved use of both phenotypic and co-twin control designs to assess associations between loneliness and methylation at CpG sites within CTRA genes. A limitation of this study was small sample size (and therefore limited power to detect effects).

This study provided an initial assessment of associations between loneliness and DNA methylation, but much work remains to fully elucidate how loneliness relates to methylation and how methylation associated with loneliness may contribute to gene expression or other outcomes. Future work evaluating relations between loneliness and genome-wide DNA methylation and links between loneliness and CpG or gene-specific methylation at sites not examined in this study is needed, as is work exploring how altered DNA methylation associated with loneliness within particular genes relates to altered gene expression. Results from this study revealed associations between DNA methylation at 2 of the 3 CpG sites examined and domain specific cognitive performance and/or change. These findings suggest that further work should examine the extent to which methylation at other CpG sites or within particular genes relate to mid and late-life performance and change in multiple specific domains of cognitive function and explore whether methylation at particular sites mediates associations between environmental exposures and these cognitive outcomes.

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Table 3.1

Descriptive Statistics for Loneliness and Covariates

	M	***SD***	***Min***	***Max***
Baseline				
Age	68.96	9.66	48	94
Loneliness	-1.62	2.34	-3.14	5.77
Social Isolation	0.88	1.00	0	3
Years of Education	8.55	2.55	6	16
Across Waves				
Age	72.67	9.70	48	99
Loneliness	-1.64	2.33	-3.14	5.77
Social Isolation	0.93	1.03	0	3

Table 3.2

Descriptive Statistics for Cognitive Measures by Age Group at Baseline

Baseline	***Baseline (Overall)***		***<60 years***		***60-69 years***		***70-79 years***		***80+ years***	
	M	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***
Symbol Digit	52.08	12.16	60.79	8.43	54.16	10.58	48.07	11.44	37.33	10.48
Block Design	53.98	11.97	61.38	10.02	56.24	11.62	49.21	11.13	47.64	8.51
Digits Backward	51.46	10.44	53.05	8.18	52.49	12.11	50.61	10.16	47.75	9.28
Synonyms	53.80	9.36	54.82	7.89	54.37	9.81	52.95	9.44	52.81	11.15

Table 3.3

Partial Correlations Between Key Study Variables at Baseline for the Analysis Sample Adjusting for Age and Sex

	Loneliness	Block Design	Symbol Digit	Digits Backward	Synonyms
Loneliness	1.00	-.13*	-.10^{t}	-.06	-.09^{t}
	(*n* = 375)	(*n* = 349)	(*n* = 343)	(*n* = 360)	(*n* = 347)
Age	.16**	-.45***	-.55***	-.13	-.10^{t}
	(*n* = 375)	(*n* = 349)	(*n* = 343)	(*n* = 360)	(*n* = 347)
Sex	.13*	-.08	.01	-.08	-.07
	(*n* = 375)	(*n* = 349)	(*n* = 343)	(*n* = 360)	(*n* = 347)
Social Isolation	.32***	-.07	-.06	-.02	-.02
	(*n* = 345)	(*n* = 324)	(*n* = 319)	(*n* = 335)	(*n* = 323)
Education	-.12*	.32***	.32***	.19**	.38***
	(*n* = 375)	(*n* = 349)	(*n* = 343)	(*n* = 360)	(*n* = 347)

Note. Correlations with education were done for the cognitive analysis sample (*n* = 361). Age and sex were adjusted for in all correlations except when one of these variables was being correlated. Pearson correlations were computed for continuous variables. Spearman correlations were computed for associations with sex.

Table 3.4

CpGs with Effects of Baseline Loneliness on Methylation Intercept or Slope at z ≥ |1.96| and Their Associated Genes

CpG	Gene	CpG	Gene	CpG	Gene	CpG	Gene
cg05307957	*ARID1A*	cg14673932	*DVL3*	cg26889367	*KIAA0101*	cg02382320	*SLC12A7*
cg04699519	*ATXN1*	cg09395034	*EGR1*	cg13472900	*LGALS8*	cg04114636	*SLC12A7*
cg04975376	*ATXN1*	cg13009654	*EGR1*	cg00452400	*MAN2C1*	cg04213775	*SLC12A7*
cg07109965	*ATXN1*	cg23951277	*EGR1*	cg00461978	*MAN2C1*	cg06637017	*SLC12A7*
cg10581503	*ATXN1*	cg07082452	*EGR3*	cg05525867	*MAN2C1*	cg08351607	*SLC12A7*
cg19185641	*ATXN1*	cg16854466	*EP400*	cg20639218	*MAN2C1*	cg10601043	*SLC12A7*
cg07475232	*BHLHB2*	cg20474144	*EP400*	cg00090767	*MAX*	cg11235297	*SLC12A7*
cg00733150	*C22orf8*	cg24789136	*EP400*	cg04318212	*MAX*	cg11962947	*SLC12A7*
cg03953157	*C22orf8*	cg23803468	*EPB42*	cg20040285	*MAX*	cg13301368	*SLC12A7*
cg10788213	*C22orf8*	cg19226017	*FKBP5*	cg07659624	*MSCP*	cg15597069	*SLC12A7*
cg14466896	*C22orf8*	cg05023151	*FOSB*	cg17797797	*MSCP*	cg17568547	*SLC12A7*
cg07921777	*CBFB*	cg12265810	*FOSB*	cg13516655	*MYBL1*	cg18997983	*SLC12A7*
cg10233691	*CBFB*	cg11414921	*GP1BB*	cg05176211	*MYST3*	cg19086001	*SLC12A7*
cg01948190	*CD164*	cg08703818	*H2AFV*	cg04722914	*NEDD5*	cg23503101	*SLC12A7*
cg26009195	*CDC25B*	cg08018179	*HGD*	cg16787284	*NEDD5*	cg24886748	*SLC12A7*
cg02953912	*CDKN1C*	cg16218610	*HIST1H2AC*	cg23888423	*NEDD5*	cg26439015	*SLC12A7*
cg22865058	*CDKN1C*	cg19213665	*HIST1H2AC*	cg04843801	*NKTR*	cg12894336	*SMARCC1*
cg26155475	*CLIC4*	cg25307277	*HIST1H2AC*	cg17250947	*NKTR*	cg20685352	*SMARCC1*
cg26838747	*CLIC4*	cg05070742	*HIST1H3H*	cg02530824	*PF4*	cg01085225	*STAT1*
cg00267296	*CLN2*	cg00747152	*HNRPL*	cg05509609	*PF4*	cg13186228	*STX16*
cg00929658	*COL6A2*	cg05464534	*HNRPL*	cg06834998	*PF4*	cg08667148	*TNFAIP3*
cg10435849	*COL6A2*	cg09352155	*HNRPL*	cg16072462	*PF4*	cg25971086	*TNFAIP3*
cg01446576	*COPA*	cg13353472	*HNRPL*	cg21043213	*PF4*	cg18485955	*TNFRSF17*
cg08015496	*COPA*	cg03634777	*IGF2R*	cg20357806	*PPBP*	cg09793001	*TOP2B*
cg00619097	*CPT1B*	cg16111231	*IGF2R*	cg00403457	*PTPN12*	cg19472303	*TOP2B*
cg00872628	*CSPG6*	cg21178851	*IGF2R*	cg03887471	*PTPN12*	cg15084758	*TYMS*

cg06470552	*CSPG6*	cg10677697	*IGFBP3*	cg12262427	*PTPN12*	cg22618219	*VNN1*
cg04197449	*CTTN*	cg22403266	*IGFBP3*	cg00851732	*RPH3A*	cg07103517	*ZNFN1A1*
cg08914150	*CTTN*	cg05468843	*IL10RA*	cg05793409	*SFPQ*	cg16697214	*ZNFN1A1*
cg13096351	*CTTN*	cg26661481	*IL10RA*	cg00420510	*SLC12A7*	cg10844760	*cig5*
cg25587405	*CTTN*	cg07016356	*IL8RB*	cg00551954	*SLC12A7*	cg18201077	*cig5*
cg16774942	*DDX17*	cg13739417	*IL8RB*	cg00600029	*SLC12A7*		
cg22147449	*DDX17*	cg09214993	*KIAA0101*	cg02295574	*SLC12A7*		

Table 3.5

Regression Weights (b) and Effect Sizes (d) for CpGs with the Largest Unstandardized Effects for Baseline Loneliness on Methylation Level and Change

CpG	Gene Name	b_{I70}	z_{I70}	b_S	z_S	*SD*	d_{I70}	d_S
cg02530824	*PF4*	0.07	2.27	-0.00	-0.24	1.38	0.05	---
cg05509609	*PF4*	0.06	2.36	-0.02	-0.92	1.19	0.05	---
cg06834998	*PF4*	0.07	2.43	-0.02	-0.83	1.26	0.06	---
cg16072462	*PF4*	0.05	2.16	-0.01	-0.71	0.96	0.05	---
cg21043213	*PF4*	0.05	2.22	-0.00	-0.28	1.00	0.05	---
cg05468843	*IL10RA*	-0.03	-1.37	0.04	2.14	1.12	---	0.04

Note. I70 = intercept reflecting CpG level at age 70; S = linear slope reflecting change in CpG across age. Effect sizes (*d*) were computed for each CpG by dividing the unstandardized regression weight for the intercept or slope by the standard deviation for methylation values at each site. The *d* for slope quantifies change across a 1-year period associated with loneliness. Effect sizes are only shown for effects that reached nominal significance.

Table 3.6

Regression Weights (b) and Effect Sizes (d) for CpGs with Effects of Baseline Loneliness on Both Intercept and Slope at $z \geq |1.96|$

CpG	Gene Name	*SD*	b_{I70}	z_{I70}	d_{I70}	b_S	z_S	d_S
cg26009195	*CDC25B*	0.37	0.02	2.27	0.05	-0.01	-2.14	-0.03
cg22147449	*DDX17*	0.20	-0.01	-3.49	-0.05	0.01	2.65	0.05
cg01085225	*STAT1*	0.13	-0.01	-3.06	-0.08	0.01	2.42	0.08
cg19472303	*TOP2B*	0.18	-0.01	-1.96	-0.06	0.01	2.35	0.06

Note. I70 = intercept reflecting CpG level at age 70; S = linear slope reflecting change in CpG across age. The *d* for slope quantifies change across a 1-year period associated with loneliness.

Table 3.7

Regression Weights (b) and Effect Sizes (d) for CpGs with Effects of Baseline Loneliness on Methylation Intercept or Slope at z ≥ |3|

CpG	Gene Name	*SD*	b_{I70}	z_{I70}	d_{I70}	b_{S}	z_{S}	d_{S}
cg01085225	*STAT1*	0.13	-0.01	-3.06	-0.08	0.01	2.42	---
cg16787284	*NEDD5*	0.09	0.01	3.03	0.11	-0.00	-1.73	---
cg20357806	*PPBP*	0.54	0.03	3.15	0.05	-0.01	-1.06	---
cg22147449	*DDX17*	0.20	-0.01	-3.49	-0.05	0.01	2.65	---
cg26439015	*SLC12A7*	0.12	0.01	3.15	0.08	-0.00	-1.49	---
cg26661481	*IL10RA*	0.47	-0.01	-1.52	---	0.03	3.13	0.06

Note. I70 = intercept reflecting CpG level at age 70; S = linear slope reflecting change in CpG across age. The *d* for slope quantifies change across a 1-year period associated with loneliness. Effect sizes are only shown for effects that reached nominal significance.

Table 3.8

Regulatory Feature Types for 81 of the 130 CpGs with $z \geq |1.96|$ in the Baseline Growth Analysis

Feature Type	**Frequency (%)**
Promoter	68 (52.3%)
Open chromatin	6 (4.6%)
Promoter Flanking Region	4 (3.1%)
CTCF Binding Site	1 (0.8%)
Enhancer	1 (0.8%)
TF binding site	1 (0.8%)

Note. Regulatory feature types were available in the Ensembl database for 81 of the 130 CpGs.

Table 3.9

Regression Weights (b) and Effect Sizes (d) for 32 CpGs with Effects of Loneliness at $z \geq |1.96|$ *in Both the Baseline and Time-Varying Analyses*

		Baseline					***Time-Varying***					
Cpg	**Gene**	**I70 (*b*)**	**z**	**S (*b*)**	***Z***	***d* (I70, S)**	**I70 (*b*)**	**Z**	**S (*b*)**	**Z**	***d* (I70, S)**	***SD***
cg04975376	*ATXN1*	-0.017	-2.55	0.002	0.34	-0.05, 0.006	-0.014	-2.25	-0.00523	-0.91	-0.04, -0.01	0.35
cg10581503	*ATXN1*	0.0169	2.21	-0.005	-0.68	0.04, -0.01	0.010	1.54	-0.01259	-1.97	0.03, -0.03	0.37
cg07475232	*BHLHB2*	-0.0057	-1.13	0.009	2.02	-0.02, 0.04	-0.009	-2.06	0.00634	1.46	-0.04, 0.03	0.25
cg00733150	*C22orf8*	-0.0168	-1.09	0.026	2.78	-0.02, 0.03	-0.006	-0.75	0.01483	2.07	-0.008, 0.02	0.77
cg10233691	*CBFB*	-0.0034	-1.55	0.005	2.71	-0.03, 0.05	-0.004	-2.01	0.00088	0.46	-0.04, 0.008	0.11
cg10435849	*COL6A2*	0.0138	1.30	-0.026	-2.86	0.03, -0.05	0.027	2.96	-0.02481	-2.94	0.05, -0.05	0.50
cg00619097	*CPT1B*	-0.0117	-1.03	0.028	2.65	-0.02, 0.05	-0.026	-2.53	**0.04078**	**4.22**	-0.04, 0.07	0.59
cg22147449	*DDX17*	**-0.0138**	**-3.49**	0.010	2.65	-0.07, 0.05	-0.010	-2.70	0.00422	1.24	-0.05, 0.02	0.20
cg16854466	*EP400*	0.0172	2.61	-0.007	-1.08	0.05, -0.02	**0.021**	**3.38**	-0.00776	-1.36	0.06, -0.02	0.35
cg08018179	*HGD*	0.0126	1.98	-0.006	-1.09	0.04, -0.02	0.012	2.11	-0.00001	-0.00	0.04, $-2.73E^{-05}$	0.33
cg13353472	*HNRPL*	-0.0049	-1.53	0.008	2.81	-0.03, 0.05	-0.006	-1.97	0.00688	2.61	-0.04, 0.04	0.16
cg26661481	*IL10RA*	-0.0138	-1.52	**0.026**	**3.13**	-0.03, 0.06	-0.017	-1.98	0.01533	1.94	-0.04, 0.03	0.47

		Baseline					***Time-Varying***					
Cpg	**Gene**	**I70 (*b*)**	**z**	**S (*b*)**	***Z***	***d* (I70, S)**	**I70 (*b*)**	**Z**	**S (*b*)**	**Z**	***d* (I70, S)**	***SD***
cg09214993	*KIAA0101*	-0.0194	-2.39	0.002	0.27	-0.05, 0.005	-0.016	-2.16	0.00104	0.15	-0.04, 0.002	0.41
cg26889367	*KIAA0101*	-0.0003	-0.07	-0.009	-2.58	-0.001, -0.05	0.003	0.83	-0.00826	-2.77	0.01, -0.04	0.18
cg13472900	*LGALS8*	0.0160	2.24	0.002	0.34	0.04, 0.006	0.015	2.25	-0.00276	-0.46	0.04, -0.008	0.36
cg00452400	*MAN2C1*	-0.0187	-1.56	0.024	2.20	-0.03, 0.04	-0.011	-0.99	0.02214	2.11	-0.02, 0.04	0.62
cg00461978	*MAN2C1*	-0.0188	-2.35	0.005	0.68	-0.04, 0.01	-0.015	-1.98	0.00617	0.90	-0.04, 0.02	0.41
cg04722914	*NEDD5*	-0.0071	-2.94	0.001	0.67	-0.06, 0.01	-0.006	-2.75	0.00190	0.92	-0.05, 0.02	0.12
cg16787284	*NEDD5*	**0.0050**	**3.03**	-0.003	-1.73	0.06, -0.03	0.004	2.91	-0.00133	-0.90	0.05, -0.01	0.09
cg20357806	*PPBP*	**0.0343**	**3.15**	-0.010	-1.06	0.06, -0.02	0.020	2.04	-0.01259	-1.39	0.04, -0.02	0.54
cg00403457	*PTPN12*	-0.0301	-2.89	0.005	0.52	-0.05, 0.009	-0.021	-2.17	0.00719	0.79	-0.04, 0.01	0.56
cg12262427	*PTPN12*	-0.0045	-2.15	0.001	0.68	-0.04, 0.01	-0.005	-2.63	0.00393	2.20	-0.05, 0.04	0.11
cg00420510	*SLC12A7*	0.0193	2.34	-0.012	-1.58	0.05, -0.03	0.009	1.25	-0.01421	-2.05	0.02, -0.03	0.42
cg02382320	*SLC12A7*	0.0128	1.61	-0.017	-2.27	0.03, -0.04	0.016	2.25	-0.01186	-1.72	0.04, -0.03	0.40
cg04213775	*SLC12A7*	0.0183	2.33	-0.006	-0.76	0.04, -0.01	0.019	2.69	-0.01157	-1.70	0.05, -0.03	0.40
cg11235297	*SLC12A7*	-0.0241	-2.49	0.003	0.35	-0.05, 0.006	**-0.027**	**-3.05**	0.00979	1.21	-0.05, 0.02	0.49

		Baseline					*Time-Varying*					
Cpg	**Gene**	**I70 (*b*)**	**z**	**S (*b*)**	***Z***	***d* (I70, S)**	**I70 (*b*)**	**Z**	**S (*b*)**	**Z**	***d* (I70, S)**	***SD***
cg13301368	*SLC12A7*	0.0225	2.17	-0.005	-0.51	0.04, -0.009	0.019	1.99	-0.00082	-0.09	0.03, -0.001	0.55
cg26439015	*SLC12A7*	**0.0072**	**3.15**	-0.003	-1.49	0.06, -0.02	0.006	2.98	-0.00233	-1.17	0.05, -0.02	0.12
cg01085225	*STAT1*	**-0.0078**	**-3.06**	0.006	2.42	-0.06, 0.04	-0.006	-2.79	0.00319	1.44	-0.05, 0.02	0.13
cg09793001	*TOP2B*	-0.0133	-2.10	0.002	0.39	-0.04, 0.007	-0.012	-1.99	-0.00064	-0.12	-0.04, -0.002	0.32
cg19472303	*TOP2B*	-0.0068	-1.96	0.008	2.35	-0.04, 0.04	-0.006	-2.03	**0.00942**	**3.15**	-0.04, 0.05	0.18
cg22618219	*VNN1*	0.0194	2.52	-0.002	-0.24	0.05, -0.004	0.015	2.12	-0.00909	-1.36	0.04, -0.02	0.40

Note. I70 = intercept reflecting CpG level at age 70; S = linear slope reflecting change in CpG across age. Effect sizes (*d*) were computed for each CpG by dividing the unstandardized regression weight for the intercept or slope by the standard deviation for methylation values at each site. Effect sizes for effects that did not reach nominal significance are in gray. Regression weights with *z*-values $\geq$ |3| are in bold.

Table 3.10

Summary of Results for Baseline and Time-Varying Growth Analyses

Analysis	N_b **Nominally Significant at .05**	N_b **Sig. at .05 Adj. for Multiple Testing**	***b* Range (I70)**	***b* range (S)**
Baseline	130 (88 I70, 46 S)	0	-.05 to .07	-.03 to .04
Time-Varying	32 (26 I70, 10 S)	1 (b = 0.04, z = 4.22)	-.03 to .03	-.02 to .04

Note. I70 = intercept reflecting CpG level at age 70; S = linear slope reflecting change in CpG across age. Significant effect of time-varying loneliness (correcting for multiple testing) was on methylation slope.

Table 3.11

Between and Within-Pair Effects of Loneliness on Methylation Intercept and Slope for cg26661481 and cg13009654

CpG (Gene)	I70				S			
	B	**DZW**	**MZW**	**MZ/DZ**	**B**	**DZW**	**MZW**	**MZ/DZ**
cg00403457 (*PTPN12*)	-.049*	-.042*	-.005	.126	.006	.006	.005	.904
cg26661481 (*IL10RA*)	-.012	-.015	-.018	1.19	.032*	.038*	.024	.632

*$z \geq |1.96|$ *Note*. I70 = intercept reflecting CpG level at age 70; S = linear slope reflecting change in CpG across age; B = between-pair effect; W = within-pair effect. (Note that fixed effects for MZ twins estimated deviation of each effect for MZ twins from that for DZ twins; MZ effects were computed by adding estimated effects for MZ and DZ twins.)

Table 3.12

Fit Statistics for Symbol Digit Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***P***
Unconditional						
A. Age	343	5986.4	6000.4	---	---	---
B. Age + Age^2	343	5958.9	5978.9	27.5	4	< .0001
B. Age + Age^2 ♦	343	5963.6	5979.6	22.8	1	< .0001
C. AgeC70A + AgeC70B	343	5957.1	5977.1	29.3	4	< .0001
C. AgeC70A + AgeC70B♦	343	5959.4	5975.4	27.0	1	< .0001
Conditional Quadratic						
D. Model B♦ + Covariates	343	5929.0	5949.0	34.6	2	< .0001
E. Model D + Loneliness	343	5927.3	5951.3	1.7	2	.4274
F. Model E + cg00403457 (*PTPN12*)	343	5918.8	5946.8	8.5	2	.0143
G. Model E + cg26661481 (*IL10RA*)	343	5926.4	5954.4	0.9	2	.6376
Conditional Spline						
D. Model C♦ + Covariates	343	5924.8	5944.8	34.6	2	< .0001
E. Model D + Loneliness	343	5923.3	5947.3	1.5	2	.4724
F. Model E + cg00403457 (*PTPN12*)	343	5918.9	5944.8	4.4	2	.1108
G. Model E + cg26661481 (*IL10RA*)	343	5922.4	5950.4	0.9	2	.6376

Note. Model C (spline) slopes AgeC70A and AgeC70B refer to linear change prior to and at/after age 70, respectively.
♦ = The covariance parameter for linear change (quadratic model; Model B) or linear change before age 70 (spline model; Model C) for individuals within twin pairs hit a boundary of 0 and were removed from each model. As no random effects were modeled for linear change in quadratic models or linear change before age 70 in spline models, interactions with these terms were excluded from later models.

Table 3.13

Regression Weights (b) for Symbol Digit Models

Fixed Effects	**Model B♦**	**Model D**	**Model E**	**Model F**	**Model G**	**Model H**
Level						
Performance (age 70)	51.77**	53.97**	53.47**	53.49**	53.61**	53.52**
Sex	---	1.31	1.40	1.41	1.28	1.37
Education	---	1.25**	1.23**	1.24**	1.23**	1.24**
Loneliness	---	---	-0.16	-0.15	-0.17	-0.15
cg00403457 (*PTPN12*)	---	---	---	---	**-1.44****	---
cg26661481 (*IL10RA*)	---	---	---	---	---	0.49
Linear Change						
Linear slope (age 70)	-0.59**	-0.58**	-0.58**	-0.58**	-0.58**	-0.58**
Loneliness	---	---	---	---	---	---
cg00403457 (*PTPN12*)	---	---	---	---	---	---
cg26661481 (*IL10RA*)	---	---	---	---	---	---
Quadratic Change						
Quadratic slope (age 70)	-0.01**	-0.01**	-0.01**	-0.01**	-0.01**	-0.01**
Loneliness	---	---	0.00011	0.00007	0.00035	0.00008
cg00403457 (*PTPN12*)	---	---	---	---	**0.0079***	---
cg26661481 (*IL10RA*)	---	---	---	---	---	-0.0008

**$p < .01$, * $p < .05$ *Note*. No linear random effects/interaction terms were modeled. Significant effects of methylation on cognition are in bold.

Table 3.14

Fit Statistics for Block Design Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***P***
Unconditional						
A. Age	349	6009.5	6023.5	---	---	---
B. Age + Age^2	349	5979.7	6001.7	29.8	4	< .0001
C. AgeC70A + AgeC70B	349	5982.6	6004.6	26.9	4	< .0001
Conditional						
D. Model B + Covariates	349	5947.1	5973.1	32.6	2	< .0001
E. Model D + Loneliness	349	5941.9	5973.9	5.2	3	.1577
F. Model E + cg00619097 (*CPT1B*)	349	5940.8	5978.8	1.1	3	.7771
G. Model E + cg00403457 (*PTPN12*)	349	5941.0	5979.0	0.9	3	.8254
H. Model E + cg26661481 (*IL10RA*)	349	5939.7	5977.7	1.1	3	.7771

Note. Model C (spline) slopes AgeC70A and AgeC70B refer to linear change prior to and at/after age 70, respectively.

Table 3.15

Regression Weights (b) for Block Design Models

Fixed Effects	**Model B**	**Model D**	**Model E**	**Model F**	**Model G**	**Model H**
Level						
Performance (age 70)	53.88**	60.22**	59.81**	59.81**	59.81**	59.84**
Sex	---	-1.41	-1.35	-1.37	-1.34	-1.35
Education	---	1.19**	1.17**	1.17**	1.17**	1.18**
Loneliness	---	---	-0.11	-0.11	-0.10	-0.10
cg00403457 (*PTPN12*)	---	---	---	---	0.27	---
cg26661481 (*IL10RA*)	---	---	---	---	---	0.57
Linear Change						
Linear slope (age 70)	-0.40**	-0.39**	-0.36**	-0.36**	-0.36**	-0.35**
Loneliness	---	---	0.01	---	---	---
cg00403457 (*PTPN12*)	---	---	---	---	0.01	---
cg26661481 (*IL10RA*)	---	---	---	---	---	0.04
Quadratic Change						
Quadratic slope (age 70)	-0.009**	-0.008**	-0.010**	-0.010**	-0.010**	-0.011**
Loneliness	---	---	-0.001	-0.001	-0.001	-0.001
cg00403457 (*PTPN12*)	---	---	---	---	-0.003	---
cg26661481 (*IL10RA*)	---	---	---	---	---	-0.005

**$p < .01$, * $p < .05$ *Note*. Significant effects of methylation on cognition are in bold.

Table 3.16

Fit Statistics for Synonyms Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***p***
Unconditional						
A. Age	347	5599.4	5613.4	---	---	---
B. Age + Age^2	347	5576.7	5598.7	22.7	4	< .0001
C. AgeC70A + AgeC70B	347	5579.4	5601.4	20.0	4	< .0001
Conditional						
D. Model B + Covariates	347	5534.8	5560.8	41.9	2	< .0001
E. Model D + Loneliness	347	5533.8	5565.8	1.0	3	.8012
F. Model E + cg00619097 (*CPT1B*)	347	5532.7	5570.7	1.1	3	.7771
G. Model E + cg00403457 (*PTPN12*)	347	5529.9	5567.9	3.9	3	.2725
H. Model E + cg26661481 (*IL10RA*)	347	5527.7	5565.7	6.1	3	.1068

Note. Model C (spline) slopes AgeC70A and AgeC70B refer to linear change prior to and at/after age 70, respectively.

Table 3.17

Regression Weights (b) for Synonyms Models

Fixed Effects	Model B	Model D	Model E	Model F	Model G	Model H
Level						
Performance (age 70)	54.00**	58.87**	58.64**	58.60**	58.67**	58.73**
Sex	---	-0.41	-0.37	-0.34	-0.38	-0.43
Education	---	1.24**	1.23**	1.24**	1.24**	1.23**
Loneliness	---	---	-0.09	-0.09	-0.10	-0.08
cg00403457 (*PTPN12*)	---	---	---	---	-0.33	---
cg26661481 (*IL10RA*)	---	---	---	---	---	-0.22
Linear Change						
Linear slope (age 70)	-0.12**	-0.10**	-0.11**	-0.11**	-0.11**	-0.11**
Loneliness	---	---	-0.004	-0.004	-0.006	-0.003
cg00403457 (*PTPN12*)	---	---	---	---	-0.04	---
cg26661481 (*IL10RA*)	---	---	---	---	---	**0.09***
Quadratic Change						
Quadratic slope (age 70)	-0.006**	-0.006**	-0.005*	-0.005*	-0.005*	-0.005*
Loneliness	---	---	0.0004	0.0004	0.0007	0.0002
cg00403457 (*PTPN12*)	---	---	---	---	**0.004**^{t}	---
cg26661481 (*IL10RA*)	---	---	---	---	---	0.001

**$p < .01$, * $p < .05$ *Note*. Model fit did not significantly improve when methylation at these sites was added in separate models. Single parameters that achieved significance or trend significance are in bold.

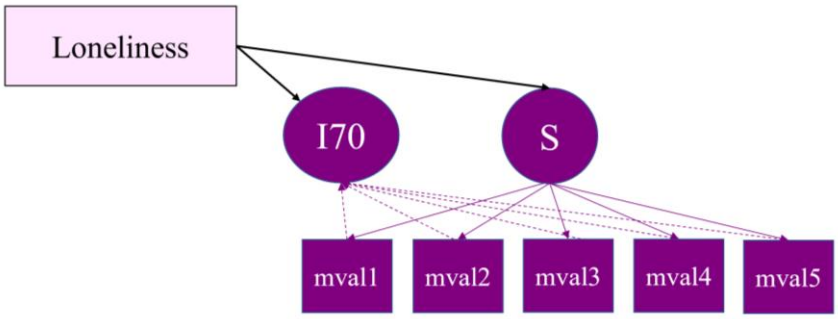

Figure 3.1. Growth model fitted to longitudinal methylation data with (a) baseline and (b) time-varying loneliness as predictors of methylation level at age 70 (I70) and subsequent change in methylation across age (S). Note: not all paths are shown.

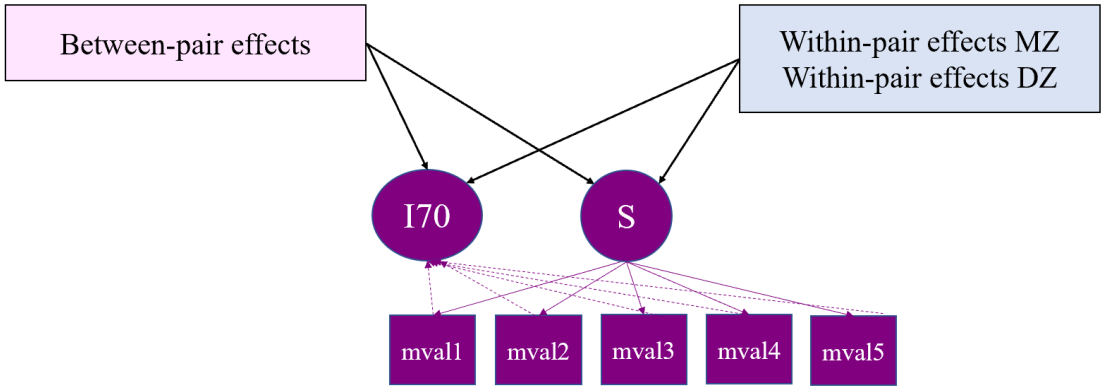

Figure 3.2. Growth model fitted to longitudinal methylation data with (a) average baseline loneliness scores for each twin pair, and (b) baseline loneliness difference scores for individual twins within pairs as predictors of methylation level at age 70 (I70) and subsequent change in methylation across age (S). Note: not all paths are shown.

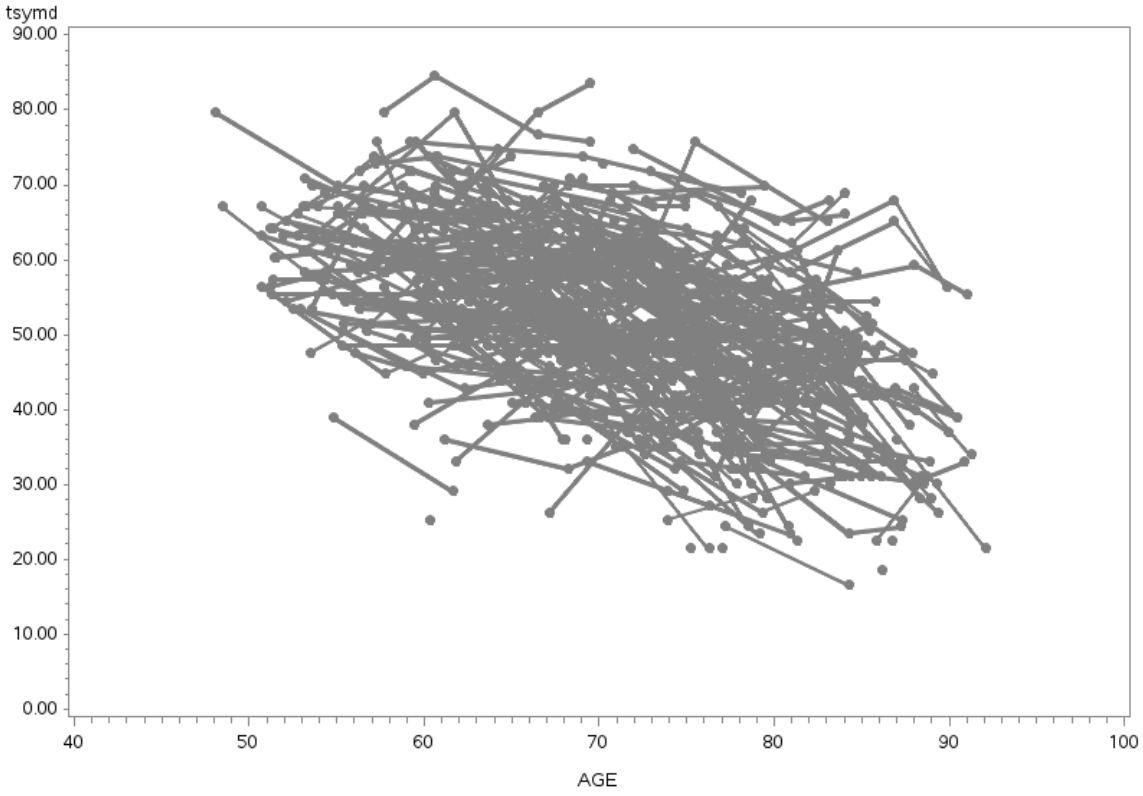

Figure 3.3. Trajectory plot for the SATSA methylation subsample of Symbol Digit *T*-scores (y-axis) by age. Observations corresponding with waves at or after dementia diagnosis are not shown.

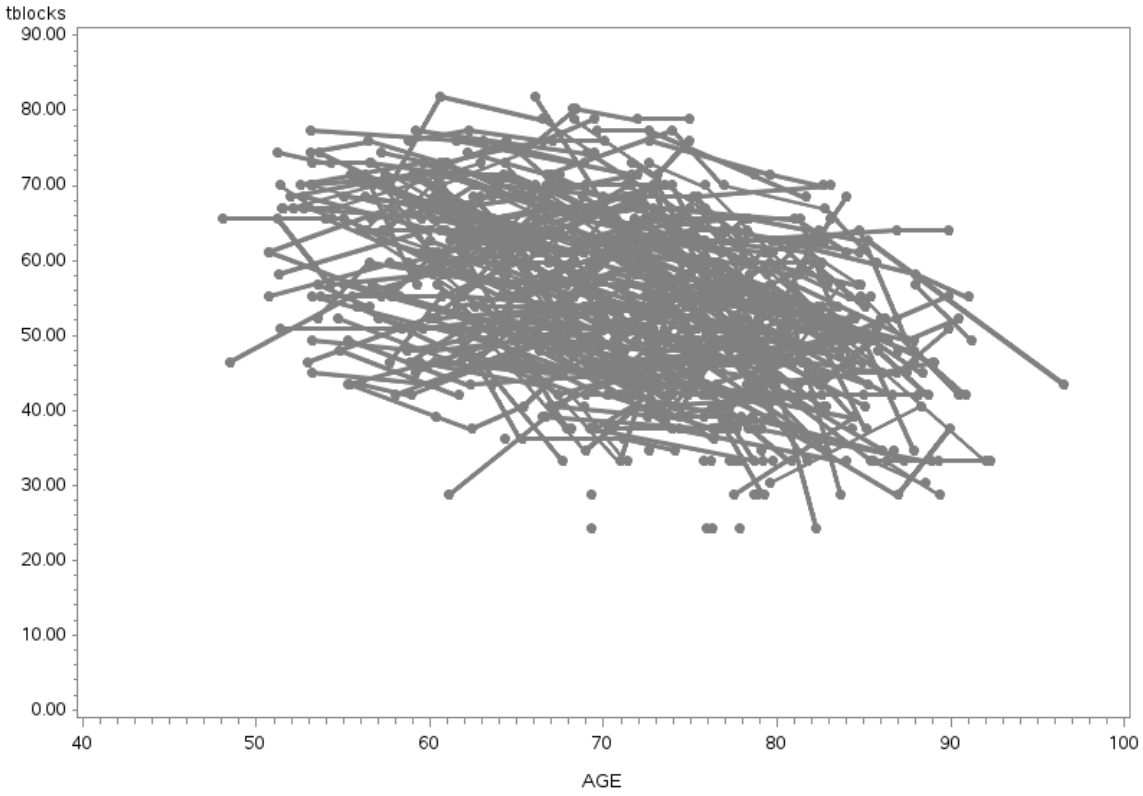

Figure 3.4. Trajectory plot for the SATSA methylation subsample of Block Design *T*-scores (y-axis) by age. Observations corresponding with waves at or after dementia diagnosis are not shown.

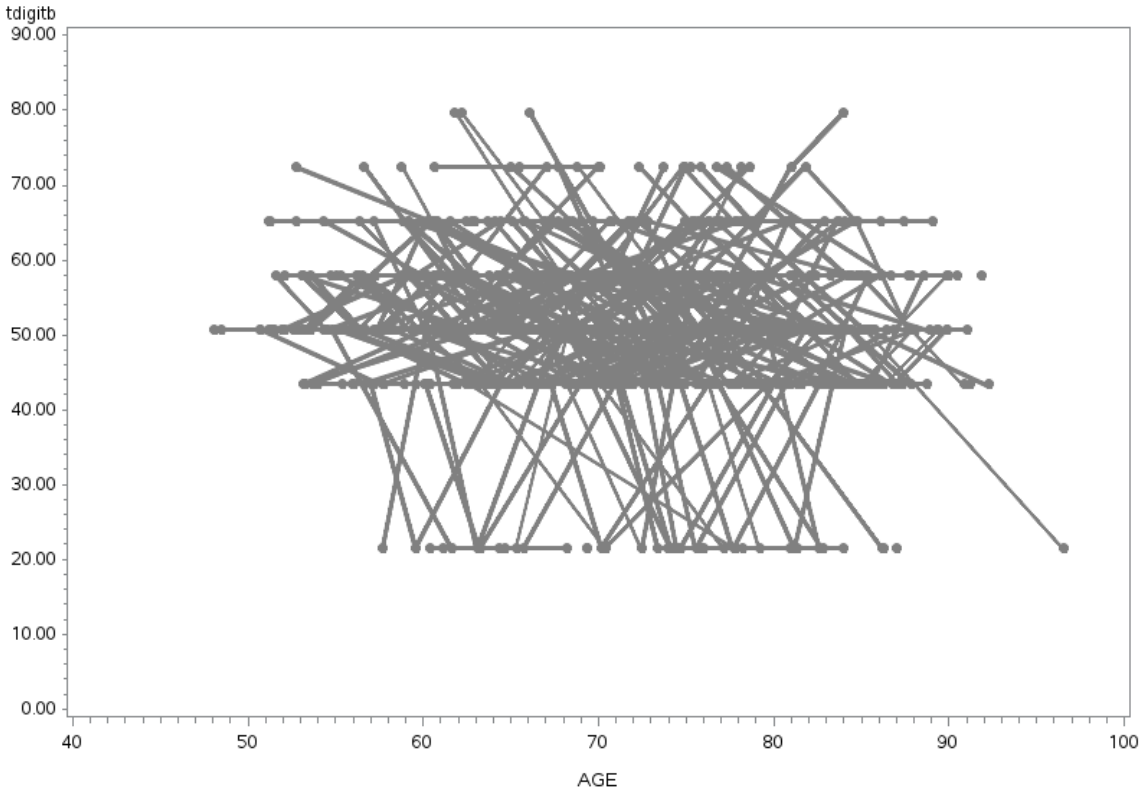

Figure 3.5. Trajectory plot for the SATSA methylation subsample of Digits Backward *T*-scores (y-axis) by age. Observations corresponding with waves at or after dementia diagnosis are not shown.

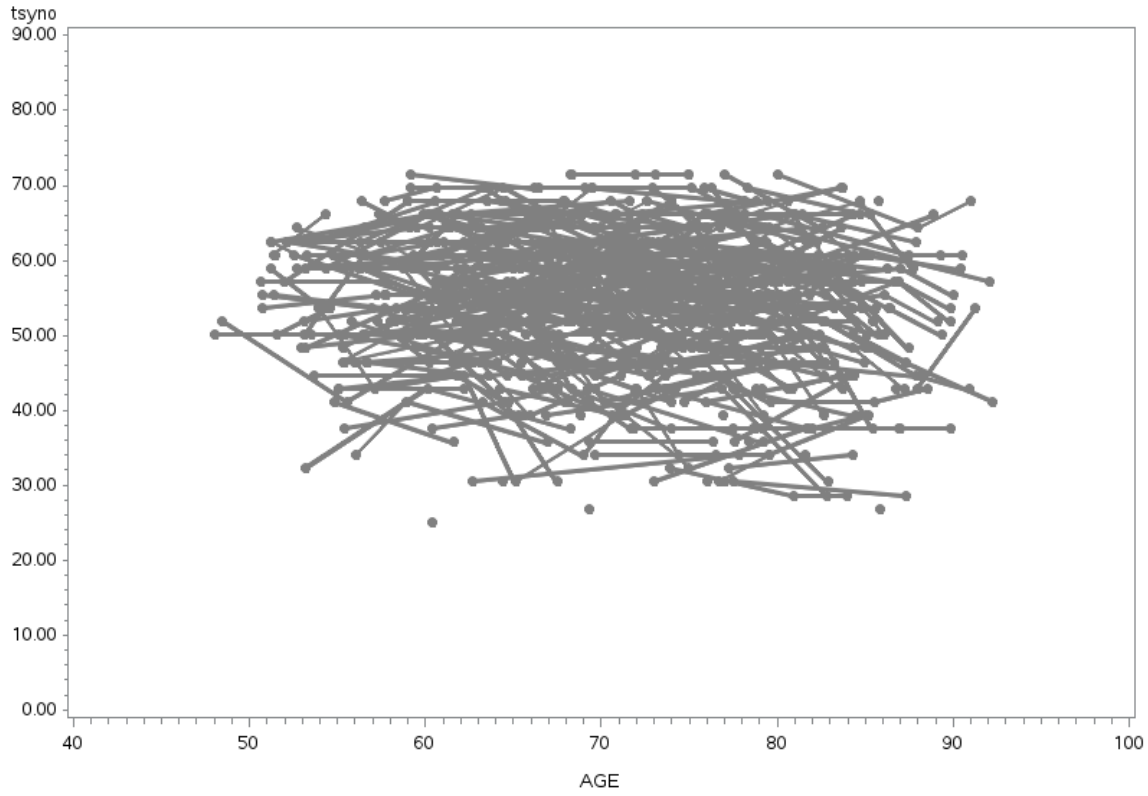

Figure 3.6. Trajectory plot for the SATSA methylation subsample of Synonyms *T*-scores (y-axis) by age. Observations corresponding with waves at or after dementia diagnosis are not shown.

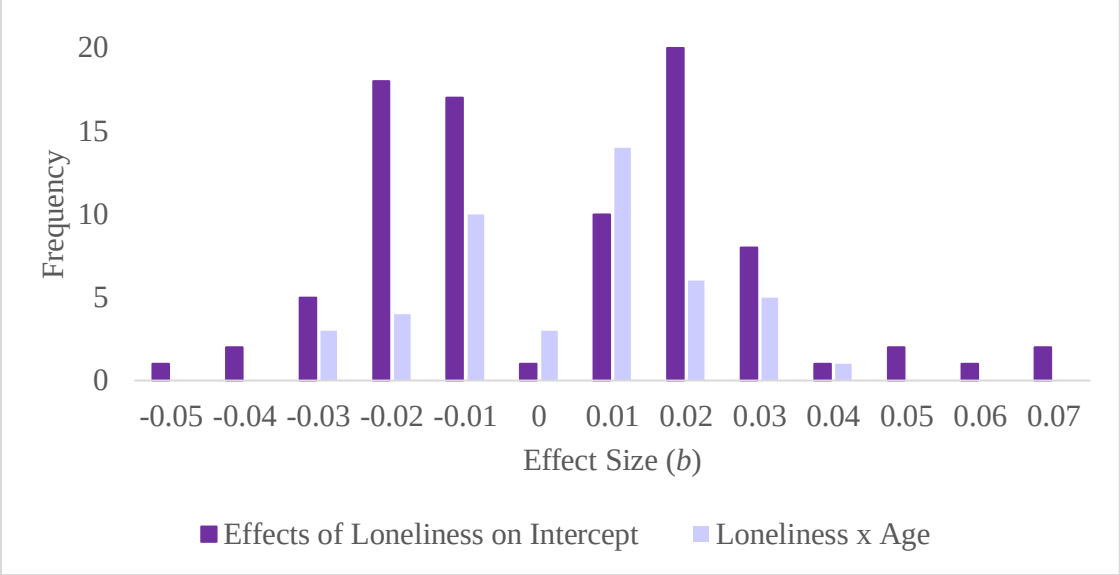

Figure 3.7. Frequency distribution of regression weights (*b*) for the 88 CpGs with nominally significant effects of baseline loneliness on methylation level at age 70 and the 46 CpGs with effects of baseline loneliness on change in methylation with age.

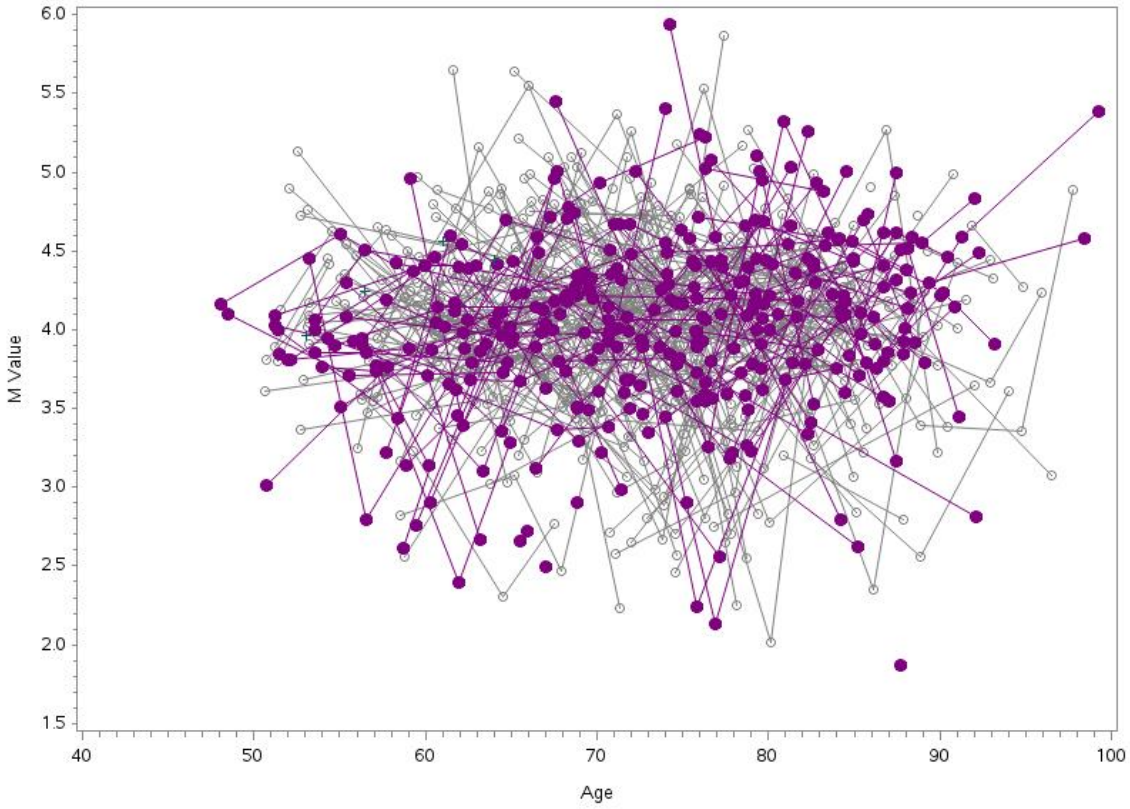

Figure 3.8. Longitudinal plot of *M* values by baseline loneliness for cg00619097 in *CPT1B*. Purple = lonely at first assessment; gray = not lonely at first assessment. Participants were considered lonely if they reported feeling lonely '*some or a little of the time*', '*occasionally or a moderate amount of time*', or '*most or all of the time*'.

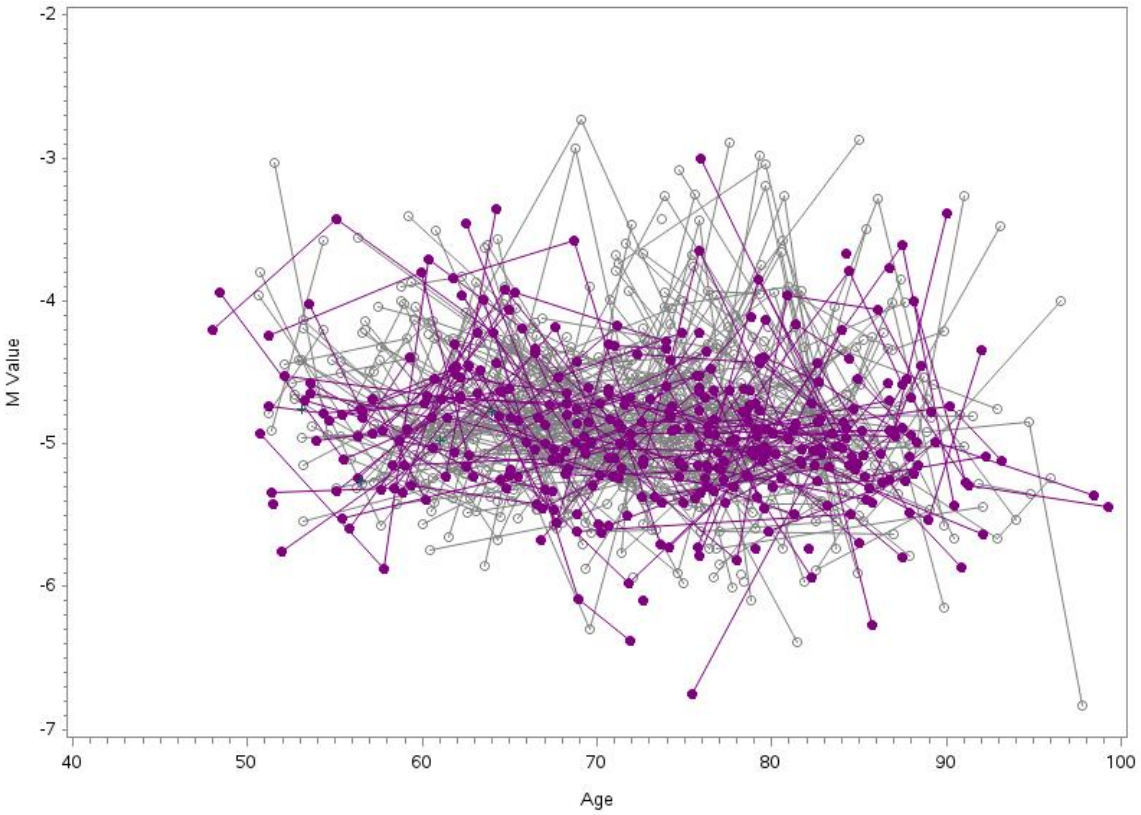

Figure 3.9. Longitudinal plot of *M* values by baseline loneliness for cg00403457 in *PTPN12*. Purple = lonely at first assessment; gray = not lonely at first assessment. Participants were considered lonely if they reported feeling lonely '*some or a little of the time*', '*occasionally or a moderate amount of time*', or '*most or all of the time*'.

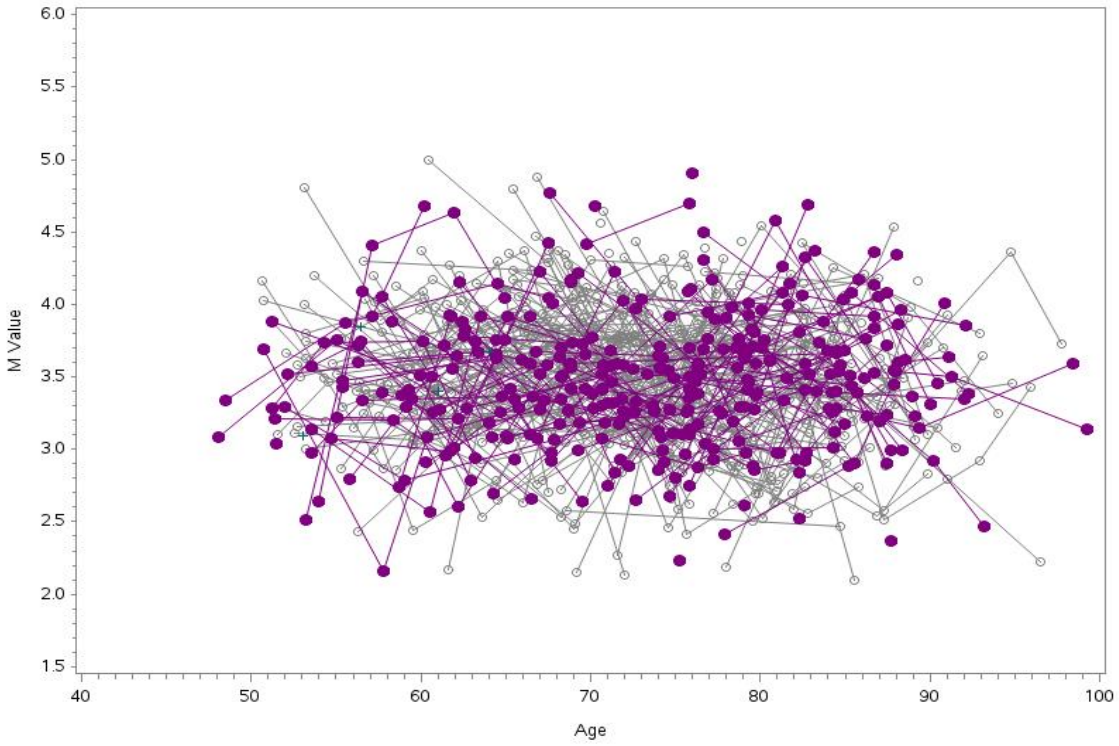

Figure 3.10. Longitudinal plot of *M* values by baseline loneliness for cg26661481 in *IL10RA*. Purple = lonely at first assessment; gray = not lonely at first assessment. Participants were considered lonely if they reported feeling lonely '*some or a little of the time*', '*occasionally or a moderate amount of time*', or '*most or all of the time*'.

GENERAL DISCUSSION

Cognitive development occurs within a social context, and social interaction has been shown to be important to cognition throughout the life span (Hughes, Waite, Hawkley, & Cacioppo, 2004). Feelings of loneliness have been shown to detract from healthy cognitive aging in late life, with associations reported between loneliness and poorer performance and faster decline in both global cognitive functioning and specific domains of cognition (Holwerda et al., 2012; O'Luanaigh et al., 2012; Tilvis et al., 2004; Wilson et al., 2007; Zhong, Chen, Tu, & Conwell, 2017) and elevated dementia risk (Rafnsson et al., 2017; Wilson et al., 2007). Mechanisms of these associations remain unknown, however, as loneliness has been associated with elevated stress (Cacioppo, Hawkley, & Berntson, 2003), it has been hypothesized that feeling lonely results in adverse physiological changes that accumulate over time (Hawkley & Capitanio, 2015) which may detract from healthy cognitive aging. As such changes associated with loneliness likely unfold over time, it has been proposed that shorter periods of loneliness are not likely to result in enduring adverse physiological changes (Hawkley & Capitanio, 2015) likely to detract from healthy aging, however, such assertions have yet to be explored empirically for associations between loneliness and cognition. The purpose of this dissertation was to assess relations between both baseline and longitudinal loneliness and domain-specific cognitive performance and change and to explore whether DNA methylation at CpG sites within genes linked with the conserved transcriptional response to adversity (CTRA) might be one pathway by which feelings of loneliness detract from

cognitive health over time. Two studies were conducted to investigate four research questions.

1.1 Do baseline loneliness, time-varying loneliness, or geometric means for loneliness across time predict performance or change in specific cognitive abilities?

1.2 Is longitudinal loneliness (i.e., time-varying, geometric mean) more strongly associated with cognitive performance and change than baseline loneliness?

2.1 Does loneliness predict level or change in methylation of blood leukocyte DNA at CpG sites associated with CTRA genes for (a) individuals in the overall sample (i.e., not by zygosity group), (b) dizygotic (DZ) twin pairs, and (c) monozygotic (MZ) twin pairs? If observed, do patterns of associations suggest confounding of the loneliness—methylation relationship by genetic or common environmental factors or a potentially causal association?

2.2 Is there a potential mediational role for DNA methylation at CpG sites associated with CTRA genes in the association between loneliness and performance or decline in four specific cognitive abilities?

The conceptual model shown in Figure 1.1 in Chapter 1 depicts the associations that were explored in this dissertation. In Study 1 and Study 2, direct associations between loneliness and domain-specific cognitive performance and change were examined. In Study 2, a potential indirect pathway linking loneliness to cognition vis-à-vis methylation at specific CpG sites within CTRA genes was also explored. Loneliness may directly impact cognition through adverse altered perceptions of social interactions

that lead to behavior changes that impede positive social interaction (Cacioppo & Hawkley, 2009). Such behavior changes have been posited to decrease exposure of lonely persons to stimulating social interaction and social support, both which have been shown to support healthy cognitive aging (Ayalon, Shiovitz-Ezra & Roziner, 2016; Hawkley & Cacioppo, 2009). Loneliness may also indirectly impact cognition by inducing physiological changes such as increased inflammation (Cole et al., 2007; Jaremka et al., 2013) or altered immune function (Cole et al., 2007), which may, in part, be explained by epigenetic changes previously associated with loneliness (Cole et al., 2007). This model posits that loneliness is associated with cognition both directly and indirectly, through altered methylation at CpG sites within CTRA genes.

Summary of General Findings

Study 1.

In Study 1, a large multinational sample was drawn from eight twin studies participating in the Consortium on Interplay of Genes and Environment Across Multiple Studies (IGEMS) to examine and compare associations between baseline and longitudinal loneliness and performance and change in processing speed, spatial ability, working memory, and verbal comprehension, adjusting for baseline objective social isolation, baseline depression, sex, and country of residence. Mixed-effects growth curve models were fitted to the cognitive data to explore the extent to which baseline loneliness, time-varying loneliness, and geometric means for loneliness each predicted cognitive performance at age 65 and age-associated change in cognition across up to 28 years of follow-up. For processing speed, both greater baseline loneliness and greater longitudinal

loneliness were associated with *better* performance at age 65, but greater longitudinal loneliness predicted faster linear and quadratic decline. This pattern of results remained after adjusting for years of education. For spatial ability, loneliness was not related to performance at age 65. However, higher baseline loneliness and higher longitudinal loneliness each predicted faster age-associated decline, with geometric means for loneliness predicting faster linear decline and baseline and time-varying loneliness predicting faster acceleration in decline with age. Adjusting for education did not alter these effects. No relation was found between baseline loneliness and performance or change in verbal comprehension. Greater time-varying loneliness was associated with faster quadratic decline with age for this domain, although this association was attenuated when education was added to the model. Preliminary correlational results indicated a very small association between loneliness and working memory, and models were not fitted for this task. Overall our hypotheses were supported regarding the adverse influence of perceived loneliness, particularly continued loneliness, on cognitive change across the second half of the life-span even while accounting for indices of social isolation. The effect sizes for predicted loneliness on spatial and speed trajectories calculated across a 15-year period between ages 65 to 80 were small albeit provide a picture of how loneliness effects may play out on average with no intervention. Implications are discussed further below.

Study 2.

In Study 2, data from a subsample from the Swedish Adoption Twin Study of Aging (SATSA) were used to explore longitudinal associations between loneliness and

DNA methylation at 1,586 CpG sites within 105 CTRA genes using both phenotypic and co-twin control approaches and to assess whether a potential mediational role for DNA methylation at sites associated with loneliness may exist. Bayesian mixed-effects linear growth models were fitted to the longitudinal methylation data to explore associations between loneliness and methylation level at age 70 and age-associated change in methylation at these sites, adjusting for sex and time-varying social isolation. Associations between loneliness and cognition were assessed using mixed effects quadratic growth models (using maximum likelihood) fitted to the cognitive data, with (a) loneliness, and (b) loneliness and methylation as predictors of cognitive performance at age 70 and age-associated change, adjusting for fixed effects of sex and years of education on the intercept.

Phenotypic results showed a significant effect of time-varying loneliness on age-associated change in methylation for cg00619097 in *CPT1B*, with faster increase in methylation with age associated with loneliness. Co-twin control results revealed nominally significant effects of baseline loneliness on methylation, with greater loneliness associated with reduced methylation at age 70 at cg00403457 in *PTPN12* and faster age-related increase in methylation for cg26661481 in *IL10RA*. Co-twin control analyses indicated partial confounding of the effect for cg26661481 in *IL10RA* and near complete confounding of the observed effect for cg00403457 in *PTPN12*. Model-fitting analyses assessing loneliness, methylation, cognition associations showed no significant associations between loneliness and cognition for the domains assessed, however, methylation at cg00403457 in *PTPN12* significantly predicted reduced processing speed

performance at age 70 and dampened the average quadratic trend of accelerating change with age. Overall, results suggest a nominal role for perceived loneliness on altered methylation level or change across time with only one site achieving significance after multiple correction. Moreover, correlations between loneliness and cognitive performance while similar to the larger analysis in Study 1, did not bear out significant associations in growth analyses, thus the selected methylation sites in *PTPN12* or *IL10RA* did not mediate loneliness on cognition. However, indirect associations of perceived loneliness to methylation of cg00403457 *PTPN12* to change in speed of processing warrant some additional follow-up work.

Implications

The population of aging adults is expected to grow rapidly across the next few decades, with a worldwide 3-fold increase expected by 2050 (World Health Organization, 2011). The number of persons with declining cognitive functioning or dementia are also expected to increase along with the aging population (World Health Organization, 2011), and identification of potentially modifiable factors associated with increased risk of these adverse cognitive outcomes is critical. Results from prior work assessing associations between feelings of loneliness and late-life cognition suggest that loneliness may detract from healthy cognitive aging, with associations reported between greater loneliness and poorer cognitive performance (O'Luanaigh et al., 2012; Wilson et al., 2007; Zhong, Chen, Tu, & Conwell, 2017), faster cognitive decline (Holwerda et al., 2012; Tilvis et al., 2004; Wilson et al., 2007), and greater dementia risk (Rafnsson et al., 2017; Wilson et al., 2007). However, additional longitudinal work is needed to fully understand how

loneliness relates to performance and change in specific domains of cognitive functioning with age in mid and late life, as limited longitudinal work exists on loneliness and domain-specific cognitive function and relatively few to no studies of loneliness and cognition have used age-based analyses. Although recent work has begun to explore relations between longitudinal loneliness and cognition, how different patterns of loneliness across time relate to cognition remains poorly understood. Additionally, pathways by which loneliness may adversely impact cognition remain undetermined.

The prevalence of loneliness has been shown to rise in late life (Luhmann & Hawkley, 2016; Nicolaisen & Thorsen, 2014; Qualter et al., 2015). Although aging is accompanied by increasing likelihood of social losses and physical limitations such as frailty, chronic health conditions, and reduced mobility that increase likelihood of loneliness in older adults (D'Augustino & Canli, 2018; Nicolaisen & Thorsen, 2014; Qualter et al., 2015), the strength and vulnerability integration (SAVI) model (Charles, 2010) posits that aging is also linked with increasing attentional preference for positive information (i.e., the positivity effect; Charles, 2010), increasing investment of time and energy on nurturing close relationships with family members and/or friends (i.e., socioemotional selectivity theory; Carstensen, Fung, & Charles, 2003), and employment of more effective strategies for regulating negative emotions (Charles, 2010), all of which likely enhance satisfaction with one's social relationships and reduce likelihood of experiencing loneliness. These protective factors associated with aging are unlikely to effectively protect older adults from feelings of loneliness, however, under extreme circumstances such as losing a spouse or other members of their close social network

(Charles, 2010). As loneliness has been linked with elevated stress including hypervigilance to threat and altered HPA function shown to contribute to inflammatory processes (Carroll et al, 2013; Hawkley & Capitanio, 2015; Lin, Epel, & Blackburn, 2012), reduced capability of older persons compared to younger persons to regulate physiological arousal associated with stress (also posited by the SAVI model; Charles, 2010) suggests that intense or sustained loneliness experienced when the protective factors linked with aging discussed above are ineffective at preventing loneliness may pose a more immediate risk to health and cognition in late life compared to earlier ages.

This dissertation makes several contributions to the current literature. First, it includes one of the first longitudinal explorations of associations between loneliness and domain-specific cognitive functioning in late life using age-based (rather than time-based) analyses. Assessment of how age-related performance and change in domain-specific cognition relate to loneliness is an important step toward understanding how feelings of loneliness relate to cognition in mid and late adulthood, as it is change with age rather than time that is more relevant in studies of cognition (c.f., Grimm, Ram, & Estabrook, 2017; Reynolds et al., 2005). This dissertation also includes one of the first studies to compare effects of baseline and longitudinal loneliness on domain-specific cognitive performance and change. Loneliness theorists have proposed that chronic stable loneliness is likely to lead to lasting adverse physiological changes (which may detract from healthy cognitive aging), while transient loneliness is not (Hawkley & Capitanio, 2015). It has also been proposed that such physiological changes associated with loneliness may dissipate even following longer periods of loneliness (Hawkley &

Capitanio, 2015). Results from initial work assessing how transient loneliness and longer-term loneliness (assessed at two waves, 3 years apart) each relate to global cognition support these claims—those who reported feeling lonely at both waves had worse global cognitive function than those who only reported feeling lonely at a single wave (Zhong et al., 2016). However, much remains to be understood about how different patterns of loneliness across time relate to both global and domain-specific cognition. Finally, this dissertation serves as an initial exploration of associations between loneliness and CpG specific DNA methylation of sites within CTRA genes and relations between such methylation and domain-specific cognitive performance and change. This is the one of the first explorations of a potential mechanism for the observed loneliness—cognition association.

Cross-sectional and time-based longitudinal analyses assessing relations between loneliness and domain-specific cognitive performance and change have indicated associations between loneliness and performance on executive function (Shankar et al., 2013), working memory (Wilson et al., 2007) episodic memory (Shankar et al., 2013; Wilson et al., 2007), semantic memory (Wilson et al., 2007), visual memory (O'Luanaigh et al., 2012), visuospatial ability (Wilson et al., 2007), and processing speed (O'Luanaigh et al., 2012; Wilson et al., 2007) tasks, although how loneliness relates to these outcomes across age has not been previously explored. Limited prior work also indicates that longer periods of loneliness may be associated with worse cognitive outcomes than shorter periods of loneliness (Zhong et al., 2016). It was therefore predicted that both baseline and longitudinal loneliness would be associated with performance and change in

processing speed, spatial ability, working memory, and verbal comprehension, and that effects would be stronger for measures of longitudinal loneliness than for baseline loneliness. The results of longitudinal model-fitting analyses from Study 1 have important implications for future work on loneliness and cognition in mid to late life.

In Study 1, both baseline and longitudinal loneliness predicted faster age-associated decline in spatial ability, however this hypothesis was only partially supported for processing speed, as both baseline and longitudinal loneliness predicted *better* processing speed at age 65, while longitudinal loneliness was associated with faster linear and quadratic decline. This hypothesis was not supported for verbal comprehension or working memory. For verbal comprehension, greater time-varying loneliness was associated with slightly faster acceleration in decline, however, after adjusting for education this effect was attenuated and time-varying loneliness predicted slightly reduced performance at age 65 at trend significance. No associations were observed for baseline loneliness for this domain.

Effects of loneliness on performance at age 65 and change across age were small and tended to be observed for tasks tapping more fluid abilities (i.e., processing speed and spatial ability) and for change rather than level (although exceptions to this were observed). The implication of these results is that experiencing feelings of loneliness in mid to late life may lead to slightly faster decline in fluid abilities with age. For those who have reduced or declining cognitive functioning, even slightly faster decline in these domains may have adverse impacts on cognition that may affect quality of life. This is especially relevant considering the finding that losses in processing speed may precede

losses in other cognitive domains (Finkel, Reynolds, McArdle, & Pedersen, 2005; Finkel & Pedersen, 2004; Verhaeghen & Salthouse, 1997).

Standardized effects of loneliness on age-associated change between ages 65 and 80 were larger for longitudinal measures of loneliness than for baseline loneliness for processing speed and verbal comprehension, albeit not for spatial ability. For spatial ability, effects were largest for baseline loneliness and loneliness geometric means, and smaller for time-varying loneliness. Importantly, for verbal comprehension, standardized effects on change were very small, and unstandardized estimates indicated significant effects for time-varying loneliness only, with no effects observed for baseline loneliness or loneliness geometric means. The implication of these results is not clear, however, they appear to indicate that comparison of baseline loneliness and different measures of longitudinal loneliness may show different patterns of associations both within and across cognitive domains, suggesting that the loneliness measure selected for use in studies of loneliness and cognition has important implications for the results. Additional work is needed to further clarify how loneliness across age relates to performance and change in specific domains of cognition.

Although our results indicate that effects of loneliness on cognitive change are small, they suggest that experiencing loneliness may lead to meaningful differences in cognition over time that could adversely impact daily functioning and quality of life for older persons. For individuals with poorer cognitive performance, feelings of loneliness may result in earlier dependence on others for tasks associated with daily living due to earlier cognitive impairment or dementia onset and increased utilization of health

services. Consequently, interventions aimed at preventing loneliness in older adults, if effective, may result in longer maintenance of normative cognitive function and independence, thus enhancing quality of life. Research pointing to harmful effects of loneliness on health and cognition is gaining attention in multiple countries; for example, policy was recently implemented in the United Kingdom aimed at reducing loneliness to deter negative physiological and cognitive outcomes associated with this phenotype (HM Government, 2018).

In Study 2, phenotypic and co-twin control analyses indicated associations between loneliness and methylation at 3 of the 1,586 sites for which associations were assessed, with greater loneliness predicting faster increase in age-associated change in methylation at cg00619097 in *CPT1B* and cg26661481 in *IL10RA*, and slightly reduced methylation at age 70 at cg00403457 in *PTPN12*. Effects of loneliness on methylation were small, suggesting hints of altered methylation associated with loneliness at these sites. These results link loneliness with age-associated increases in methylation at two sites within genes whose transcripts act in a protective manner against inflammation and inflammatory disorders (*IL10RA*; National Center for Biotechnology Information, 2019b) and cellular damage (*CPT1B*; Henique et al., 2010; Karlic et al., 2003; National Center for Biotechnology Information, 2019a). To the extent that increased methylation at these sites may result in reduced expression of these genes, such patterns of altered change in methylation with age associated with loneliness may reduce protective effects of these gene products with age. Non-significant results for 5 other CpG sites within *IL10RA* also showed the same direction of positive effects of loneliness on slope for 4 CpGs. One

nominally significant positive effect of loneliness on slope was observed for a 6th CpG within this gene (b = 0.04, z = 2.14). For *CPT1B*, however, non-significant results for 17 other CpG sites within this gene showed positive associations between loneliness and methylation slope for only 3 CpGs, suggesting that the observed association between loneliness and methylation slope for cg00619097 may not reflect patterns of associations at other sites within this gene. These results also link loneliness with reduced methylation at age 70 at a CpG site within a tumor suppressor gene whose transcripts have been shown to protect against tumor formation (*PTPN12*; e.g., Luo et al., 2014; Xunyi, Xhentao, Dandan, & Funian, 2012). To the extent that decreased methylation at this site corresponds with increased expression of this gene, loneliness may be linked with potential protective effects with respect to expression of this gene at age 70. For *PTPN12*, non-significant results for 23 additional CpGs within this gene also showed negative associations between loneliness and methylation intercept at 18 sites. Future work assessing associations between loneliness and methylation at these sites using a gene-wide approach may provide additional insights.

Co-twin control results indicated that the observed association between loneliness and methylation level at cg00403457 in *PTPN12* at age 70 was almost completely confounded, and the association between loneliness and age-associated change in methylation at cg26661481 in *IL10RA* was partially confounded by genetic and/or environmental factors shared within twin pairs. The implication of this finding is that a portion of the systematic co-variance between loneliness and methylation may be explained in part by direct alterations of methylation at these sites as a result of

experiencing feelings of loneliness (although causal effects in the opposite direction are also possible), however, a portion of the systematic co-variation observed between loneliness and methylation at these sites results from possible third factors (e.g., particular genotypes which may contribute to both social perceptions or behaviors and altered methylation at these sites associated with loneliness), suggestive of potential regulation of both individual differences in adaptation to the experience of loneliness and methylation at these sites by particular genetic factors.

Results from model-fitting analyses assessing relations between loneliness, cognition, and methylation indicated that there were no significant direct associations between loneliness and cognition and that methylation at particular CpG sites was differentially related to performance and change across cognitive domains. Although no direct associations between loneliness and cognition reached significance in Study 2, an indirect pathway was observed between loneliness and processing speed vis-à-vis methylation at cg00403457 in *PTPN12*. Although it has been proposed that mediation should not be examined for factors that are not significantly directly associated, it has also been argued that a direct association is not a prerequisite for mediation, and that significant indirect effects may exist even when a direct effect is not observed (MacKinnon & Fairchild, 2009). The implication of these findings is that further work exploring gene-environment dynamics in the association between loneliness and cognition is warranted and may illuminate pathways that, in part, explain this association by furthering understanding of how loneliness relates to epigenetic changes important to cognition. Results showed significant effects of methylation at one of the two CpG sites

assessed on cognition. The implication of this result is that genes involved in the CTRA may be a promising set for exploration of associations between methylation and cognition. CTRA genes play roles in immune and inflammatory processes (Cole, 2013) and altered methylation at these sites may affect cognition.

Future Directions

One limitation of the current work was that we were restricted in how we could characterize longitudinal patterns of loneliness across age given heterogeneity in the number of waves of data collected across the IGEMS studies used (which ranged from 1 to 10) and long follow up periods between waves in the longitudinal studies. We were unable to successfully use latent profile growth analysis (Asparouhov & Muthén, 2014; Jung & Wickrama, 2008) to classify individuals into groups based on patterns of loneliness across age for the Study 1 sample, and therefore were unable to explore how loneliness trajectory shape relate to cognitive performance and change and how such associations compare with those for other measures of longitudinal loneliness and those for baseline loneliness. The long follow-up periods between waves in these studies also limited our ability to truly distinguish transient loneliness from other loneliness subtypes. Consequently, we recommend that future work on loneliness and cognition further examine how different patterns of loneliness (e.g., transient, intermittent, chronic, late-onset, or earlier enduring loneliness that is later overcome) across age relate to cognitive outcomes. Moreover, to distinguish transient and intermittent loneliness from other loneliness subtypes, or to measure duration of loneliness, we recommend that future work assess loneliness both frequently across shorter periods of time and across longer periods

of time (e.g., measurement burst design; Nesselroade, 1991; Sliwinski, 2008). Such work can answer important questions such as how duration of loneliness relates to cognition and whether effects of loneliness on cognition might lessen or subside once loneliness is overcome. Moreover, loneliness has been reported to predict adverse health outcomes such as inflammation (Jaremka et al., 2013) and cardiovascular disease (Caspi, Harrington, Moffitt, Milne, & Poulton, 2006) which have also been linked with poorer cognition in mid and late adulthood (Elwan et al., 2003; Komulainen et al. 2007; Ravaglia et al., 2005; Schram et al., 2007; Stampfer, 2006; Rafnsson et al., 2007; Teunissen et al., 2003). Such findings indicate that a more holistic approach considering concurrent links between loneliness, physiological changes, and physical and cognitive health is warranted and may shed light on mechanisms of observed associations between loneliness and cognition.

The current results suggest a potential indirect pathway via methylation at cg00403457 in *PTPN12* linking loneliness and processing speed. As mentioned earlier, based on these findings we recommend that future studies explore gene-environment dynamics in the association between loneliness and cognition, as such work has potential to further understanding of the mechanisms behind the loneliness—cognition association. The current results also suggest that genetic factors and/or environmental factors shared within twin pairs were associated with both loneliness and methylation at the sites associated with loneliness in the current work, and, in part, explained the observed associations between loneliness and methylation. Therefore, we recommend that future work examine the extent to which overlapping genetic and environmental factors

contribute to both loneliness and cognition, as the current results indicate that such factors may increase likelihood of both loneliness and epigenetic changes associated with cognition.

Finally, the current results revealed a significant relation between DNA methylation at cg00403457 in *PTPN12* and processing speed. As associations between methylation and cognition were observed for 1 of 2 CpGs for which associations were explored, we recommend that further work be conducted investigating whether and how methylation at other CpG sites within CTRA genes relate to cognitive performance and change in mid and late life. To the extent that methylation at these sites is associated with cognition, we also advocate assessment of whether methylation at these sites mediates associations between environmental exposures and these cognitive outcomes, and investigation of whether observed associations are potentially causal in nature or are confounded by genetic or common environmental factors using a co-twin control approach.

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Appendices

Appendix 1. ***Computation of Loneliness Person Measure Scores Using Rasch Analysis (Study 1 and Study 2)***

Rasch measurement analyses, which estimate single scores on a latent trait based on responses to multiple items assessing the trait (Boone et al., 2014), were used to compute harmonized loneliness scores across 12 IGEMS studies using all available loneliness data from each study. In each study, loneliness was assessed using one or more questionnaire items (range = 1 to 7). See Table A1 for a list of the 17 loneliness items and item response options from each study used for the Rasch analysis.

The loneliness items from each study were combined into a single questionnaire that also included a 10-item version of the UCLA Loneliness Scale (ULS; Russell et al., 1980) and six easy vocabulary items that were added to flag participants who responded indiscriminately to survey items. The questionnaire was administered to a "crosswalk" sample of N = 888 individuals who filled out the questionnaire either in pencil and paper format or online using Mechanical Turk. For the crosswalk sample, those with missing values on most or all of the loneliness items (n = 10) and those with vocabulary scores between 1 and 4 (n = 18) were dropped prior to analysis. The resulting analysis sample (n = 860) was 46.3% male (n = 398) and 53.7% female (n = 462). Age was assessed for the crosswalk sample with an item that asked individuals to report whether they were (a) younger than 60 years or (b) 60 years or older. 50.1% of the sample reported that they were younger than 60 (n = 431), and 49.9% of the sample reported that they were 60 or older (n = 429).

Longitudinal loneliness data from the IGEMS samples and cross-sectional data from the crosswalk sample were pooled for analysis. All loneliness items were coded in the same direction, such that higher scores corresponded with greater reported loneliness.

Partial-credit Rasch models (Boone et al., 2014) were fitted using Winsteps v. 3.92.1. Eleven sets of analyses were run. The first analysis was conducted using data from the two IGEMS studies with the richest loneliness data (GENDER, 7 items, OCTO-Twin, 6 items) and the crosswalk sample. In each subsequent analysis, one additional IGEMS study was added to the analysis to assess whether adding any individual IGEMS study to the analysis resulted in unexpected changes to item difficulties or fit. The analysis yielded latent loneliness scores called "person measures" for each participant that quantify each participant's reported loneliness at each wave based on their responses to individual loneliness items and the item difficulty (i.e., a measure of how likely each loneliness item was to be endorsed in comparison to the other items) of items they endorsed (Boone et al., 2014).

Item outfit statistics were examined for the final analysis with all 13 samples to assess how well the data fit the model. Item outfit statistics quantify model fit for each item, and values between .5 and 1.5 signify that data for an item fit the model reasonably well (Boone et al., 2014). For 13 of the 17 items, outfit statistics fell within this range. For two items (which asked participants '*do you often feel lonely?*' *and 'do you feel at the present moment you are very lonely, fairly lonely, or not at all lonely?*') item outfit was less than the cutoff value of .5. For these items, the low outfit values of .25 and .48, respectively, indicate that these items did not contribute as much as others to the construction of the latent scores, but also did not add extra noise to or distort the model (Boone et al., 2014). For two items (which asked '*if you ever have personal problems or are in trouble, do you have someone you can talk to?*' and '*do you often feel lonely even*

when you are together with others?'), item outfit was above the cutoff value of 1.5. For these items, high outfit values of 1.75 and 1.59, respectively, indicate that these items did not contribute to the construction of latent scores due to unexplained variance remaining after fitting the model. However, since both values were less than 2, they did not distort the model (Boone et al., 2014). The computed harmonized loneliness scores were in logit units.

Table A1

Loneliness Items Used to Compute Person Measures for Each IGEMS Study

Loneliness Item	**IGEMS Studies/Waves**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
CES-D item 14:	SATSA	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪
I felt lonely	OCTO-Twin	▪	▪	▪	▪	▪					
	GENDER	▪	▪	▪							
	TOSS	▪									
	VETSA	▪	▪								
	MTSADA	▪		▪							
CAMDEX item 12:	LSADT	▪	▪	▪	▪	▪	▪				
Have you felt lonely lately?	MADT	▪	▪								
Are you ever troubled by feelings of loneliness?	SATSA		▪								
Do you often feel lonely even when you are together with others?	SATSA		▪								
Do you suffer from feelings of loneliness nowadays?	OCTO-Twin	▪	▪	▪	▪	▪					
	GENDER	▪	▪	▪							
Have you got friends with whom you can talk?	OCTO-Twin	▪	▪	▪	▪	▪					
	GENDER	▪	▪	▪							
Do you feel you are a part of a set of friends?	OCTO-Twin	▪	▪	▪	▪	▪					
	GENDER	▪	▪	▪							
Do you lack company?	OCTO-Twin	▪	▪	▪	▪	▪					
	GENDER	▪	▪	▪							
Do you feel abandoned?	OCTO-Twin	▪	▪	▪	▪	▪					
	GENDER	▪	▪	▪							
NHP scale Item 9: I feel lonely	GENDER	▪	▪	▪							
If you have personal problems or are in trouble, do you have someone you can talk to?	MADT	▪									
I often feel lonely	MTSADA	▪		▪							
LSIA scale Item 21: I often experience periods of loneliness	MTSADA	▪		▪							
Do you often feel lonely?	A50	▪									
I feel lonely, even in the presence of other people.	A50	▪									

Loneliness Item	IGEMS Studies/Waves	1	2	3	4	5	6	7	8	9	10
During the past 30 days how much of the time did you feel lonely?	MIDUS	▪									
Do you feel at the present moment you are very lonely, fairly lonely, or not at all lonely?	FTC	♦									
10-Item UCLA Loneliness Scale	Crosswalk Only	♦									

Note: CES-D scale = Center for Epidemiologic Studies Depression scale (Radloff, 1977); CAMDEX = Cambridge Mental Disorders of the Elderly Examination (Roth et al., 1986); NHP Scale = Nottingham Health Profile (Hunt, McEwen, & McKenna, 1985); LSIA scale = Life Satisfaction Index-A (Neugarten, Havighurst, & Tobin, 1961). ♦ = Given to crosswalk sample; data from FTC not included in Rasch analysis. A50 = Australia Over 50's Twin Study; MIDUS = Midlife in the United States: A National Study of Health and Well-Being (MIDUS) (Kessler, Gilman, Thornton, & Kendler, 2004); FTCS = Finnish Twin Cohort Study (Kaprio, 2013).

Appendix 2: ***Harmonization of Depression Across IGEMS Studies (Study 1)***

Table A2

Conversion Table for Converting Total Scores on the CAMDEX to CES-D Units

CAMDEX Score	PM (Ɵ)	CES-D Score	CAMDEX Score	PM (Ɵ)	CES-D Score
0	-5.57	.991	20	1.06	38.861
1	-4.31	2.482	21	1.28	40.722
2	-3.53	4.284	22	1.52	42.623
3	-3.04	5.953	23	1.76	44.379
4	-2.66	7.605	24	2.03	46.173
5	-2.34	9.272	25	2.32	47.885
6	-2.05	11.015	26	2.66	49.616
7	-1.79	12.769	27	3.06	51.297
8	-1.55	14.547	28	3.57	52.954
9	-1.31	16.471	29	4.37	54.692
10	-1.08	18.441	30	5.65	56.093
11	-.86	20.426			
12	-.64	22.493			
13	-.43	24.525			
14	-.21	26.695			
15	.00	28.783			
16	.21	30.868			
17	.42	32.928			
18	.63	34.944			
19	.84	36.898			

Note. Harmonized depression scores were computed excluding single loneliness items from each scale. A CAMDEX item that asked whether participants '*preferred to be more on their own recently*' was also excluded for harmonization.

Table A3

Conversion Table for Converting Total Scores on the CES-D to CAMDEX Units

CES-D Score	PM (Ɵ)	CAMDEX Score	CES-D Score	PM (Ɵ)	CAMDEX Score
0	-5.41	0.061	29	.04	15.14
1	-4.19	0.298	30	.11	15.58
2	-3.47	0.752	31	.19	16.08
3	-3.04	1.272	32	.27	16.58
4	-2.73	1.829	33	.34	17.02
5	-2.48	2.42	34	.42	17.52
6	-2.27	3.03	35	.50	18.02
7	-2.09	3.63	36	.58	18.52
8	-1.93	4.24	37	.66	19.02
9	-1.79	4.82	38	.75	19.57
10	-1.66	5.40	39	.83	20.05
11	-1.53	6.02	40	.92	20.58
12	-1.42	6.57	41	1.01	21.11
13	-1.31	7.14	42	1.11	21.68
14	-1.21	7.68	43	1.21	22.23
15	-1.11	8.22	44	1.31	22.77
16	-1.02	8.73	45	1.42	23.33
17	-.92	9.30	46	1.53	23.88
18	-.84	9.77	47	1.66	24.48
19	-.75	10.30	48	1.79	25.05
20	-.67	10.77	49	1.94	25.66
21	-.58	11.31	50	2.10	26.25
22	-.50	11.80	51	2.28	26.84
23	-.43	12.22	52	2.48	27.42
24	-.35	12.71	53	2.73	28.02
25	-.27	13.20	54	3.05	28.61
26	-.19	13.70	55	3.48	29.17
27	-.12	14.14	56	4.20	29.66
28	-.04	14.63	57	5.42	29.93

Note. As noted above, harmonized depression scores were computed excluding single loneliness items from each scale. A CAMDEX item that asked whether participants '*preferred to be more on their own recently*' was also excluded for harmonization.

Appendix 3: *Model-Fitting Results for Spline Models (Study 1)*

Loneliness and Processing Speed (Symbol Digit)

The covariance parameter estimate for individuals within twin pairs for the linear age term at or after age 65 (slope B) hit a boundary of 0 in the unconditional spline model and was removed from all subsequent spline models. Model fit significantly improved when covariates were added to this reduced unconditional spline model ($\Delta\chi^2(15) = 545.7$, $p < .0001$). As was seen for quadratic models fitted to the Symbol Digit data, adding baseline loneliness to this model improved model fit at trend significance ($\Delta\chi^2(3) = 7.1$, $p = .0688$), and adding geometric means for loneliness across waves significantly improved model fit ($\Delta\chi^2(3) = 14.2$, $p = .0026$). As observed for the quadratic models, effects of loneliness on the intercept were small and positive ($b = 0.21$, $p = .0347$ for baseline, $b = 0.14$, $p = .0310$ for time-varying, and $b = 0.25$, $p = .0372$ for loneliness geometric means) indicating that higher loneliness was associated with higher performance on this task at age 65. Loneliness was negatively associated with linear slope at and after age 65. For baseline loneliness, a trend significant effect of loneliness on slope B was observed ($b = -0.013$, $p = .0853$). For time-varying loneliness ($b = -0.018$, $p = .0001$) and loneliness geometric means ($b = -0.030$, $p = .0007$) effects were significant at .01. These results suggest a link between longitudinal loneliness and faster decline in Symbol Digit scores with age at and after age 65.

After adjusting for educational attainment, the effect of baseline loneliness on intercept was attenuated and non-significant ($b = 0.13$, $p = .3695$), while effects of time-varying loneliness and loneliness geometric means were larger in magnitude ($b = 0.19$, $p = .0284$ and $b = 0.27$, $p = .1274$, respectively), although the effect for loneliness

geometric means was no longer significant. Regression weights were also larger for effects of loneliness on linear slope at age 65 after adjusting for educational attainment. For baseline loneliness, the increase in the magnitude of the regression weight was minimal (b = -0.014, p = .1805) and trend significance was no longer observed. For time-varying loneliness (b = -.025, p = .0001) and loneliness geometric means (b = -0.036, p = .0023), the increase in effect size was larger and effects remained significant. After adjusting for practice effects, effects of loneliness on the intercept were attenuated and non-significant, effects of loneliness on linear slope at/after age 65 were slightly attenuated, and the effect of baseline loneliness was non-significant. See Tables A4 and A5 for model fit statistics and regression weights (b) for spline models for the Symbol Digit task.

Effect sizes (d) for Symbol Digit spline models showed no effect of baseline loneliness on change in Symbol Digit scores between ages 65 and 80 (d = 0.00). A small negative effect of time-varying loneliness (d = -0.24) was observed. The effect of loneliness geometric means was negative and larger in magnitude (d = -0.39). These effect sizes suggest that effects of the longitudinally-informed measures of loneliness on change in Symbol Digit scores between ages 65 and 80 were small and negative, with faster decline in task performance associated with time-varying loneliness and loneliness geometric means. As was seen for the Symbol Digit quadratic model, the effect was largest in magnitude for loneliness geometric means. Figure A1 shows predicted Symbol Digit scores by age for high, intermediate, and low loneliness.

Loneliness and Spatial Ability (Block Design)

Adding covariates to the unconditional spline model significantly improved model fit ($\Delta\chi^2(15) = 165.0$, $p < .0001$). When covariates were added to the model, the covariance parameter estimates for both twin pairs and individuals within twin pairs for the linear age term prior to age 65 (slope A) hit a boundary of 0. These covariance parameters were dropped from subsequent models. Since no random effects were modeled on this linear term, no interactions with the slope A term were included in subsequent models. Adding baseline loneliness to this reduced spline model improved model fit at trend significance ($\Delta\chi^2(2) = 5.9$, $p = .0523$); adding geometric means for loneliness significantly improved model fit ($\Delta\chi^2(2) = 8.9$, $p = .0117$). No effects of loneliness on the intercept were observed. Time-varying loneliness was negatively associated with linear change across age at/after age 65 ($b = -0.01$, $p = .0471$), suggestive that higher loneliness scores across waves were related to faster decline in task performance after age 64. The magnitude of the effect remained the same after adjusting for educational attainment ($b = -.01$, $p = .0546$), although significance dropped to trend level. Model fit statistics and regression weights (*b*) for the Block Design spline models are shown in Tables A6 and A7.

Effect sizes (*d*) for the Block Design spline models indicated that effects of loneliness on change in Block Design performance between ages 65 and 80 were small and negative and did not vary for baseline loneliness, time-varying loneliness, and loneliness geometric means ($d = -0.13$). This finding suggests slightly faster decline in Block Design scores was associated with loneliness between ages 65 and 80 that was

consistent across loneliness predictors. Figure A2 shows predicted Block Design scores by age for high, intermediate, and low loneliness.

Loneliness and Verbal Comprehension (Synonyms)

The covariance parameter estimate for twin pairs for the linear age effect prior to age 65 (slope A) hit a boundary of 0 in the unconditional spline model and was removed from subsequent spline models. Adding covariates to this reduced unconditional model resulted in a significant improvement in model fit ($\Delta\chi^2(12) = 34.0$, $p = .0034$). When covariates were added to the model, the covariance parameter estimates for twin pairs for the linear age term at or after age 65 (slope B) hit a boundary of 0 and was subsequently excluded from spline models. Adding baseline loneliness ($\Delta\chi^2(3) = 5.2$, $p = .1577$) and loneliness geometric means ($\Delta\chi^2(3) = 3.8$, $p = .2839$) to the model did not significantly improve fit. No significant or trend significant effects of loneliness on the intercept or linear slope prior to age 65 were observed. For time-varying loneliness, a very small negative effect of loneliness on linear slope at/after age 65 was observed ($b = -.0112$, $p = .0404$), suggestive that higher loneliness scores were associated with faster linear decline in Synonyms scores with age. This effect was slightly attenuated and trend significant after adjusting for educational attainment ($b = -.0093$, $p = .0897$) and practice effects ($b = -.0097$, $p = .0784$). Tables A8 and A9 display model fit statistics and regression weights (b) for spline models for the Synonyms task.

Effect sizes (d) for Synonyms spline models showed a very small positive effect of baseline loneliness on change in Synonyms scores between ages 65 and 80 ($d = 0.07$). A small negative effect of time-varying loneliness was observed ($d = -0.15$). There was

no effect of loneliness geometric means on change in Synonyms scores between ages 65 and 80 (d = 0.00). Figure A3 shows predicted Synonyms scores by age for high, intermediate, and low loneliness. Effect sizes (d) for loneliness on change in cognitive scores between ages 65 and 80 for spline models are shown in Table A10 for each cognitive task.

Table A4

Model Fit Statistics for Symbol Digit Spline Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***p***
Unconditional						
A. Intercept Only	9,042	134484.8	134492.8	---	---	---
B. Age	9,042	129419.4	129437.4	5,065.4	5	< .0001
C1. Age(<65) + Age(≥65)	9,042	129184.6	129214.6	234.8	7	< .0001
C1♦: Age(<65) + Age(≥65)	9,042	129217.3	129243.3	202.1	4	< .0001
Model C1♦ + Practice	9,042	128961.6	128989.6	255.7	1	< .0001
Conditional						
D: Model C1♦ + Covariates	9,042	128671.6	128727.6	545.7	15	< .0001
E1: Model D + Baseline Loneliness	9,042	128664.5	128726.5	7.1	3	.0688
E2: Model D + Time-Varying Loneliness	9,032	127889.4	127951.4	---	---	---
E3: Model D + Loneliness Geometric Means	9,042	128657.4	128719.4	14.2	3	.0026
Sensitivity (Education)						
Model E1 + Education	2,897	46867.1	46933.1	---	---	---
Model E2 + Education	2,897	46423.4	46489.4	---	---	---
Model E3 + Education	2,897	46858.6	46924.6	---	---	---
Sensitivity (Practice)						
Model E1 + Practice	9,042	128414.4	128478.4	---	---	---
Model E2 + Practice	9,032	127645.2	127709.2	---	---	---
Model E3 + Practice	9,042	128410.1	128474.1	---	---	---

Note. Model C1♦ = Unconditional spline model with the covariance parameter estimate for individuals within twin pairs for the linear age term (for ≥65 years) removed. This parameter hit a boundary of 0 in the unconditional spline model and was removed from subsequent models.

Table A5

Unstandardized Parameters (b) for Symbol Digit Spline Models

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**	**Model E1 + Pract**	**Model E2 + Pract.**	**Model E3 + Pract.**
Level											
Perf. (age 65)	52.36**	54.95**	55.72**	55.29**	55.74**	55.85**	55.96**	56.22**	54.76**	54.29**	54.57**
Sex	---	1.71**	1.71**	1.64**	1.68**	2.92**	2.83**	2.84**	1.62**	1.58**	1.61**
Country	---	2.71**	2.66**	2.46**	2.54**	7.34**	7.00**	7.21**	3.04**	2.86**	2.97**
Marital	---	0.21	0.11	0.14	0.13	0.62	0.49	0.60	0.32	0.34	0.34
Live Alone	---	-0.99	-1.05	-0.96	-1.03	-1.23	-1.03	-1.20	-0.70	-0.60	-0.67
Depression	---	-0.25**	-0.28**	-0.27**	-0.28**	-0.21**	-0.21**	-0.22**	-0.25**	-0.24**	-0.25**
Baseline Loneliness	---	---	**0.21***	---	---	0.13	---	---	0.15	---	---
Time-Varying Loneliness	---	---	---	**0.14***	---	---	**0.19***	---	---	0.05	---
Loneliness Geomeans	---	---	---	---	**0.25***	---	---	0.27	---	---	0.11
Education	---	---	---	---	---	1.11**	1.12**	1.12**	---	---	---
Practice	---	---	---	---	---	---	---	---	2.10**	2.09**	2.09**
Linear Change (< 65)											
Linear slope (< 65)	-0.41**	-0.47**	-0.48**	-0.49**	-0.48**	-0.61**	-0.58**	-0.57**	-0.58**	-0.58**	-0.59**
Sex	---	-0.05^{t}	-0.06^{t}	-0.06*	-0.06^{t}	-0.16**	-0.16**	-0.16**	-0.06*	-0.06*	-0.06*
Country	---	-0.25**	-0.25**	-0.26**	-0.25**	-0.70**	0.66**	0.69**	-0.20**	-0.21**	-0.20**

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**	**Model E1 + Pract**	**Model E2 + Pract.**	**Model E3 + Pract.**
Marital status	---	-0.06	-0.05	-0.05	-0.05	0.05	0.04	0.04	-0.02	-0.03	-0.02
Live Alone	---	0.087	0.082	0.086	0.085	-0.012	-0.001	-0.011	0.086	0.091	0.090
Depression	---	-0.0026	-0.0024	-0.0018	-0.0027	0.0015	0.0004	0.0004	-0.0007	-0.0010	-0.0006
Baseline Loneliness	---	---	-0.0021	---	---	0.0014	---	---	-0.0051	---	---
Time-Varying Loneliness	---	---	---	-0.0014	---	---	0.0106	---	---	-0.0041	---
Loneliness Geomeans	---	---	---	---	-0.0008	---	---	0.0131	---	---	-0.0087
Education	---	---	---	---	---	0.03**	0.03**	0.03**	---	---	---
Linear Change (≥ 65)											
Linear slope (≥ 65)	-0.72**	-0.68**	-0.73**	-0.73**	-0.78**	-0.56**	-0.59**	-0.63**	-0.80**	-0.80**	-0.84**
Sex	---	0.03	0.03	0.04	0.03	-0.04	-0.02	-0.03	0.04	0.04^{t}	0.04
Country	---	0.08**	0.08**	0.09**	0.09**	-0.12	-0.09	-0.11	0.11**	0.12**	0.11**
Marital Status	---	-0.15**	-0.15**	-0.15**	-0.15**	-0.15*	-0.15^{t}	-0.16*	-0.15**	-0.15**	-0.15**
Live Alone	---	0.07	0.07	0.07	0.08	0.07	0.05	0.07	0.06	0.06	0.06
Depression	---	-0.0001	0.0013	0.0018	0.0027	0.0025	0.0036	0.0043	0.0007	0.0011	0.0017
Baseline Loneliness	---	---	**-0.013^{t}**	---	---	-0.014	---	---	-0.009	---	---
Time-Varying Loneliness	---	---	---	**-0.018****	---	---	**-0.025****	---	---	**-0.013****	---

Fixed Effects	**Model C1♦**	**Model D**	**Model E1**	**Model E2**	**Model E3**	**Model E1 + Educ.**	**Model E2 + Educ.**	**Model E3 + Educ.**	**Model E1 + Pract**	**Model E2 + Pract.**	**Model E3 + Pract.**
Loneliness											
Geomeans	---	---	---	---	**-0.030****	---	---	**-0.036****	---	---	**-0.020***
Education	---	---	---	---	---	0.004	0.004	0.004	---	---	---

**$p < .01$, * $p < .05$, $^{t} = p < .10$ *Note*. Educ. = years of education. Pract. = Practice effect. Significant and trend significant effects of loneliness are in bold. Model C1♦ = Unconditional spline model with the covariance parameter estimate for individuals within twin pairs for the linear age term (for ≥65 years) removed. This parameter hit a boundary of 0 in the unconditional spline model and was removed from subsequent models.

Table A6

Model Fit Statistics for Block Design Spline Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***P***
Unconditional						
A. Intercept Only	2,263	41853.3	41861.3	---	---	---
B. Age	2,263	40669.5	40687.5	1,183.8	5	< .0001
C1. Age(<65) + Age(≥65)	2,263	40569.2	40601.2	100.3	7	< .0001
Model C1 + Practice	2,263	40527.3	40557.3	41.9	1	< .0001
Conditional						
D: Model C1 + Covariates	2,263	40404.2	40462.2	165.0	15	< .0001
D♦: Model C1 + Covariates	2,263	40471.8	40511.8	97.4	4	< .0001
E1: Model D♦ + Baseline Loneliness	2,263	40465.9	40509.9	5.9	2	.0523
E2: Model D♦ + Time-Varying Loneliness	2,263	39971.4	40015.4	---	---	---
E3: Model D♦ + Loneliness Geometric Means	2,263	40462.9	40506.9	8.9	2	.0117
Sensitivity (Education)						
Model E2 + Education	2,210	39398.1	39446.1	---	---	---

Note. Model D♦ = Conditional spline model with the covariance parameter estimates for (a) twin pairs, and (b) individuals within twin pairs for the linear age term prior to age 65 (AgeC65A) effect removed. These parameters hit a boundary of 0 when covariates were added to the unconditional spline model and were removed from subsequent spline models, as were interaction terms for the AgeC65A term.

Table A7

Unstandardized Parameters (b) for Block Design Spline Models

Fixed Effects	**Model C1**	**Model D**	**Model D♦**	**Model E1**	**Model E2**	**Model E3**	**Model E2 + Educ.**
Level							
Performance (age 65)	53.78**	53.20**	55.93**	55.70**	56.00**	55.34*	54.12**
Sex	---	-1.28*	-1.64**	-1.63**	-1.62**	-1.62**	-1.04[t]
Country	---	7.52**	0.83	0.66	0.71	0.49	13.92**
Marital Status	---	-0.25	-0.71	-0.66	-0.78	-0.57	-0.92
Live Alone	---	0.003	0.17	0.18	0.23	0.18	0.43
Depression	---	-0.27**	-0.21**	-0.21**	-0.22**	-0.20**	-0.21**
Baseline Loneliness	---	---	---	-0.06	---	---	---
Time-Varying Loneliness	---	---	---	---	0.05	---	0.04
Loneliness Geomeans	---	---	---	---	---	-0.18	---
Education	---	---	---	---	---	---	1.21**
Linear Change (< 65)							
Linear slope (< 65)	-0.23**	-0.44**	-0.20**	-0.20**	-0.21**	-0.20**	-0.18**
Sex	---	0.12*	---	---	---	---	---
Country	---	0.68**	---	---	---	---	---
Marital status	---	0.09	---	---	---	---	---
Live Alone	---	-0.001	---	---	---	---	---
Depression	---	-0.006*	---	---	---	---	---
Baseline Loneliness	---	---	---	---	---	---	---
Time-Varying Loneliness	---	---	---	---	---	---	---

Fixed Effects	**Model C1**	**Model D**	**Model D♦**	**Model E1**	**Model E2**	**Model E3**	**Model E2 + Educ.**
Loneliness							
Geomeans	---	---	---	---	---	---	---
Education	---	---	---	---	---	---	---
Linear Change (≥ 65)							
Linear slope (≥ 65)	-0.47**	-0.77**	-1.08**	-1.12**	-1.11**	-1.11**	-0.96
Sex	---	0.14**	0.15**	0.15**	0.15**	0.16**	0.13**
Country	---	0.57	1.26**	1.24**	1.26**	1.23**	0.87*
Marital Status	---	-0.22**	-0.20**	-0.20**	-0.19**	-0.20**	-0.16*
Live Alone	---	0.11	0.10	0.10	0.09	0.10	0.07
Depression	---	0.004^{t}	0.002	0.003	0.003	0.003	0.002
Baseline Loneliness	---	---	---	-0.01	---	---	---
Time-Varying Loneliness	---	---	---	---	**-0.01***	---	**-0.01^{t}**
Loneliness Geomeans	---	---	---	---	---	-0.01	---
Education	---	---	---	---	---	---	-0.01*

**$p < .01$, * $p < .05$, ^{t} = $p < .10$ *Note*. Educ. = years of education. Pract. = Practice effect. Significant and trend significant effects of loneliness are in bold. Model D♦ = Conditional spline model with the covariance parameter estimates for (a) twin pairs, and (b) individuals within twin pairs for the linear age term prior to age 65 (AgeC65A) effect removed. These parameters hit a boundary of 0 when covariates were added to the unconditional spline model and were removed from subsequent spline models, as were interaction terms for the AgeC65A term.

Table A8

Model Fit Statistics for Synonyms Spline Models

Model	***N***	**-2LL**	**AIC**	**$\Delta\chi^2$**	**Δdf**	***p***
Unconditional						
A. Intercept Only	3,204	45904.3	45912.3	---	---	---
B. Age	3,204	45586.2	45602.2	318.1	5	< .0001
C1. Age(<65) + Age(≥65)	3,204	45533.3	45563.3	52.9	7	< .0001
C1♦. Age(<65) + Age(≥65)	3,204	45536.4	45562.4	49.8	4	< .0001
Model C1♦ + Practice	3,204	45520.0	45552.0	16.4	1	< .0001
Conditional						
D: Model C1♦ + Covariates	3,204	45502.4	45550.4	34.0	12	.0007
D♦: Model C1♦ + Covariates	3,204	45502.8	45548.8	33.6	10	.0002
E1: Model D♦ + Baseline Loneliness	3,204	45497.6	45549.6	5.2	3	.1577
E2: Model D♦ + Time-Varying Loneliness	3,204	45093.5	45145.5	---	---	---
E3: Model D♦ + Loneliness Geometric Means	3,204	45499.0	45551.0	3.8	3	.2839
Sensitivity (Education)						
Model E2 + Education	3,204	40591.0	40647.0	---	---	---
Sensitivity (Practice)						
Model E2 + Practice	3,204	45082.0	45136.0	11.5	---	---

Note. Model C1♦ = unconditional spline model with the covariance parameter estimate for twin pairs for the linear age effect prior to age 65 removed. This parameter hit a boundary of 0 in the unconditional spline model and was removed from subsequent spline models. Model D♦ = conditional spline model with the covariance parameter estimates for twin pairs for both linear age terms (prior to age 65, and at/after age 65) removed. The covariance parameter for the second linear age term hit a boundary of 0 when covariates were added to the model. This term was removed from subsequent spline models.

Table A9

Unstandardized Parameters (b) for Synonyms Spline Models

Fixed Effects	**Model C1♦**	**Model D**	**Model D♦**	**Model E1**	**Model E2**	**Model E3**	**Model E2 + Educ.**	**Model E2 + Practice**
Level								
Performance (age 65)	53.93**	54.51**	54.49**	53.78**	54.26**	54.07**	58.53**	54.06**
Sex	---	-0.17	-0.17	-0.17	-0.31	-0.16	0.21	-0.33
Marital Status	---	-0.54	-0.51	-0.43	-0.46	-0.48	1.09	-0.33
Live Alone	---	-1.42	-1.44	-1.40	-1.37	-1.42	-1.63	-1.19
Depression	---	0.0036	0.0046	0.0308	0.0111	0.0176	0.0005	0.0138
Baseline Loneliness	---	---	---	-0.182	---	---	---	---
Time-Varying Loneliness	---	---	---	---	-0.006	---	-0.068	-0.030
Loneliness Geomeans	---	---	---	---	---	-0.112	---	---
Education	---	---	---	---	---	---	1.50**	---
Practice	---	---	---	---	---	---	---	0.58**
Linear Change (< 65)								
Linear slope (< 65)	-0.032[t]	-0.021	-0.022	-0.012	0.008	-0.004	0.177**	-0.006
Sex	---	-0.04	-0.04	-0.03	-0.04	-0.03	-0.02	-0.04
Marital status	---	-0.005	-0.006	-0.011	0.004	-0.004	-0.123	-0.001
Live Alone	---	0.04	0.04	0.04	0.02	0.03	0.03	0.02
Depression	---	0.0015	0.0016	0.0010	0.0002	0.0007	0.0051*	0.0004
Baseline Loneliness	---	---	---	0.002	---	---	---	---
Time-Varying Loneliness	---	---	---	---	0.010	---	-0.003	0.009

	Model C1♦	**Model D**	**Model D♦**	**Model E1**	**Model E2**	**Model E3**	**Model E2 + Educ.**	**Model E2 + Practice**
Loneliness Geomeans						0.004		---
Education	---	---	---	---	---	---	0.04**	---
Practice	---	---	---	---	---	---	---	---
Linear Change (≥ 65)								
Linear slope (≥ 65)	-0.20**	-0.17**	-0.17**	-0.15**	-0.19**	-0.17**	-0.16**	-0.21**
Sex	---	0.09**	0.09**	0.09**	-0.10**	0.09**	0.09**	0.10**
Marital Status	---	-0.12	-0.12	-0.12^{t}	-0.13^{t}	-0.12^{t}	-0.10	-0.13^{t}
Live Alone	---	0.09	0.09	0.09	0.09	0.09	0.08	0.08
Depression	---	-0.002	-0.002	-0.003	-0.001	-0.002	-0.001	-0.002
Baseline Loneliness	---	---	---	0.00505	---	---	---	---
Time-Varying Loneliness	---	---	---	---	**-0.01124***	---	**-0.00927^{t}**	**-0.0097^{t}**
Loneliness Geomeans	---	---	---	---	---	0.00008	---	---
Education	---	---	---	---	---	---	0.001	---

**$p < .01$, * $p < .05$, ^{t} = $p < .10$ *Note*. Educ. = years of education. Pract. = Practice effect. Significant and trend significant effects of loneliness are in bold. Model C1♦ = unconditional spline model with the covariance parameter estimate for twin pairs for the linear age effect prior to age 65 removed. This parameter hit a boundary of 0 in the unconditional spline model and was removed from subsequent spline models. Model D♦ = conditional spline model with the covariance parameter estimates for twin pairs for both linear age terms (prior to age 65, and at/after age 65) removed. The covariance parameter for the second linear age term hit a boundary of 0 when covariates were added to the model. This term was removed from subsequent spline models.

Table A10

Effect Sizes (d) for Loneliness on Change in Cognitive Performance Between Ages 65 to 80 for Spline Models

	Baseline Loneliness (*d*)	Time-Varying Loneliness (*d*)	Loneliness Geometric Means (*d*)
Symbol Digit	0.00	-0.24	-0.39
Block Design	-0.13	-0.13	-0.13
Synonyms	0.07	-0.15	0.00

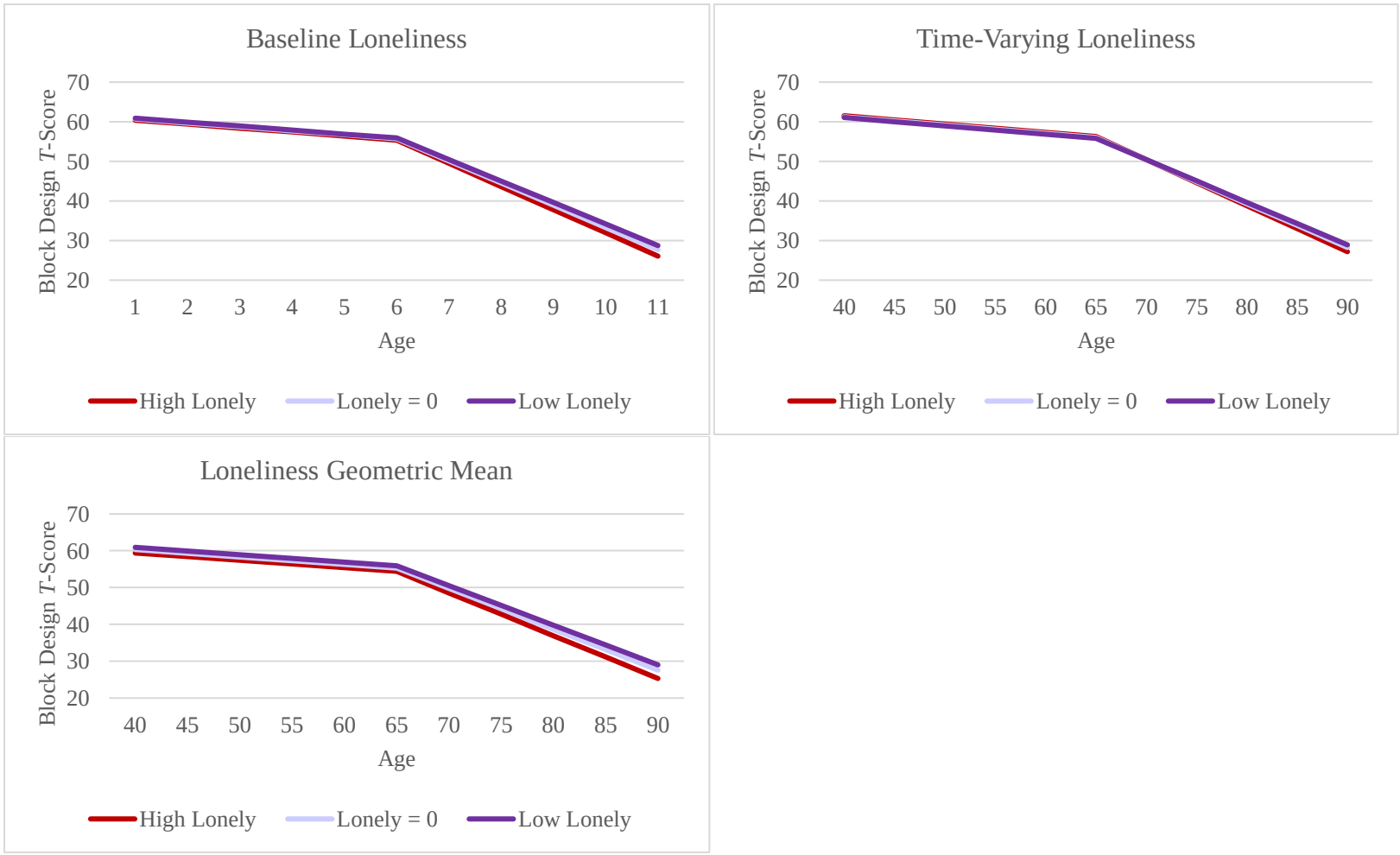

Figure A1. Expected trajectory plots by loneliness for Block Design *T*-scores estimated from spline models.

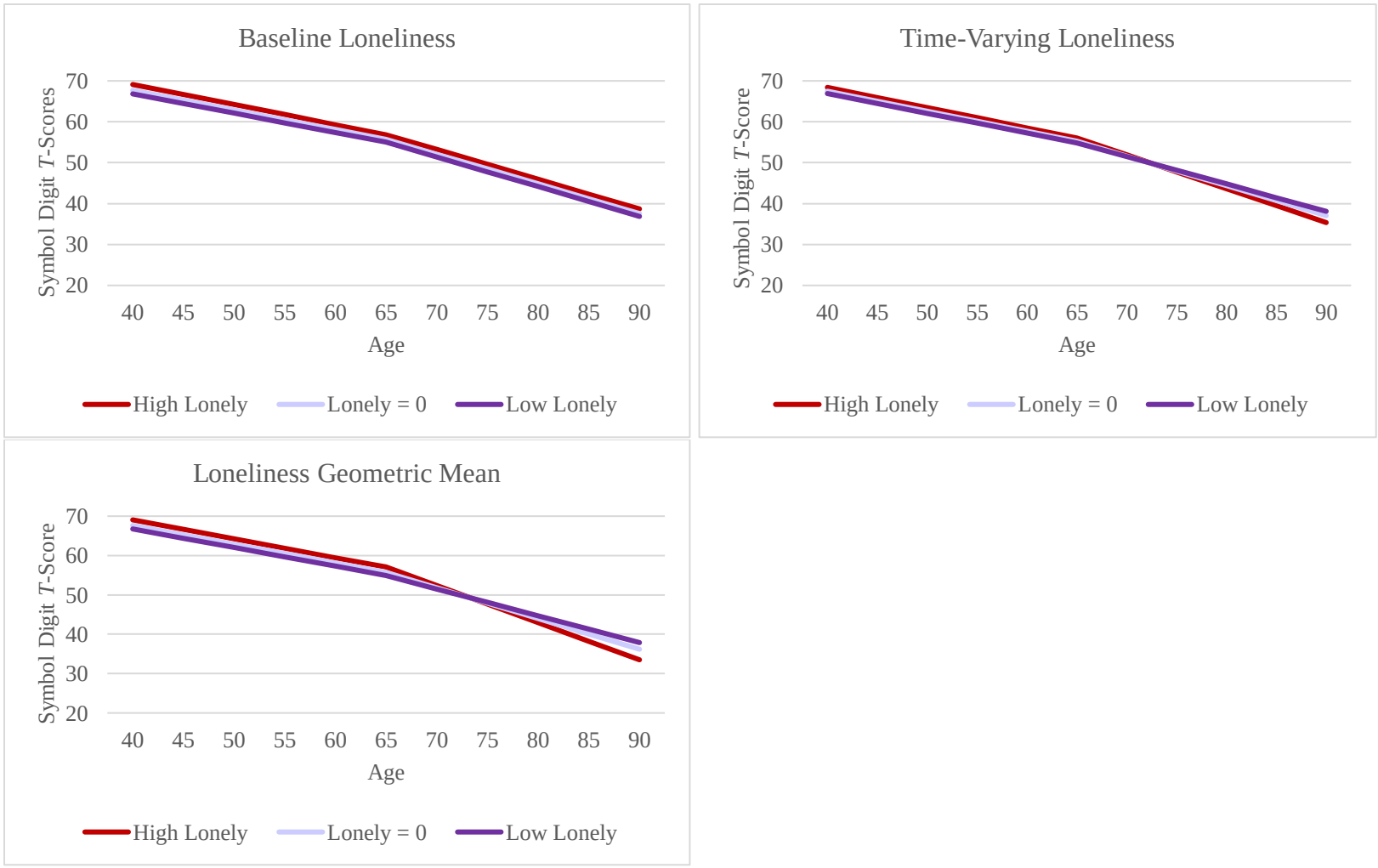

Figure A2. Expected trajectory plots by loneliness for Symbol Digit *T*-scores estimated from spline models.

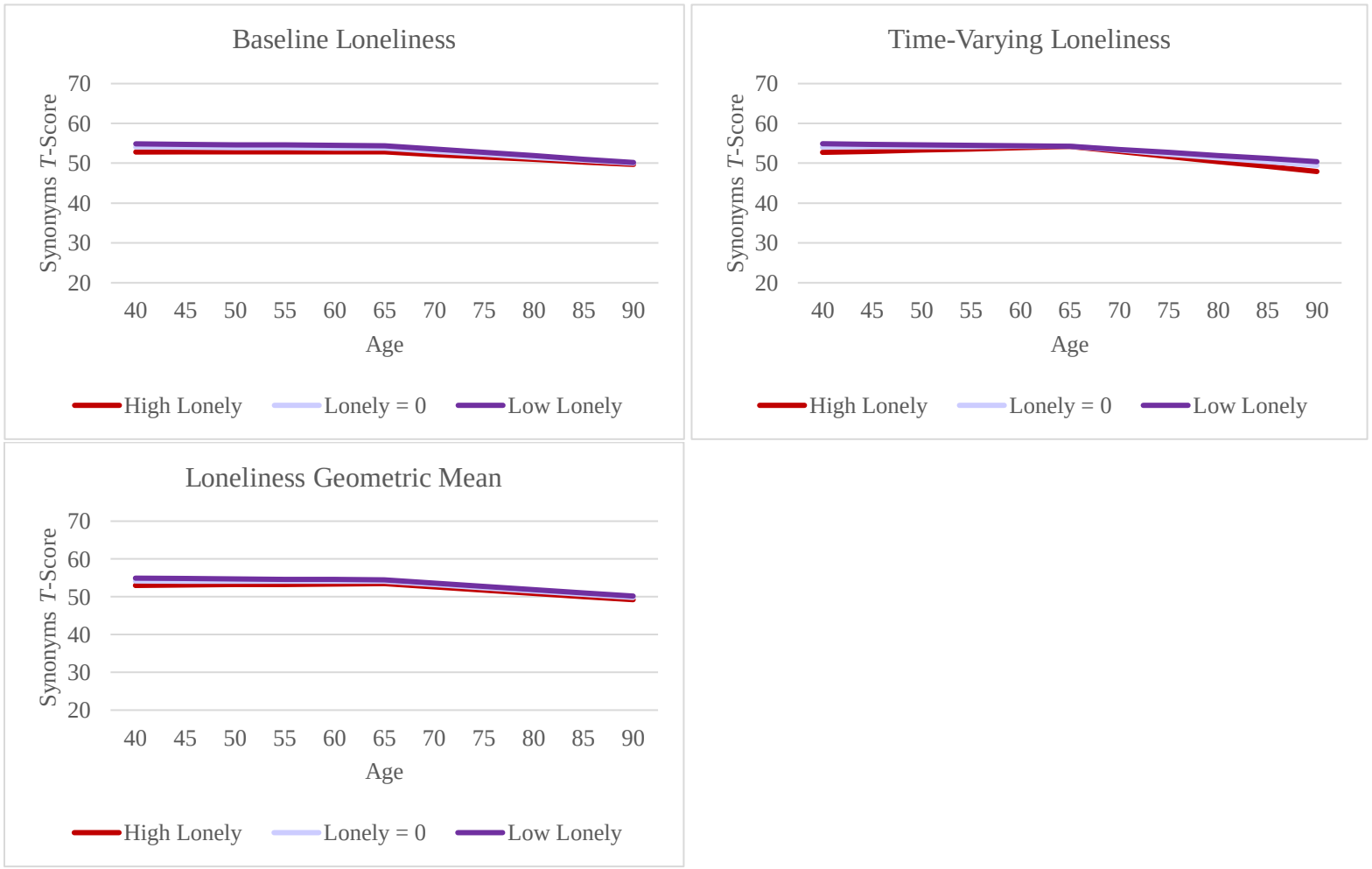

Figure A3. Expected trajectory plots by loneliness for Synonyms *T*-scores estimated from spline models.

Appendix 4: ***Genes/Chromosomes Associated with the 1,586 CpGs for Which Relations between Loneliness and Methylation were Assessed (Study 2)***

Table A11

Genes and Chromosomes Associated With the 1,586 CpGs for Which Associations Between Loneliness and Methylation Were Assessed

Gene Name	n_{CpG}	Chr	Gene Name	n_{CpG}	Chr
ARFGEF2	8 (.5%)	20	KIAA0101	8 (.5%)	15
ARID1A	22 (1.39%)	1	KIAA1033	8 (.5%)	12
ATXN1	46 (2.9%)	6	KIF21B	33 (2.08%)	1
BHLHB2	18 (1.13%)	3	LGALS8	15 (.95%)	1
BTG2	12 (.76%)	1	LR8	14 (.88%)	7
C21orf7	3 (.19%)	21	MAN2C1	21 (1.32%)	15
C22orf8	23 (1.45%)	22	MAX	13 (.82%)	14
CA2	8 (.5%)	8	MCL1	13 (.82%)	1
CBFB	14 (.88%)	16	MFAP3L	15 (.95%)	4
CCR2	2 (.13%)	3	MS4A1	2 (.13%)	11
CD164	12 (.76%)	6	MSCP	20 (1.26%)	8
CD79B	10 (.63%)	17	MTRR	9 (.57%)	5
CDC25B	13 (.82%)	20	MYBL1	12 (.76%)	8
CDKN1C	38 (2.4%)	11	MYST3	12 (.76%)	8
CLIC4	19 (1.2%)	1	NEDD5	16 (1.01%)	2
CLN2	5 (.32%)	11	NKTR	11 (.69%)	3
CLU	29 (1.83%)	8	OAS1	3 (.19%)	12
COL6A2	24 (1.51%)	21	PF4	6 (.38%)	4
COPA	12 (.76%)	1	PF4V1	5 (.32%)	4
CPT1B	18 (1.13%)	22	PI3	1 (.06%)	20
CSPG6	15 (.95%)	10	POU2AF1	12 (.76%)	11
CTTN	46 (2.9%)	11	PPAT	12 (.76%)	4
DCTN1	15 (.95%)	2	PPBP	1 (.06%)	4
DDX17	13 (.82%)	22	PRKAR1A	9 (.57%)	17
DEFA1	4 (.25%)	8	PTGDR	14 (.88%)	14
DUSP2	9 (.57%)	2	PTGS2	12 (.76%)	1
DVL3	17 (1.07%)	3	PTPN12	24 (1.51%)	7
EGR1	14 (.88%)	5	RAB1A	8 (.5%)	2
EGR3	19 (1.2%)	8	RHCE	4 (.25%)	1
EP400	74 (4.67%)	12	RPH3A	20 (1.26%)	12
EPB42	10 (.63%)	15	RPS26	5 (.32%)	12
FKBP5	23 (1.45%)	6	RRM2	14 (.88%)	2
FOSB	19 (1.2%)	19	SDPR	9 (.57%)	2
G0S2	12 (.76%)	1	SFPQ	16 (1.01%)	1
G1P3	12 (.76%)	1	SFRS6	15 (.95%)	20
GP1BB	11 (.69%)	22	SLC12A7	147 (9.27%)	5
H2AFV	13 (.82%)	7	SMARCC1	12 (.76%)	3

Gene Name	n_{CpG}	**Chr**	**Gene Name**	n_{CpG}	**Chr**
HCA112	1 (.06%)	7	SNAP23	7 (.44%)	15
HGD	6 (.38%)	3	SPARC	11 (.69%)	5
HIST1H2AC	12 (.76%)	6	STAT1	12 (.76%)	2
HIST1H2BG	6 (.38%)	6	STX16	8 (.5%)	20
HIST1H3H	6 (.38%)	6	TCN1	2 (.13%)	11
HLA-DQB1	6 (.38%)	6	TNFAIP3	22 (1.39%)	6
HNRPL	20 (1.26%)	19	TNFRSF17	3 (.19%)	16
IER2	17 (1.07%)	19	TNFSF10	4 (.25%)	3
IFI27	7 (.44%)	14	TOP2B	11 (.69%)	3
IGF2R	54 (3.4%)	6	TUBB1	12 (.76%)	20
IGFBP3	32 (2.02%)	7	TYMS	12 (.76%)	18
IGLL1	6 (.38%)	22	VNN1	2 (.13%)	6
IL10RA	7 (.44%)	11	XCL2	2 (.13%)	1
IL1B	3 (.19%)	2	ZNFN1A1	26 (1.64%)	7
IL8RB	22 (1.39%)	2	Cig5	4 (.25%)	2
KCNJ15	10 (.63%)	21	**Total**	**1,586**	

Appendix 5: ***Regression Weights (b) for the 130 CpGs with Effects of Baseline Loneliness on Methylation Intercept or Slope at z ≥ |1.96| (Study 2)***

Table A12

Regression Weights (b) for the 130 CpGs with Effects of Baseline Loneliness on Methylation Intercept or Slope at z ≥ |1.96|

CpG	Gene Name	b_{I70}	z_{I70}	b_S	z_S
cg05307957	*ARID1A*	-0.04	-2.28	-0.01	-0.93
cg04699519	*ATXN1*	-0.05	-2.97	0	-0.01
cg04975376	*ATXN1*	-0.02	-2.55	0	0.34
cg07109965	*ATXN1*	0.03	2.2	0.01	0.88
cg10581503	*ATXN1*	0.02	2.21	0	-0.69
cg19185641	*ATXN1*	0.01	2.61	0	-1.07
cg07475232	*BHLHB2*	-0.01	-1.13	0.01	2.02
cg00733150	*C22orf8*	-0.02	-1.09	0.03	2.78
cg03953157	*C22orf8*	0	1.32	0	2.29
cg10788213	*C22orf8*	0	0.48	-0.01	-2.15
cg14466896	*C22orf8*	0.01	2.02	0	0.35
cg07921777	*CBFB*	-0.01	-1.61	0.01	2.03
cg10233691	*CBFB*	0	-1.55	0.01	2.71
cg01948190	*CD164*	-0.02	-1.52	0.02	2
cg26009195	*CDC25B*	0.02	2.27	-0.01	-2.14
cg02953912	*CDKN1C*	0.02	2.01	-0.01	-1.74
cg22865058	*CDKN1C*	0	0.48	-0.02	-2.85
cg26155475	*CLIC4*	0	1.79	0	-2.39
cg26838747	*CLIC4*	-0.02	-2.83	-0.01	-0.82
cg00267296	*CLN2*	-0.01	-2.05	0	-0.42
cg00929658	*COL6A2*	0	0	0.03	2.1
cg10435849	*COL6A2*	0.01	1.3	-0.03	-2.86
cg01446576	*COPA*	0	-0.56	-0.01	-2.52
cg08015496	*COPA*	0.03	2.3	0.02	1.59
cg00619097	*CPT1B*	-0.01	-1.03	0.03	2.65
cg00872628	*CSPG6*	-0.01	-2.19	0	0.85
cg06470552	*CSPG6*	-0.01	-1.98	0	0.41
cg04197449	*CTTN*	0.02	2.28	0	-0.55
cg08914150	*CTTN*	0	-0.26	-0.01	-2.15
cg13096351	*CTTN*	0.02	2.11	0	-0.33
cg25587405	*CTTN*	0.02	2.38	-0.01	-1.31
cg16774942	*DDX17*	-0.01	-2.2	0	0.99
cg22147449	*DDX17*	-0.01	-3.49	0.01	2.65
cg14673932	*DVL3*	0	-0.66	0.01	2.07
cg09395034	*EGR1*	-0.01	-2.02	0	0.35
cg13009654	*EGR1*	0	0.23	-0.01	-2.67
cg23951277	*EGR1*	0	0.48	0.01	2.19

CpG	Gene Name	b_{I70}	z_{I70}	b_S	z_S
cg07082452	*EGR3*	0	-0.18	0.01	2.24
cg16854466	*EP400*	0.02	2.61	-0.01	-1.08
cg20474144	*EP400*	0	-0.42	-0.01	-1.97
cg24789136	*EP400*	0.02	2.35	0	0.23
cg23803468	*EPB42*	-0.02	-2.59	0	0.11
cg19226017	*FKBP5*	-0.02	-2.8	0.01	0.77
cg05023151	*FOSB*	0.03	2.56	-0.01	-1.08
cg12265810	*FOSB*	-0.01	-0.36	0.02	2.18
cg11414921	*GP1BB*	-0.01	-0.39	-0.03	-2.08
cg08703818	*H2AFV*	0.03	2.21	0.02	1.5
cg08018179	*HGD*	0.01	1.98	-0.01	-1.09
cg16218610	*HIST1H2AC*	-0.02	-2.1	-0.01	-1.1
cg19213665	*HIST1H2AC*	-0.02	-2.53	0.01	1.51
cg25307277	*HIST1H2AC*	0.01	2.45	0	-0.38
cg05070742	*HIST1H3H*	-0.02	-1.97	0	0.02
cg00747152	*HNRPL*	-0.04	-2.54	0	-0.2
cg05464534	*HNRPL*	-0.02	-2.07	0	-0.09
cg09352155	*HNRPL*	0	-0.05	0.01	2.1
cg13353472	*HNRPL*	0	-1.53	0.01	2.81
cg03634777	*IGF2R*	-0.02	-2.57	0.01	1.73
cg16111231	*IGF2R*	0	-0.81	0.01	2.64
cg21178851	*IGF2R*	0	-0.24	-0.01	-2.08
cg10677697	*IGFBP3*	0.01	1.96	-0.01	-1.23
cg22403266	*IGFBP3*	0.02	1.1	-0.03	-2.08
cg05468843	*IL10RA*	-0.03	-1.37	0.04	2.14
cg26661481	*IL10RA*	-0.01	-1.52	0.03	3.13
cg07016356	*IL8RB*	0.01	1.3	-0.01	-2.14
cg13739417	*IL8RB*	0.01	1.73	-0.01	-2.27
cg09214993	*KIAA0101*	-0.02	-2.39	0	0.27
cg26889367	*KIAA0101*	0	-0.07	-0.01	-2.58
cg13472900	*LGALS8*	0.02	2.24	0	0.34
cg00452400	*MAN2C1*	-0.02	-1.56	0.02	2.2
cg00461978	*MAN2C1*	-0.02	-2.35	0	0.68
cg05525867	*MAN2C1*	-0.03	-2.39	0.02	1.87
cg20639218	*MAN2C1*	-0.02	-2.2	0	0.15
cg00090767	*MAX*	-0.02	-1.78	0.02	1.97
cg04318212	*MAX*	-0.01	-2.05	0.01	0.93
cg20040285	*MAX*	-0.01	-2.03	0	-0.02
cg07659624	*MSCP*	-0.01	-2.05	0	-0.51
cg17797797	*MSCP*	0	0.2	0.03	2.51
cg13516655	*MYBL1*	-0.03	-2.32	0	0.27
cg05176211	*MYST3*	-0.03	-2.25	0	-0.15

CpG	Gene Name	b_{I70}	z_{I70}	b_S	z_S
cg04722914	*NEDD5*	-0.01	-2.94	0	0.67
cg16787284	*NEDD5*	0.01	3.03	0	-1.73
cg23888423	*NEDD5*	-0.02	-2.18	0	-0.45
cg04843801	*NKTR*	-0.01	-2.08	0	1.24
cg17250947	*NKTR*	-0.01	-1.87	0.01	2.13
cg02530824	*PF4*	0.07	2.27	0	-0.24
cg05509609	*PF4*	0.06	2.36	-0.02	-0.92
cg06834998	*PF4*	0.07	2.43	-0.02	-0.83
cg16072462	*PF4*	0.05	2.16	-0.01	-0.71
cg21043213	*PF4*	0.05	2.22	0	-0.28
cg20357806	*PPBP*	0.03	3.15	-0.01	-1.06
cg00403457	*PTPN12*	-0.03	-2.89	0	0.52
cg03887471	*PTPN12*	0	-0.1	-0.02	-2.06
cg12262427	*PTPN12*	0	-2.15	0	0.69
cg00851732	*RPH3A*	0.03	1.99	0.01	0.5
cg05793409	*SFPQ*	-0.02	-2.15	0.01	1.88
cg00420510	*SLC12A7*	0.02	2.34	-0.01	-1.59
cg00551954	*SLC12A7*	0.02	2.05	0	0.24
cg00600029	*SLC12A7*	0.02	2.5	0	0.13
cg02295574	*SLC12A7*	0.04	2.17	-0.01	-0.65
cg02382320	*SLC12A7*	0.01	1.61	-0.02	-2.27
cg04114636	*SLC12A7*	0.01	1.98	0	-0.18
cg04213775	*SLC12A7*	0.02	2.33	-0.01	-0.76
cg06637017	*SLC12A7*	0	0.09	0.02	2.21
cg08351607	*SLC12A7*	0	0.04	0.02	2.1
cg10601043	*SLC12A7*	0.02	2.15	-0.01	-1.45
cg11235297	*SLC12A7*	-0.02	-2.49	0	0.35
cg11962947	*SLC12A7*	-0.01	-1.96	0.01	2.04
cg13301368	*SLC12A7*	0.02	2.17	0	-0.51
cg15597069	*SLC12A7*	0.02	2.02	0.01	0.83
cg17568547	*SLC12A7*	0.02	2.02	0	0.4
cg18997983	*SLC12A7*	0	0.11	-0.02	-2.96
cg19086001	*SLC12A7*	0.03	2.27	0.01	0.76
cg23503101	*SLC12A7*	0.02	2.04	0	0.24
cg24886748	*SLC12A7*	0.01	2.13	-0.01	-1.83
cg26439015	*SLC12A7*	0.01	3.15	0	-1.49
cg12894336	*SMARCC1*	-0.01	-1.98	0	1.19
cg20685352	*SMARCC1*	-0.01	-2.18	0	0.28
cg01085225	*STAT1*	-0.01	-3.06	0.01	2.42
cg13186228	*STX16*	0	-0.58	0	2.08
cg08667148	*TNFAIP3*	0.02	2.02	0.01	0.69
cg25971086	*TNFAIP3*	-0.02	-2.29	0.01	0.85
cg18485955	*TNFRSF17*	0.03	2.01	0.01	0.73

CpG	Gene Name	b_{I70}	z_{I70}	b_S	z_S
cg09793001	*TOP2B*	-0.01	-2.1	0	0.39
cg19472303	*TOP2B*	-0.01	-1.96	0.01	2.35
cg15084758	*TYMS*	-0.01	-2.18	0	1.51
cg22618219	*VNN1*	0.02	2.52	0	-0.24
cg07103517	*ZNFN1A1*	-0.02	-1.99	0.01	0.92
cg16697214	*ZNFN1A1*	0.01	2.03	-0.01	-0.97
cg10844760	*cig5*	-0.02	-2	0	-0.14
cg18201077	*cig5*	-0.03	-2.1	0.01	0.87