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Exploring cortical proteins underlying the relation of neuroticism to cognitive resilience

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Some individuals maintain cognitive health despite neuropathology. Targets impacting “cognitive resilience” may provide interventions for preventing dementia without decreasing neuropathology. Neuroticism represents the tendency to experience negative emotions, and is related to worse cognitive resilience. Exploring proteins associated with cognitive resilience risk factors, such as neuroticism, could yield new protein targets. We identified (i) proteins associated with both neuroticism and cognitive resilience, and (ii) proteins statistically mediating relations of neuroticism to cognitive resilience. We found two proteins, 40S ribosomal proteinS3 (RPS3) and branched chain keto acid dehydrogenase E1, subunit beta (BCKDHB), ranked in the top 1% of smallest p-values in parallel linear regression models of neuroticism to protein levels, and protein levels to cognitive decline resilience. In mediation models, RPS3 and BCKDHB accounted for 25% (p = 0.005) of the relation of neuroticism to cognitive resilience. Our sample size is modest, thus results may be due to chance (p-values did not meet Bonferroni significance) and will require further confirmation; however, investigating biologic mediators of associations of risk factors to cognitive resilience may help discover targets to promote cognitive resilience and reduce dementia.

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I n t r o d u c t i o n

Approximately 50 million adults have dementia worldwide [1]. To date, research on interventions to prevent or treat Alzheimer’s dementia has focused on modifying Alzheimer’s disease pathology. While such efforts continue, identifying alternate approaches for intervention is critical, as multiple agents aimed at reducing amyloid pathology have not yet been successful [2].

There is growing interest in the concept of cognitive reserve [3], also termed cognitive resilience [4]. This concept of cognitive resilience is rooted in observations of consistent discrepancies in some individuals between levels of neuropathology and levels of cognitive functioning [3,5]; cognitive resilience is then defined as the ability to cope with pathologic changes and maintain higher cognitive

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function with aging. Cognitive resilience has been extended in recent years to encompass the full spectrum of both better as well as worse than expected cognitive function, given levels of neuropathology [4]. Thus, carefully evaluating risk factors and biologic mechanisms of enhanced or degraded cognitive resilience can provide a novel path towards reducing or delaying the onset of cognitive impairment and dementia, without altering neuropathology [6].

Several behavioral and experiential risk factors have been established which are related to cognitive resilience, including education, occupation, cognitive activity and physical activity [7, 8]. Although these are largely modifiable factors, all can be challenging to effectively change on either an individual or a population level. Substantially less research has investigated biologic drivers of cognitive resilience, which might lead to pharmacologic targets for intervention. In a recent large GWAS [9], the genetic predictors of resilience generally did not overlap with those of Alzheimer’s dementia, confirming that molecular signatures of resilience are distinct from those of dementia, and that further investigating the biology of resilience could be critical to discovering new approaches for dementia intervention or therapy.

In our own previous research, we have investigated both behavioral and biological factors related to cognitive resilience (which we operationalize using residual approaches [4], or the cognitive trajectories that remain after regressing out effects of common brain pathologies) [10–12]. In particular, we have consistently identified neuroticism as a risk factor for cognitive resilience [10]. Neuroticism is a classic “Big Five” personality trait, and is the tendency to experience negative emotions, including anxiety and distress; we (and others) report strong and highly significant relations of neuroticism to dementia and cognitive decline [13–16]. Yet, we have also clearly found [16–18] that neuroticism was not related to Alzheimer Disease neuropathology or other common brain pathologies; indeed, neuroticism was significantly associated with higher risk of dementia after controlling for multiple neuropathologic indices, indicating that neuroticism degrades cognitive resilience. In parallel, in biologic research, we previously conducted a proteome-wide association study (PWAS) of over 8,000 cortical proteins in relation to cognitive resilience [12]. We identified 8 proteins which appeared to confer resilience. Given the critical importance of expanding knowledge regarding mechanisms underlying cognitive resilience, we extend our research here by presenting broader approaches to biologic discovery than either single risk factor research or purely agnostic ‘omics association studies.

Specifically, we explore here proteins associated both with risk factors for cognitive resilience, as well as with cognitive resilience itself; that is, we investigate pathways leading from neuroticism to cognitive resilience. We present a three-step approach, ascertaining cortical proteins that: (i) strongly relate to neuroticism; (ii) strongly relate to cognitive resilience; and (iii) formally mediate the relation of neuroticism to cognitive resilience. Because we use multiple integrated and converging steps to nominate proteins of interest, we simultaneously impose less conserva-

Aims of the study

Given the potential consequences for dementia prevention of identifying underlying mechanisms of cognitive resilience, our overall aim here is to enlarge the platform of research investigating biologic drivers of resilience. By expanding this platform, we support and encourage future research in other cohorts and settings. In particular, our research here aims to present strategies for biologic discovery when formal replication may be problematic or impractical; this includes the field of cognitive resilience, where studies and sample sizes can be restricted by the breadth, complexity and expense of data required to define resilience in a biologically meaningful and tractable way (i.e., neuropsychologic data plus pathologic data from imaging or biospecimens). Nonetheless, we also note that because our sample of participants is modest, any results may be chance findings that will require replication and confirmation in future research.

Materials and methods

Study populations

The Religious Orders Study was initiated in 1994 [20], and includes older priests, nuns and brothers from across the US, free of known dementia at the time of enrollment. Participants agreed to annual neurological exams, neuropsychological testing, and blood draw, and signed an informed consent and Anatomic Gift Act to donate their brains at death. Over 1,490 participants completed a baseline evaluation. The follow-up rate and autopsies exceed 90%. The Rush Memory and Aging Project was established in 1997 [20], and includes older men and women from across the Chicago metropolitan area, without known dementia at enrollment; over 2,190 participants completed a baseline evaluation to date. The follow-up rate exceeds 90% and the autopsy rate exceeds 80%. Both cohorts have virtually identical design and data collection. Both studies were approved by an Institutional Review Board of Rush University Medical Center. For the work described here, we leveraged participants of ROSMAP who had completed a neuroticism scale at study recruitment and had proteomics available from frozen post-mortem tissue samples obtained from the dorsolateral prefrontal cortex (DLPFC). These and other ROSMAP data can be requested at https://www.radc.rush.edu.
In the brain specimens, we used a multiplex mass spectrometry based proteomics approach with tandem mass tag (TMTs) to analyze frozen tissue samples in the DLPFC. Briefly, 100 mg frozen sections were thawed on ice, with the gray matter dissected from the white matter, as previously described in detail [12]. Details of the mass spectrometry–based proteomics, database searches, and quality control have been previously described [12]. To summarize, the samples were homogenized, and the protein concentration was determined. After protein digestion, isobaric TMT peptide labeling and high pH fractionation were performed. Fractions were then analyzed by liquid chromatography-mass spectrometry. The resulting mass spectrometry spectra were searched against the UniProt human protein database, with individual protein abundance checked against the global internal standard. An additional data process included regressing out technical confounders. A total of 8356 proteins in 391 persons passed the final quality control (9 participants were excluded who did not pass quality control). These proteomics data are available in the AD Knowledge Portal (https://adknowledgeportal.synapse.org).

Assessment of neuroticism

We assessed neuroticism at or near baseline. Neuroticism is a classic “Big Five” personality trait, and is the tendency to experience negative emotions, including anxiety, anger, and distress. We measured neuroticism using 12 items from the NEO Five-Factor Inventory [21]. Participants rated agreement with each item (e.g., “I often feel inferior to others”) on a 5-point Likert scale, ranging from strongly disagree to strongly agree. Negatively worded items were flipped so that higher scores on all items indicated greater neuroticism. Individual items were scored from 0 to 4, and then summed to yield a composite score ranging from 0 to 48. Cronbach’s coefficient alpha was 0.8 for the neuroticism measure, indicating adequate internal consistency.

Assessment of cognitive function

Participants underwent an annual, uniform structured clinical evaluation, which included detailed cognitive testing [20]. The annual cognitive evaluation includes 19 cognitive performance tests that are in common between the two cohorts, Religious Orders Study and Memory and Aging Project. The Mini–Mental State Examination is used for descriptive purposes, and the Complex Ideational Material from the Boston Diagnostic Aphasia Examination is used only in diagnostic classification. The remaining 17 tests are combined into a composite measure of global cognition. Briefly, individual test scores were converted to z scores using the baseline mean (SD) value and then averaged to obtain the composite score. The global cognitive score was utilized in estimating cognitive resilience to neuropathology (see below).

Assessment of neuropathology

In quantifying resilience to neuropathology, we considered 9 neuropathologic indices, previously described in detail [12]. Briefly, the neuropathologic evaluations systematically assessed common neurodegenerative and cerebrovascular conditions, including: Alzheimer’s disease pathology, Lewy bodies, transactive response DNA binding protein (TDP)-43, hippocampal sclerosis, chronic macroscopic and microinfarcts, cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis. The neuropathologic evaluations were conducted by examiners blinded to all clinical data.

Statistical analysis

We used a multi-stage approach for discovery of candidate proteins which may mediate the relation of neuroticism to cognitive decline resilience (Fig. 1); in part, this multi-stage approach may promote identification of results outside the context of “traditional” correction for multiple comparisons. In our first step, we performed a proteome-wide association analysis to examine the relations of neuroticism with individual proteins. We conducted 8356 parallel linear regression models, with neuroticism as the independent variable and protein abundance as the dependent variable, controlling for age at death, sex (male/female), educational attainment (years), and 9 neuropathologic indices; we controlled for the neuropathologic indices because our interest was focused on proteins which are independent of pathology. Given our small sample size, and our multi-step approach, rather than setting statistical significance conservatively at an α level of $6 \times 10^{-6}$ for 8,356 tests, we ranked p-values for the 8,356 proteins from smallest to largest. We then considered proteins of interest among the top 1% of all proteins examined (or the 84 smallest p-values).

Next, as part of this first step, we also leveraged our previously published proteome-wide association study of cognitive decline resilience [11]; in that research, we defined cognitive resilience as change in the global cognitive composite score (described above) over follow-up before death, above and beyond the effect of 9 neuropathologic indices. Briefly, to identify proteins related to cognitive resilience, we had implemented 8356 parallel linear mixed effects models, with each protein as the independent variable and annual global cognitive scores as the longitudinal dependent variable, controlling for age at death, sex, education (years), the 9 neuropathologic indices described above, and the interaction of each covariate with time. For the purposes of the work here, we ranked the proteins according to their p-values, from smallest to largest.

In the second step (Fig. 1), we then identified overlapping proteins from the neuroticism PWAS and the previous cognitive resilience PWAS. To be somewhat flexible in our definition of “overlap”, we considered any of our top 1% of proteins in the neuroticism PWAS that also ranked in the top 1% of proteins in relation to cognitive resilience.

While we appreciate that these criteria are arbitrary, we also included a third step (Fig. 1), in which we formally tested whether any of these overlapping proteins specifi-
cally mediated the relation of neuroticism to cognitive resilience. Mediation analysis simultaneously requires strong associations of the risk factor to the mediator, the mediator to the outcome, and the risk factor to the outcome [22]; thus, identification of significant mediation may be considered compelling support of a nominated protein/mediator. Specifically, for mediation analyses, the total “effect” of neuroticism on cognitive resilience was decomposed into direct and indirect effects via the proteins of interest, following a causal mediation approach. This allowed us to estimate the proportion of the effect of neuroticism mediated by the protein(s), including p-values for both the direct and indirect/mediated effects.

To more easily model relations of neuroticism to cognitive resilience, we extracted the person-specific cognitive resilience estimates, defined as slopes of cognitive decline not attributable to demographics or common neuropathologies, and then used the slope as the dependent variable in a linear regression model, controlled for age, sex, and educational attainment.

### Results

Among the 355 participants with data on both neuroticism and on DLPFC proteomics (Table 1), mean age at baseline was 79.5 years (SD 6.7), and mean age at death was 89.3 years (SD 6.4). Approximately one-third of participants were male, over 95% were white, and on average, participants completed 16 years (SD 3.5) of education. At the baseline neuropsychologic evaluation, mean score on the Mini-Mental State Exam (MMSE) was 28.5, and at the evaluation proximate to death, mean MMSE score had decreased to 22.9. The prevalence of mild cognitive impairment was extremely low at baseline (1 participant had MCI), although increased to 27% prevalence at the end of follow-up. Mean score on the neuroticism items from the NEO-5 at baseline was approximately 16 (SD 6.5).

### Neuroticism and the human brain proteome

In the 8,356 parallel models of neuroticism in relation to protein abundance (controlled for demographic factors and neuropathologic indices), no proteins achieved formal significance after Bonferroni correction (Fig. 2). When considering the top 1% of proteins examined, in terms of p-values, these top 50 smallest p-values ranged from $7 \times 10^{-4}$ to $8 \times 10^{-3}$. We then identified three of these 50 proteins that also ranked in the top 1% of smallest p-values within the previously published CR PWAS (Fig. 2): RPS3, or 40S ribosomal protein S3; BCKDHB, or branched chain keto acid dehydrogenase E1, subunit beta; and ATXN1L, or ataxin 1-like protein.

Specifically, in quantifying relations of these three proteins with (i) neuroticism and (ii) cognitive resilience (Table 2), we found that for two of the proteins, greater neuroticism was related to lower protein abundance. For RPS3, the mean difference in protein abundance with each 1 SD increment in neuroticism = $0.010$ (p = 0.008) and for BCKDHB, the mean difference in protein abundance with each 1 SD increment in neuroticism = $0.019$ (p = 0.001). As expected, higher abundance of these two proteins was correspondingly related to better resilience (RPS3: mean difference in resilience with each 1 unit increment in pro-
tein abundance = 0.217, p = 0.0003; and BCKDHB: mean difference = 0.142, p = 0.0003). In contrast, for the third protein, greater neuroticism was related to higher protein abundance (ATXN1L: mean difference = 0.029, p = 0.004), and higher protein abundance was related to worse resili-

ece (mean difference = −0.175, p = 0.0003).

For our third step, we examined the extent to which these proteins mediated the relation of neuroticism to cog-
nitive resilience (Fig. 3). We estimated the “total effects” of neuroticism on cognitive resilience, and further divided the total effects into the “direct effects” of neuroticism and “indirect effects” of the protein mediators, using pathway analysis. We excluded ATXN1L since fewer participants had adequate data for both neuroticism and cognitive resi-

Table 2 Cortical Proteins Related to Both Neuroticism and to Cognitive Resilience.¹

<table>
<thead>
<tr>
<th>Protein</th>
<th>Relation of neuroticism to protein²</th>
<th>Relation of protein to cognitive resilience³</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean Difference (β) p-value</td>
<td>Mean Difference (β) p-value</td>
</tr>
<tr>
<td>RPS3</td>
<td>−0.010  0.008</td>
<td>0.217  0.0003</td>
</tr>
<tr>
<td>BCKDHB</td>
<td>−0.019  0.001</td>
<td>0.142  0.0003</td>
</tr>
<tr>
<td>ATXN1L</td>
<td>0.029  0.004</td>
<td>−0.175  0.0003</td>
</tr>
</tbody>
</table>

¹ These proteins were in the group with the top 1% of smallest p-values for relations of each of the 8,356 proteins to neuroticism, as well as the top 1% smallest p-values for relations of the proteins to cognitive resilience.

² The beta and p-value were derived from a linear regression model with protein abundance as the dependent variable, and neuroticism as the independent variable, with covariates including age, sex, education, and nine neuropathologic indices. Sample size was n = 355, except for ATXN1L, where valid protein data were available in n = 192 participants.

³ The beta and p-value were derived from a linear mixed effects regression model with annual cognitive scores prior to death as the longitudinal dependent variable, and protein abundance as the independent variable, with covariates including age, sex, education, nine neuropathologic indices, and interactions of time with each covariate. The beta and p-value presented are for the interaction of the protein by time. These results were previously published (reference [12]).
indirect effects of the protein mediators (p = 0.005). Although we focused on the mediating effects of the two proteins together, so that we could more validly estimate the overall direct and indirect effects, we also found that each protein alone accounted for approximately half of the indirect effects. Specifically, when modeled separately, RPS3 individually mediated 16% of the relation of neuroticism to cognitive resilience (p = 0.03) and BCKDHB mediated 17% (p = 0.02); the limited overlap of the extent of mediation by each protein suggests they are acting on different pathways to resilience.

Discussion

We utilized a three-step approach, requiring a convergence of multiple lines of evidence, and identified two cortical proteins which strongly mediated the association of greater neuroticism with degraded cognitive resilience. These proteins could eventually provide targets for research to enhance resilience in those with neuroticism, or potentially to enhance resilience more broadly in older persons. However, findings should be confirmed in future research. Overall, our multi-step approach provides alternate pathways to discovery for biologic factors potentially involved in cognitive resilience, and may be especially advantageous in contexts, such as the cognitive resilience field, where the sample size does not accommodate “traditional”, full, statistical correction for multiple comparisons. A goal of our research here is to expand the platform of biologic discovery in CR, thereby motivating further research. Indeed, an arsenal of approaches will certainly be required to enable the discovery of promising proteins/biologic factors which can be tested to reduce dementia in the absence of agents capable of modifying neuropathology.

The two proteins nominated here, RPS3 or 40S ribosomal protein S3, and BCKDHB or branched chain keto acid dehydrogenase E1 subunit beta, are of interest. As a ribosomal protein, RPS3 is highly conserved and forms part of the 40S subunit domain, where mRNA translation is initiated. Beyond its central role in translation, the RPS3 protein may be essential in DNA repair. Specifically in cultured neurons, RPS3 can increase DNA repair and promote neuron survival [26]. Further, in mitochondria, RPS3 protein decreases cellular concentrations of reactive oxygen species, and reduces mitochondrial DNA damage [24]. Mitochondrial damage has been implicated in the etiology of Alzheimer’s dementia [25] and has also been linked with mood and anxiety conditions [27]. Thus, besides our preliminary findings here, there is scientific support for a possible role of RPS3 in cognitive health, and in neuroticism.

The BCKDHB enzyme complex is involved in the catabolism of branched-chain amino acids and is thus central to energy production; it is particularly active in mitochondria. Evidence also suggests that BCKDHB may regulate glutamate availability in the brain [28]. Glutamate is the most abundant excitatory neurotransmitter in the central nervous system [29], and glutamate signaling has been implicated in neurodegenerative diseases such as AD. In parallel, initial research indicates the glutamate system may be involved in stress response, and underlie anxiety disorders as well [30]. Further, in rodents, traumatic brain
injury decreases BCKDHB levels in brain tissue, and BCKDHB appears to be involved in cognitive impairment after traumatic brain injury [31,32]. Finally, mutations in the BCKDHB gene result in BCKD deficiency, which is associated with maple syrup urine disease, a rare disorder leading to neurologic dysfunction [33]. Overall, an array of mechanisms suggest that BCKDHB may merit further investigation to preserve cognition.

We further emphasize that these proteins were measured in the prefrontal cortex, which may be especially relevant in understanding pathways from neuroticism to cognitive health. The amygdala-frontal cortex circuitry is central to integration of emotion and cognition, and in studies using functional MRI, higher neuroticism has been specifically linked with less activation in the prefrontal cortex [35]. Thus, the anatomy of neuroticism suggests particular influences on prefrontal cortex.

Nonetheless, we appreciate significant limitations in our work. As noted earlier, our sample of participants with data on both neuroticism and cortical proteins was small. Thus, compared to standard Bonferroni correction, we applied somewhat arbitrary and less conservative thresholds for our protein discovery; our results for RPS3 and BCKDHB may be chance findings. Future research to replicate and confirm our results will be needed. For example, we have previously tested findings from proteome-wide research by using a quantitative targeted protein pipeline (ie, selective reaction monitoring) [23]. This pipeline enables large-scale, lower-cost measurement of specific proteins; thus, next steps might incorporate these technologies to measure RPS3 and BCKDHB in a larger sample of independent specimens in our cohorts. Other confirmatory approaches could test the proteins in animal models as well [34].

Further, since the proteins were measured cross-sectionally in post-mortem samples, we cannot establish directionality or the time sequence of possible pathways from neuroticism to cognitive resilience; it is also possible that some third factor leads to both higher neuroticism and higher protein levels, rather than that the proteins mediate relations of neuroticism to cognitive resilience. Important strengths of this research include the deep level of phenotyping in participants, with information on neuroticism, demographic factors, cognitive decline, 9 different neuropathology indices, and over 8,000 cortical proteins evaluated in post-mortem tissue. We identified two proteins which were strongly related to both neuroticism and to cognitive resilience, and which appeared to significantly mediate the relation of neuroticism to cognitive resilience. In doing so, we broadly demonstrated the potential value in further investigating biologic mediators of the associations of risk factors to cognitive resilience, as an approach for discovery of novel targets which may promote cognitive resilience and thus reduce dementia.

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**Role of funding source**

The funding sources had no role in in study design; the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

**Data availability**

The proteomics data are available in the AD Knowledge Portal [https://adknowledgeportal.synapse.org]. All other data can be requested at [https://www.radc.rush.edu].

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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