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Relations among *CRFR1* and *FKBP5* genotype, cortisol, and cognitive function in aging humans: A Project FRONTIER study

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ABSTRACT

Here we use the glucocorticoid cascade hypothesis framework to address the role of baseline cortisol on changes in cognitive function over a 3-year span in non-demented rural Americans. We also determine if genotype at 4 different single nucleotide polymorphisms (SNPs) relates to change in cognitive function. We predicted 1) over time, increases in baseline cortisol will be associated with decline in cognitive function, 2) individuals homozygous for 3 *CRFR1* SNP rare alleles (AA rs110402, TT rs7209436, and TT rs242924 vs. others) will show less cognitive decline and this will be particularly pronounced in those with lower baseline cortisol, and 3) *FKBP5* T carriers (TT or CT vs. CC homozygotes) will have decreased cognitive performance and this will be particularly pronounced in individuals with higher baseline cortisol. Collectively, our data do not robustly support the glucocorticoid cascade hypothesis. In several cases, higher baseline cortisol related to better cognitive performance over time, but within individuals, increased cortisol over time related to decreased performance on some cognitive domains over time. Contrary to our predictions, individuals with the rare *CRFR1* haplotype (AA, TT, TT) performed worse than individuals with the common haplotype across multiple domains of cognitive function. *FKBP5* genotype status had minimal impacts on cognitive outcomes. Genotype effects were largely not dependent on cortisol. The Project FRONTIER dataset is supported by Texas Tech University Health Sciences Center Garrison Institute on Aging.

1. Introduction

Aging is associated with cognitive decline [1,2], and in humans, both longitudinal and cross-sectional data show that multiple domains of cognitive performance are lower in aged vs. young adults [3–5]. However, cognitive decline is not wholly universal in healthy (non-demented) adults [6]. Longitudinal studies indicate substantial interindividual variation in rate and type of decline, suggesting inter-individual attributes, independent of age, impact cognitive outcomes [5,7]. The exact physiological mechanisms that contribute to, or exacerbate, cognitive changes in non-demented individuals are largely unknown, but data suggest that psychosocial and environmental factors, including stress, are important [3,8–12].

Perceived stress, and the hormones associated with the hypothalamic-pituitary-adrenal (HPA) axis (i.e., glucocorticoids; cortisol in humans), can negatively impact cognitive performance and memory, particularly aspects of declarative memory and attention [8–10,13–18]. The glucocorticoid cascade hypothesis captures these relationships and posits a feed-forward cycle where aging (i.e., neuronal and receptor loss) in the hippocampus leads to reduced negative feedback effectiveness resulting in dysregulation of HPA axis function. This occurs with concomitant age-associated increases in baseline and post-stressor glucocorticoids, further brain aging, and impaired cognition, especially in declarative memory as that construct is regulated by the hippocampus and medial temporal lobe [19–22]. Although this hypothesis was originally proposed from work with rats [19], data from several lines of work in humans support it. Baseline and post-stress glucocorticoid levels increase with age [23–26], and natural

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age-related increases in baseline cortisol are associated with hippocampal atrophy and/or decreased memory outcomes [27–30]. Cushing's syndrome, a hypercortisolemic disorder, is associated with decreased cognitive function across a range of domains [31]. Even small differences in baseline cortisol can matter, however, as higher morning cortisol in dementia-free, community dwelling adults (mean age: 48.5 yrs) was associated with smaller brain volume and lower cognitive performance on four difference tasks [32]. In another study, higher salivary cortisol, both baseline and mean over the testing period, was associated with poorer performance on 6 domains of cognitive function [33].

Data, however, do not always (fully) support the glucocorticoid cascade hypothesis [20,34]. Relationships among glucocorticoids, aging, and cognitive function are variable among individuals [29,35]. Namely, interindividual differences are noted in the magnitude of change in glucocorticoids over time and the relationship among glucocorticoids and measures of cognitive function. Several potential factors could explain this variation, including rates of aging, age at sampling, duration of time between samples, or type of cognitive tests used. Additionally, circulating levels of glucocorticoids do not tell the full story as several factors, such as genotype, can alter the functional impacts of hormones [36–39]. Thus, incorporating measures of genotype status may help explain differences in results obtained across studies and can lead to a better understanding of how stressors and cortisol impact changes in health over time [40].

Here, we examine the role of baseline cortisol and genotype on changes in cognitive function over time in a non-demented population of community dwelling rural Americans. We analyzed single nucleotide polymorphisms (SNPs) in 2 different genes: *CRFR1* and *FKPB5*, as these SNPs influence stress reactivity (*CRFR1*) and tissue-level actions of glucocorticoids (*FKBP5*), and both have been implicated in cognitive function [41–46]. Using a longitudinal dataset from the Project FRONTIER database, maintained by Garrison Institute on Aging at Texas Tech University Health Sciences Center, we address several questions. First, we test the glucocorticoid cascade hypothesis – do changes in baseline cortisol over time relate to cognitive decline over time? Second, we determine if 4 different SNPs impact the relationship between cortisol, aging, and cognitive function. Specifically, we predict that: 1) over time, increases in baseline cortisol will be associated with decline in multiple domains of cognitive function; 2) individuals homozygous for *CRFR1* SNP rare alleles (AA rs110402; TT rs7209436; TT rs242924) will perform better on cognitive tests at each time point and that genotype would interact with cortisol such that those with rare SNPs would have lower cortisol and better cognitive scores (2a), and that baseline cortisol would predict change in cognitive scores over time with the rare allele carriers having lower cortisol and therefore less decline (2b); 3) *FKBP5* SNP homozygotes (CC rs1360780) will perform better on cognitive tests at each time point and that genotype would interact with cortisol such that CC individuals would have lower cortisol and better cognitive scores (3a), and that baseline cortisol would predict change in cognitive scores over time with CC individuals having lower cortisol and therefore less decline (3b). This study will test the glucocorticoid cascade hypothesis and will help elucidate which aspects of interindividual variation in HPA axis function and regulation are related to cognitive performance during aging in community dwelling, non-demented humans.

2. Methods

2.1. Participants

Data were obtained from the Texas Tech University Health Sciences Project FRONTIER (Facing Rural Obstacles to healthcare Now through Intervention, Education, and Research) database. This program is a longitudinal epidemiological study examining the natural course of chronic disease and cognitive changes (https://www.ttuhsc.edu/centers-institutes/garrison-aging/project-frontier.aspx). Project FRONTIER began in 2006 as the Cochran County Aging Study and expanded to include residents from multiple rural Texas counties. It is currently funded by Texas Tech University Health Sciences Center Garrison Institute on Aging and is ongoing. Participants were community dwelling at time of data collection. Study coordinators arrange appointments at study participant's rural county hospital every 3 years for cognitive testing, interviews, medical exams, and clinical labs. Whole blood is collected by a trained phlebotomist at each visit. All data are collected by Project FRONTIER personnel with patient consent and TTUHSC Institutional Review Board approval was obtained by Project FRONTIER coordinators. Data are archived on a secure computer or in biobank freezers. Authors of this study were not involved in any planning or coordination of Project FRONTIER and did not directly interact with any of the participants.

For this analysis, we obtained the following de-identified items from the Project FRONTIER database: frozen serum, frozen whole blood, neurocognitive assessment data, marital status, last grade level completed in school, high school completion status (diploma or GED), highest educational certificate, current household income, work status (retired or not), sex, age, race (White, Black, or other), ethnicity (Hispanic or not Hispanic) and dementia status at time of visit (yes or no).

Demographic information for 193 participants who met criteria for our data request (baseline and 3-yr serum samples and cognitive testing; one blood sample) are as follows: 92.2% White, 50.5% Hispanic, and 72.5% female. Marital status of participants at Time 1 was: married (74.1%), divorced (9.8%), separated (3.1%), widowed (9.8%), and never married (3.1%). Self-reported highest grade completed in school averaged 10.1 (range: 0–19; median 12); half of participants received a high school diploma (50.3%), others passed high school equivalency test or got a GED (6.7%), and the remaining did not get a diploma or GED (43.0%). Post-high-school certification percentages were: Master's degree (2.1%), Bachelor's degree (7.8%), vocational or technical certificate (23.8%), partial college or none (65.8%), do not know (0.5%). Current household income at Time 1: 70 K or higher (8.8%), 60–70 K (5.2%), 50–60 K (6.2%), 40–50 K (7.3%), 30–40 K (6.7%), 20–30 K (20.2%), 10–20 K (22.3%), >10 K (22.3%), unknown (1%). The average age at initial interview and data collection (baseline; Time 1) was 58.3 years (range: 40–87); follow-up data were collected at an average of 2.8 years later (Time 2). Due to the span in age range, we included age as a covariate in all analyses. At Time 1, a total of 55 participants were 65 years or older and the remaining 138 were 64 years or younger (see Supplemental Table 1). The majority of participant were still working (62.7%), some were retired (34.7%), and data were not available for the remaining (2.6%). No participant in our sample was reported as having dementia. All procedures performed by the authors were approved by the Texas Tech University IRB.

2.2. DNA extraction and SNP genotyping

Whole blood (2 ml) obtained from Project FRONTIER was used for DNA extraction using a commercially available kit (FlexiGene kit # 51,206, Qiagen). Kit instructions were followed except that extracted DNA was suspended in molecular grade water instead of the storage buffer provided. Following extraction, concentration of DNA in each sample was determined by Nanodrop spectrophotometry (average: 222 ng/ul) and the 260/280 ratio was inspected for purity. Prior to genotyping assay, DNA was diluted with molecular grade water to a concentration of 10 ng/ul, aliquoted, and frozen for future use.

Commercially available TaqMan allelic discrimination SNP genotyping assays (Applied Biosystems, ThermoFisher Scientific) were used to assess genotype. Participant genotype for *FKBP5* (rs1360780 [C or T]; kit C8852038) and three *CRFR1* loci (rs110402 [A or G], kit C2544843; rs7209436 [C or T], kit C1570087; rs242924 [T or G], kit C2257689) were determined.

We predicted that individuals homozygous for the rare *CRFR1* alleles

(rs110402 [AA vs. AG or GG], rs7209436 [TT vs. CT or CC]; rs242924 [TT vs. GT or GG]) would differ from individuals with the more common alleles, and we originally intended to analyze SNPs separately. However, due to the markedly high correlations between each *CRFR1* allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95), a haplotype which combined all three *CRFR1* SNP alleles was constructed. The haplotype formed a binary variable where participants who simultaneously possessed AA, TT, and TT alleles across the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes respectively, were scored as '0' and labeled 'protective.' The haplotype was marked as 'risky' and scored as '1' if any of the *CRFR1* alleles were recorded with any other *CRFR1* allele combination not indicated in the 'protective' category; see Table 1 for genotype frequencies (see Supplemental Table 1 for genotype frequencies by age category).

2.3. Serum cortisol assay

Project FRONTIER personnel obtained serum samples from blood collected at each visit (Time 1 and Time 2). Of the 193 participant datasets requested, 191 had blood samples available. All blood samples were collected between 6:50 AM and 2:45 PM (only two samples were collected after 12 noon), and 92% of samples were collected between 7:00 and 9:30 AM. We determined serum cortisol levels via a commercially available radioimmunoassay kit (ImmuChem, 07–221,102; MP Biomedicals, Solon, OH) according to manufacturer's instructions. The standard curve ranged from 1 to 100 μg/dl. A total of 7 cortisol assays were conducted. To limit assay-induced variation, participants serum samples (i.e., Time 1 and Time 2) were analyzed in the same assay run. All samples were assayed in duplicate, and coefficient of variation (CV) values were all below 10%. Inter- and intra-assay CVs for control samples were 7.2% and 10.1%, respectively. Genotype for *CRFR1* SNPs and baseline cortisol at Time 2 data were previously published [47]; the research question and hypothesis in our previous study (relationship among cortisol, *CRFR1* genotype, *FAAH* genotype, and anxiety) is distinct and different from the questions and hypotheses being addressed here.

2.4. Project FRONTIER cognitive assessment data

Baseline (Time 1) and follow-up (Time 2) data from three different neuropsychological cognitive tests were obtained: the Controlled Oral Word Association test (FAS test), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), and the Trail Making Test (TMT). Descriptive data for variables can be found in Table 2 and a correlation matrix for variables at Time 1 and Time 2 can be found in Table 3. All neurocognitive data were obtained by trained Project FRONTIER staff. These neurocognitive tests assess forms of declarative and working memory (for classification of memory types, see [48,49], both of which are at least partially mediated by the hippocampus [22,50].

The FAS is a variant of the Controlled Oral Word Association test. It is a short exam which asks participants to name as many words as they can that start with the given letter in 1 min; participants repeat for each of the three letters (F, A, S) and the number of correct words given is summed [51,52]. FAS was developed to measure word fluency and verbal ability but is also commonly used to assess executive function (e.g., cognitive organization and maintenance of effort) and performance is sensitive to mild Alzheimer's disease [53].

The RBANS is a short, 20- to 30-minute test which measures multiple aspects of cognitive function. It consists of 12 sub-sections which are combined to create five index scores: immediate memory (list and story memory), visuospatial/constructional (line orientation and figure copy), language (semantic fluency and picture naming), attention (coding and digit span), and delayed memory (list recall, list recognition, story recall, and figure recall) [54]. Additionally, a composite index score including all 5 individuals indices can be used as an overall measure of cognitive function. This test is sensitive to cognitive decline (e.g., Alzheimer's vs. Huntington's dementia [54,55]), but see [56].

The Trail Making Test has participants sequentially connect circled numbers (Part A) or letters and numbers (Part B) on a sheet of paper. Scores from the TMT are reported as a total number of seconds to complete the task [57]; if an error is made, the participant is notified and guided back to the previous circle in real time. The task is generally used to assess visuo-conceptual and visuo-motor tracking [57,58], and is sensitive to mild Alzheimer's disease [53].

Based on previous studies of aged adults, a three-year time span is sufficient to determine changes in cognitive function. For example, perceived stress predicted cognitive decline over 2 years in women 67–96 years of age [59]; cognitive changes in 50–70 year-olds were noted in the placebo control group over a 3-year study [60]; and in those over 70 years of age, higher perceived stress was associated with decreased performance (and mild cognitive impairment) an average of 3.6 years later [61].

2.5. Statistical analysis

A summary of predictions and outcomes is presented in Table 4. For all analyses, cortisol concentration was adjusted for time of day of blood collection, to account for circadian rhythm, as well as age. The reported results present the age-adjusted analyses. Prediction 1 was unique insofar as it only considered changes in diurnal-adjusted cortisol levels without consideration of genotype variation. Latent change scores, selected for their protection from attenuation due to measurement error, were constructed in *Mplus* 7.11 [62] from each cognitive measure. For prediction 1, latent cortisol change was related to latent cognitive change scores for all participants. Each model was conducted with and without adjustment for baseline age and the results below were largely consistent across iterations. In the event of discrepant findings between models with and without age, models were conducted as just-identified (i.e., degrees of freedom = 0) for age. With respect to predictions 2a, 2b, 3a, and 3b, we assessed the relation between changes in blood serum cortisol and hypothesized genotype variations with declines in cognitive functioning across time. Predictions 2a and 3a both consisted of simple cross-sectional regressions at both Time 1 (baseline) and Time 2 (separated by three years), where the *CRFR1* alleles (2a) or *FKBP5* alleles (3a) and diurnal-adjusted cortisol were respectively regressed on a given cognitive measure after adjusting for age (main prediction); the interaction of cortisol and genotype was also included in the analysis to determine if cortisol differentially impacts cognitive performance for each genotype (interaction prediction). For predictions 2b (*CRFR1*) and 3b (*FKBP5*), both SNPs were grouped into 'protective' (coded as '0') and 'risk' (coded as '1') categories. Baseline diurnal-adjusted cortisol was then regressed onto each cognitive measure's latent change score after

Table 1
Genotyping results for participants included in the study. Genotypes are listed as 'protective' or 'risky' based on previous literature and study predictions.

Gene	rs number	n	'Protective' (n)	'Risky' (n)
FKBP5	1,360,780	188	CC (88)	TT or CT (100)
Haplotype		186	(61)	(125)
CRFR1_11	110,402	176	AA (64)	AG or GG (112)
CRFR1_24	242,924	181	TT (65)	GT or GG (116)
CRFR1_72	7,209,436	185	TT (63)	CT or CC (122)

Note: Haplotype is marked as 'protective' when each of the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes are concurrently recorded as AA, TT, and TT, respectively. The haplotype is marked as 'risky' if any of the CRFR1 alleles are recorded as follows: CRFR1_11 is recorded as AG or GG, CRFR1_24 is recorded as GT or GG, or if CRFR1_72 is scored as CT or CC. Haplotype was constructed due to markedly high correlations between each CRFR1 allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95). rs = reference SNP.

Table 2
Descriptive data for dependent variables at Time 1 (baseline, year 0) and Time 2 (year 3).

Variable	Time 1 / Time 2 [Mean (Standard Error)] Total Sample	FKBP5 - 'Protective'	FKBP5 - 'Risky'	Haplotype - 'Protective'	Haplotype - 'Risky'
Age (years)	58.27 (11.68) / 61.09 (11.66)	57.85 (12.00) / 60.60 (11.94)	58.44 (11.47) / 61.34 (11.51)	56.16 (10.18) / 59.02 (10.16)	59.52 (12.26) / 62.34 (12.25)
Serum cortisol (ug/dl)	19.12 (2.74) / 18.40 (2.35)	19.07 (2.874) / 18.59 (1.97)	19.13 (2.68) / 18.22 (2.68)	18.89 (2.62) / 18.10 (1.84)	19.27 (2.83) / 18.60 (2.59)
RBANS - T	86.22 (14.89) / 85.70 (17.40)	86.84 (15.66) / 85.97 (15.19)	85.70 (14.20) / 85.46 (19.17)	81.54 (16.06) / 79.83 (15.41)	88.51 (13.82) / 88.37 (17.47)
RBANS - M	94.01 (16.81) / 91.20 (18.37)	94.49 (17.17) / 91.72 (18.25)	93.56 (16.39) / 90.84 (18.68)	88.72 (17.55) / 85.59 (16.43)	96.50 (15.82) / 93.82 (18.73)
RBANS - VC	82.03 (16.45) / 80.39 (16.39)	80.70 (17.40) / 81.00 (15.30)	82.85 (15.58) / 80.07 (17.02)	79.97 (16.53) / 78.72 (17.06)	82.87 (16.46) / 81.07 (15.89)
RBANS - L	92.13 (11.57) / 89.27 (14.73)	92.09 (11.97) / 91.17 (13.34)	92.43 (11.23) / 87.99 (15.36)	88.98 (11.64) / 84.49 (13.68)	93.90 (11.09) / 91.25 (14.84)
RBANS - A	86.44 (21.10) / 87.34 (20.32)	89.80 (21.41) / 88.33 (19.25)	84.56 (20.38) / 86.90 (21.20)	78.75 (20.58) / 79.10 (18.89)	90.63 (20.57) / 91.76 (19.61)
RBANS - DM	93.99 (15.11) / 94.60 (14.28)	94.33 (15.26) / 94.99 (13.32)	93.26 (14.94) / 93.88 (15.19)	92.77 (13.87) / 91.98 (14.81)	94.32 (15.74) / 95.54 (14.07)
Trails Aˆ	51.97 (28.10) / 48.48 (24.43)	48.82 (19.70) / 48.65 (25.62)	52.88 (31.07) / 48.26 (23.75)	59.43 (35.44) / 51.45 (23.58)	48.94 (23.78) / 47.03 (24.96)
Trails Bˆ	125.36 (75.98) / 112.32 (68.96)	124.29 (72.09) / 111.56 (63.23)	124.71 (79.83) / 111.78 (74.34)	130.52 (76.96) / 116.12 (59.63)	123.01 (76.83) / 109.81 (72.17)
FAS - GT	28.49 (12.05) / 29.94 (12.26)	30.18 (12.08) / 30.94 (11.98)	27.20 (11.91) / 29.39 (12.46)	24.73 (12.11) / 26.02 (12.41)	30.31 (11.50) / 31.90 (11.66)

*Note: N*s ranged from 172 (Trail Making Test B) to 193 (multiple variables); FKBP5 - 'Protective' (i.e., CC allele) for FKBP5 genotype; FKBP5 – 'Risky' (i.e., either TT or CT allele) for FKBP5 genotype. Haplotype is marked as 'protective' when each of the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes are concurrently recorded as AA, TT, and TT, respectively. Cognitive assessments where greater scores reflect worse performance are indicated with a 'ˆ'; The haplotype is marked as 'risky' if any of the CRFR1 alleles are recorded as follows: CRFR1_11 is recorded as AG or GG, CRFR1_24 is recorded as GT or GG, or if CRFR1_72 is scored as CT or CC. Haplotype was constructed due to markedly high correlations between each CRFR1 allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95); RBANS – *T* = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

Table 3
Time 1 and 2 correlations between all cognitive measures, genotype groups, and cortisol.

Variable	Age	Cortisol	RBANS - T	RBANS - M	RBANS - VC	RBANS - L	RBANS - A	RBANS - DM	Trails Aˆ	Trails Bˆ	FAS - GT	*FKBP5*	*CRFR1* Haplotype
Age	1	0.72**	-0.04	-0.14	0.01	-0.02	-0.03	-0.16	0.30	0.27	-0.08	0.03	0.13
Cortisol	0.59**	0.60**	0.04	-0.02	0.04	0.06	0.05	-0.05	0.14	0.11	0.02	0.10	-0.08
RBANS – T	-0.01	0.02	0.75**	0.77**	0.76**	0.70**	0.82**	0.71**	-0.56**	-0.56**	0.56**	-0.01	0.23**
RBANS - M	-0.13	-0.14	0.71**	0.65**	0.44**	0.48**	0.53**	0.69**	-0.47**	-0.49**	0.46**	-0.02	0.21**
RBANS - VC	-0.05	0.00	0.67**	0.32**	0.65**	0.48**	0.61**	0.45**	-0.40**	-0.41**	0.37**	-0.03	0.07
RBANS - L	0.09	0.06	0.65**	0.40**	0.28**	0.66**	0.62**	0.47**	-0.51**	-0.42**	0.60**	-0.11	0.22**
RBANS - A	0.04	0.11	0.76**	0.36**	0.48**	0.52**	0.82**	0.50**	-0.59**	-0.58**	0.62**	-0.04	0.30**
RBANS - DM	-0.06	-0.01	0.73**	0.63**	0.38**	0.44**	0.41**	0.65**	-0.40**	-0.42**	0.33**	-0.04	0.12
Trails Aˆ	0.23	0.05	-0.46**	-0.32**	-0.24**	-0.45**	-0.50**	-0.30**	0.58**	0.73**	-0.47**	-0.01	-0.08
Trails Bˆ	0.30	0.05	-0.51**	-0.38**	-0.31**	-0.38**	-0.52**	-0.34**	0.63**	0.67**	-0.44**	0.00	-0.04
FAS - GT	-0.01	0.07	0.50**	0.34**	0.23**	0.53**	0.56**	0.28**	-0.41**	-0.38**	0.80**	-0.06	0.23**
FKBP5	0.03	0.04	-0.04	-0.03	0.07	0.01	-0.12	-0.04	0.08	0.00	-0.12	–	0.08
CRFR1 Haplotype	0.14	0.06	0.22**	0.22**	0.08	0.20**	0.26**	0.05	-0.17*	-0.05	0.22**	0.08	–

Note: Time 1 correlations located in gray region; Time 2 correlations located in white region; Center diagonal contains Time 1 and Time 2 correlations.
* indicates $p < 0.05$
** indicates $p < 0.01$; Cognitive assessments where greater scores reflect worse performance are indicated with a 'ˆ'; Haplotype coded 0 = 'protective,' 1 = 'risky.' CRFR1 Haplotype is marked as 'protective' when each of the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes are concurrently recorded as AA, TT, and TT, respectively. The haplotype is marked as 'risky' if any of the CRFR1 alleles are recorded as follows: CRFR1_11 is recorded as AG or GG, CRFR1_24 is recorded as GT or GG, or if CRFR1_72 is scored as CT or CC. Haplotype was constructed due to markedly high correlations between each CRFR1 allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95). FKBP5 coded 0/'Protective' = CC, 1/'Risky' = CT/TT; RBANS – *T* = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

adjusting for age. Regressions were conducted separately for both the 'protective' and 'risk' groups. These analyses allowed for the determination of baseline cortisol in relation to change in cognitive performance over time for each genotype (slope for 'protective' and 'risk' categories across each genotype; main prediction) and for the comparison of the relationship between baseline cortisol and change in cognitive function *between* genotypes (difference between slopes; interaction prediction).

3. Results

3.1. Prediction 1: baseline cortisol and cognitive performance

In line with the glucocorticoid cascade hypothesis, we predicted increases in baseline serum cortisol over time would be associated with declines in cognitive function over time. Out of the nine cognitive

Table 4
Summary of predictions and outcomes.

	Prediction 1	Prediction 2a		Prediction 2b	Prediction 3a		Prediction 3b
	GC cascade Increases in cortisol over time relate to decreased cognitive function over time	*CRFR1 Haplotype* Main: 'Protective' haplotype (vs. 'risky') carriers will perform better on cognitive tests at both time points Interaction: Haplotype will interact with cortisol (not supported; no interactions found)		*CRFR1 Haplotype* Main: For each haplotype, higher baseline cortisol will be related to decline in cognitive test performance over time Interaction: Haplotype will interact with cortisol (not supported; no interactions found)	*FKBP5 rs1360780* Main: 'Protective' FKBP5 (vs. 'risky') carriers will perform better on cognitive tests at both time points Interaction: *FKBP5* will interact with cortisol (not supported; no interactions found)		*FKBP5 rs1360780* Main: For each genotype, higher baseline cortisol will be related to decline in cognitive test performance over time Interaction: *FKBP5* will interact with cortisol (*1 interaction found*)
Cognitive Tests	**Outcome**	**Outcome Time 1 (main)**	**Outcome Time 2 (main)**	**Outcome (main)**	**Outcome Time 1 (main)**	**Outcome Time 2 (main)**	**Outcome (main; *interaction*)**
RBANS - T	**Supported**	Opposite ('risky' > 'protective')	Opposite ('risky' > 'protective')	Opposite (w/in 'risky', ↑ baseline cort, ↑ Δ performance)	no	no	Opposite (w/in each, ↑ baseline cort, ↑ Δ performance)
RBANS - M	**Supported**	Opposite ('risky' > 'protective')	Opposite ('risky' > 'protective')	Opposite (w/in 'risky', ↑ baseline cort, ↑ Δ performance)	no	no	Opposite (w/in 'risky', ↑ baseline cort, ↑ Δ performance)
RBANS - VC	no	no	no	no	no	no	no
RBANS - L	**Supported**	Opposite ('risky' > 'protective')	Opposite ('risky' > 'protective')	Opposite (w/in 'risky', ↑ baseline cort, ↑ Δ performance)	no	no	Opposite (w/in each, ↑ baseline cort, ↑Δ performance)
RBANS - A	no	Opposite ('risky' > 'protective')	Opposite ('risky' > 'protective')	no	no	no	no
RBANS - DM	no	no	no	no	no	no	no
Trails A	no	Opposite ('risky' > 'protective')	no	no	no	no	no
Trails B	no	no	no	no	no	no	Opposite (w/in 'protective' ↑ cort, ↓ Δ performance*; genotypes differed in directionality of relationship)*
FAS - GT	no	Opposite ('risky' > 'protective')	Opposite ('risky' > 'protective')	no	no	no	no

Note: Plain font formating signifies result of main prediction; result of interaction is shown in italics when present. Outcomes supported are bold face font, opposite findings are regular face font. Haplotype is marked as 'protective' when each of the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes are concurrently recorded as AA, TT, and TT, respectively. The haplotype is marked as 'risky' if any of the CRFR1 alleles are recorded as follows: CRFR1_11 is recorded as AG or GG, CRFR1_24 is recorded as GT or GG, or if CRFR1_72 is scored as CT or CC. Haplotype was constructed due to markedly high correlations between each CRFR1 allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95). FKBP5 coded 0/'Protective' = CC, 1/'Risky' = CT/TT; RBANS – *T* = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

assessments analyzed, RBANS Total ($P < 0.01$) alongside the RBANS indices for Immediate Memory ($P = 0.01$) and Language ($P < 0.01$) were the only three latent change cognitive measures found to be significantly related to latent changes in cortisol (Table 5). Additionally, even though only three measures were significantly correlated with cortisol change, six out of the nine analyses showed negative correlation estimates (see Table 5). Each of these findings suggest diminished performance over time was associated with greater increase in serum cortisol over that same period. Interestingly, based on inspection of descriptive statistics (Table 2), mean baseline cortisol concentrations did not increase over time in the overall sample, or in any of the genotype subgroups, which is not supportive of the glucocorticoid cascade hypothesis.

Table 5
Correlation of latent cortisol change score with latent cognitive change scores.

Measure	Estimate	S.E.	*p*-value
RBANS - T	-0.20	0.07	**<0.01**
RBANS - M	-0.18	0.07	**0.01**
RBANS - VC	0.01	0.04	0.89
RBANS - L	-0.26	0.07	**<0.01**
RBANS - A	-0.01	0.04	0.77
RBANS - DM	0.06	0.04	0.13
Trails A^	0.01	0.04	0.80
Trails B^	-0.02	0.04	0.58
FAS - GT	-0.06	0.04	0.19

Note. All estimates are standardized beta-weights; Cognitive assessments where greater scores reflect worse performance are indicated with a '^'; RBANS – *T* = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

3.2. Prediction 2: CRFR1 haplotype status and cognitive performance

The *CRFR1* haplotype demonstrated significant associations with several cognitive assessments at both Time 1 and Time 2 (prediction 2a, main). Interactions between haplotype and cortisol at each wave were tested to predict cognitive functioning at a given wave (prediction 2a, interaction). No interactions were statistically significant, and thus the interaction term was removed from all models (data not shown). The standardized beta weights for main effects, age, diurnal-adjusted cortisol, and haplotype are shown in Table 6.

A total of five cognitive tests showed significant relations to the

Table 6
Relations between cortisol and haplotype on cognitive functioning at Time 1 and Time 2.

Parameter	Time 1 Estimate	Time 2 Estimate
RBANS - T		
Age	-0.08	-0.19
Cortisol	0.03	0.15
Haplotype	0.22**	0.24**
RBANS - M		
Age	-0.12	-0.29**
Cortisol	-0.09	0.15
Haplotype	0.23**	0.23**
RBANS - VC		
Age	-0.11	-0.06
Cortisol	0.04	0.07
Haplotype	0.08	0.06
RBANS - L		
Age	0.03	-0.16
Cortisol	0.01	0.16
Haplotype	0.21**	0.22**
RBANS - A		
Age	-0.08	-0.18
Cortisol	0.12	0.14
Haplotype	0.26**	0.30**
RBANS - DM		
Age	-0.11	-0.29**
Cortisol	0.03	0.14
Haplotype	0.07	0.14
Trails Aˆ		
Age	0.33**	0.43**
Cortisol	-0.13	-0.15
Haplotype	-0.22**	-0.13
Trails Bˆ		
Age	0.45**	0.42**
Cortisol	-0.20*	-0.16
Haplotype	-0.11	-0.09
FAS - GT		
Age	-0.11	-0.22*
Cortisol	0.11	0.24*
Haplotype	0.23**	0.22**

Note. All estimates are standardized beta-weights
* = $p < 0.05$
** = $p < 0.01$. Cognitive assessments where greater scores reflect worse performance are indicated with a 'ˆ'; Haplotype coded 0 = 'protective,' 1 = 'risky.' Haplotype is marked as 'protective' when each of the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes are concurrently recorded as AA, TT, and TT, respectively. The haplotype is marked as 'risk' if any of the CRFR1 alleles are recorded as follows: CRFR1_11 is recorded as AG or GG, CRFR1_24 is recorded as GT or GG, or if CRFR1_72 is scored as CT or CC. Haplotype was constructed due to markedly high correlations between each CRFR1 allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95); RBANS – TS = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

CRFR1 haplotype at both waves (data presented in Table 6): The RBANS Total (Time 1 and Time 2, $P < 0.01$), RBANS Immediate Memory (Time 1 and Time 2, $P < 0.01$), RBANS Language (Time 1 and Time 2, $P < 0.01$), and RBANS Attention (Time 1 and Time 2, $P < 0.01$), alongside the FAS Grand Total (Time 1 and Time 2, $P < 0.01$). Notably, each of these findings were found to occur in the opposite direction than what was predicted, such that homozygous CRFR1 allele rare, 'protective' carriers (i.e., rs110402 [AA], rs7209436 [TT]; rs242924 [TT]) had significantly *worse* cognitive assessment scores at both time points when compared to common, 'risky' allele carriers (i.e., rs110402 [AG, GG], rs7209436 [CT, CC]; rs242924 [GT, GG]). Additionally, homozygous *CRFR1* 'protective' allele carriers demonstrated significantly greater assessment scores on the Trail Making Task – A (i.e., worse performance), but this was only found at baseline (Time 1; $P < 0.01$).

The multigroup analyses showed significant relations between baseline cortisol and changes in cognitive test performance in the 'risky' haplotype (i.e., rs110402 [AG, GG], rs7209436 [CT, CC]; rs242924 [GT, GG]) group alone (prediction 2b, main; see Table 7). However, the interaction of haplotype and cortisol was not significant (prediction 2b, interaction). Similar to the results mentioned above, the significant cognitive findings were limited to the RBANS Total ($P < 0.01$), as well as the Immediate Memory ($P < 0.01$) and Language ($P < 0.01$) subtests (Table 7), and only occurred within the 'risky' haplotype; there were no significant relations for the 'protective' haplotype. Contrary to predictions, within the 'risky' group only, higher RBANS scores over time (better performance) were associated with higher baseline cortisol (beta weights for RBANS Total, Immediate Memory, Language = 0.035, 0.031, 0.042, respectively). Notably, despite these results being restricted to the 'risky' haplotype group, the relationship between baseline cortisol and change in cognitive function for the 'risky' haplotype was not found to differ significantly from the 'protective' group in any measure. Thus, the directionality and strength of the relationship between baseline cortisol and cognitive performance change did not differ significantly by haplotype.

Table 7
Baseline cortisol on change in cognitive performance between haplotype groups.

Parameter	'Protective' Estimate	'Risky' Estimate	Cort. Diff.
RBANS - T			
Baseline Age	-0.31*	-0.24*	
Baseline Cortisol	0.20	0.35**	0.15
RBANS - M			
Baseline Age	-0.28*	-0.26*	
Baseline Cortisol	0.22	0.31**	0.09
RBANS - VC			
Baseline Age	-0.04	-0.01	
Baseline Cortisol	0.13	0.13	0.00
RBANS - L			
Baseline Age	-0.33*	-0.34**	
Baseline Cortisol	0.27	0.42**	0.15
RBANS - A			
Baseline Age	-0.24	-0.11	
Baseline Cortisol	0.04	-0.07	0.11
RBANS - DM			
Baseline Age	-0.33*	-0.17*	
Baseline Cortisol	0.04	0.09	0.05
Trails Aˆ			
Baseline Age	0.36*	0.12	
Baseline Cortisol	-0.05	-0.08	0.03
Trails Bˆ			
Baseline Age	0.30*	-0.09	
Baseline Cortisol	-0.07	0.19	0.26
FAS - GT			
Baseline Age	-0.31*	-0.11	
Baseline Cortisol	0.01	0.11	0.10

Note. All estimates are standardized beta-weights;.
* = $p < 0.05$
** = $p < 0.01$; Cort. Diff. = Cortisol difference between 'protective' and 'risky' groups; Cognitive assessments where greater scores reflect worse performance are indicated with a 'ˆ'; Haplotype coded 0 = 'protective,' 1 = 'risky.' Haplotype is marked as 'protective' when each of the CRFR1_11, CRFR1_24, and CRFR1_72 genotypes are concurrently recorded as AA, TT, and TT, respectively. The haplotype is marked as 'risky' if any of the CRFR1 alleles are recorded as follows: CRFR1_11 is recorded as AG or GG, CRFR1_24 is recorded as GT or GG, or if CRFR1_72 is scored as CT or CC. Haplotype was constructed due to markedly high correlations between each CRFR1 allele group (i.e., CRFR1_11 with CRFR1_24 = 0.94; CRFR1_11 with CRFR1_72 = 0.94; CRFR1_24 with CRFR1_72 = 0.95); RBANS – TS = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

3.3. Prediction 3: FKBP5 allele status, cognitive performance, and baseline cortisol

We predicted that *FKBP5* T carriers (TT or CT vs. CC homozygotes) would have decreased cognitive performance relative to CC homozygotes (prediction 3a, main). Interactions between *FKBP5* genotype and cortisol at each wave were again tested to predict cognitive functioning at a given wave (prediction 3a, interaction). No interactions were statistically significant, and thus the interaction term was excluded (data not shown). The main effects of age, diurnal-adjusted cortisol, and *FKBP5* SNP are shown in Table 8. None of the analyses showed significant relations between *FKBP5* SNP and a given cognitive assessment.

The multigroup analyses showed significant positive relations between diurnal-adjusted cortisol and cognitive performance (prediction 3a, main; Table 9). In contrast to predictions, higher baseline cortisol related to better test performance over time on the RBANS Total ('risky' $P < 0.01$ and 'protective' $P < 0.05$), RBANS Immediate Memory ("risky" only $P < 0.01$), and RBANS Language ('risky' $P < 0.01$ and 'protective' $P < 0.01$) subtests, and worse performance on the Trail Making Task – B for the 'protective' genotype only ($P < 0.05$; higher scores on the Trail Making Task indicate worse performance). The relationship between baseline cortisol and change in cognitive function for the 'risky' genotype differed significantly from the 'protective' genotype in Trails B (standardize beta weight protective was +0.26 and for risky was -0.12; $P < 0.05$). Namely, the relationship between baseline cortisol and Trails B performance was positive for the 'protective' genotype (higher baseline cortisol = significantly decreased performance), and for the 'risky' genotype, the relationship was negative (although not significant as a main effect), suggesting higher baseline cortisol trended toward better performance in this group.

Table 8
Relations between cortisol and FKBP5 on cognitive functioning at Time 1 and Time 2.

Parameter	Time 1 Estimate	Time 2 Estimate
RBANS - T		
Age	-0.05	-0.16
Cortisol	0.04	0.14
FKBP5	-0.04	-0.01
RBANS - M		
Age	-0.10	-0.25^
Cortisol	-0.09	0.15
FKBP5	-0.04	-0.02
RBANS – VC		
Age	-0.10	-0.03
Cortisol	0.05	0.05
FKBP5	0.06	-0.03
RBANS – L		
Age	0.07	-0.10
Cortisol	0.01	0.13
FKBP5	0.02	-0.11
RBANS – A		
Age	-0.02	-0.14
Cortisol	0.12	0.14
FKBP5	-0.13	-0.04
RBANS – DM		
Age	-0.11	-0.28^
Cortisol	0.04	0.14
FKBP5	-0.03	-0.03
Trails A^		
Age	0.30**	0.42**
Cortisol	-0.12	-0.15
FKBP5	0.07	-0.03
Trails B^		
Age	0.43**	0.40**
Cortisol	-0.19*	-0.17
FKBP5	0.01	-0.01
FAS - GT		
Age	-0.06	-0.14
Cortisol	0.11	0.21^
FKBP5	-0.12	-0.11

Note. All estimates are standardized beta-weights
^ = $p < 0.05$
** = $p < 0.01$; Cognitive assessments where greater scores reflect worse performance are indicated with a '^'; FKBP5 coded 0/'Protective' = CC, 1/'Risky' = CT/TT; RBANS – TS = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

Table 9
Cortisol on change in cognitive performance between FKBP5 groups.

Parameter	'Protective' Estimate	'Risky' Estimate	Cort. Diff.
RBANS - T			
Baseline Age	-0.24*	-0.24	
Baseline Cortisol	0.24*	0.35**	0.11
RBANS - M			
Baseline Age	-0.11	-0.39**	
Baseline Cortisol	0.17	0.42**	0.25
RBANS - VC			
Baseline Age	0.01	-0.01	
Baseline Cortisol	0.15	0.12	0.03
RBANS - L			
Baseline Age	-0.19	-0.38**	
Baseline Cortisol	0.33**	0.40**	0.07
RBANS - A			
Baseline Age	-0.19	-0.06	
Baseline Cortisol	-0.06	-0.05	0.01
RBANS - DM			
Baseline Age	-0.14	-0.25*	
Baseline Cortisol	0.01	0.13	0.12
Trails A^			
Baseline Age	0.09	0.31**	
Baseline Cortisol	0.00	-0.14	0.14
Trails B^			
Baseline Age	-0.09	0.18	
Baseline Cortisol	0.26*	-0.12	0.38#
FAS - GT			
Baseline Age	-0.20	-0.06	
Baseline Cortisol	0.07	0.03	0.04

Note. All estimates are standardized beta-weights
* = $p < 0.05$
** = $p < 0.01$; Cort. Diff. = Cortisol difference between 'protective' and 'risky' groups; Cognitive assessments where greater scores reflect worse performance are indicated with a '^'; FKBP5 coded 0/'Protective' = CC, 1/'Risky' = CT/TT. # = significant differences between the protective and risk FKBP5 groups; RBANS – TS = RBANS Total Score; RBANS – *M* = RBANS Index Score Immediate Memory; RBANS – VC = RBANS Index Score Visuospatial/Constructional; RBANS – *L* = RBANS Index Score Language; RBANS – *A* = RBANS Index Score Attention; RBANS – DM = RBANS Index Score Delayed Memory; Trails *A* = Trail Making Test A (seconds); Trails *B* = Trail Making Test B (seconds); FAS – GT = FAS Grand Total (word count).

4. Discussion

We tested three broad predictions about the relation among baseline cortisol, SNP genotype, and changes in various domains of cognitive performance over a three-year period in community dwelling human adults. We predicted that 1) increases in baseline serum cortisol over time would be associated with decline in cognitive function over time (glucocorticoid cascade hypothesis), 2) those with *CRFR1* rare, 'protective' alleles (i.e., rs110402 [AA], rs7209436 [TT]; rs242924 [TT]) would perform better on cognitive tests at each time, and over time (e.g., show less decline), and this would be particularly pronounced in those with lower baseline cortisol, and 3) *FKBP5* T carriers (TT or CT ['risky'] vs. CC homozygotes ['protective'])) would have decreased cognitive performance at each time, and over time, and this outcome would be particularly pronounced in individuals with higher baseline cortisol. We

found partial support for our predictions. In the overall sample (prediction 1, all genotypes), increase in cortisol was associated with decrease in performance in three measures of cognitive function. Across genotype-specific analyses, however, we found the most robust effects for the role of *CRFR1* genotype on cognitive outcomes. Relationships were opposite to what we predicted; namely, the 'risky' *CRFR1* haplotype performed consistently better on cognitive tests (vs. 'protective' haplotype), and when relations with baseline cortisol were found, *higher* baseline concentrations were related to *increased* cognitive performance. We found few associations with *FKBP5* genotype, and again found higher baseline cortisol was associated with increased performance over time.

Prediction 1- Glucocorticoid Cascade Hypothesis

In line with the glucocorticoid cascade hypothesis, we predicted that increases in baseline serum cortisol over time would be associated with declines in cognitive function [29,63], particularly in declarative domains (i.e., those that consist of facts and events than can be stored and consciously recalled) which are regulated, at least partially, by the hippocampus [22]. Our results partially support this hypothesis. Increased cortisol over time was associated with decreased performance over time on three of the nine measures of cognitive performance, including: RBANS Total (composite score), RBANS Immediate Memory (list learning and story memory), and RBANS Language (semantic fluency and picture naming). Interestingly, based on descriptive data, serum cortisol concentration did not increase over time in our overall sample, but the results here suggest that in some participants, cortisol did increase, and this increase was related to decrease in cognitive performance. This outcome is similar to that obtained by Lupien and colleagues [29] in that only healthy adults with the most pronounced positive cortisol slope over a 4-yr period had significantly associated declines in two out of 15 measured variables, explicit memory (cued recall) and selective attention (visual search task), whereas initial baseline cortisol did not predict change in cognitive function. In contrast to that study, we did not see any relationship between cortisol change and change in attention scores as measured by the RBANS Attention index (coding and digit span), however, Lupien and colleagues categorized digit span as a separate measure and did not find a significant relationship with cortisol and digit span. Thus, specific neurocognitive assessments used and their classification for construct operationalization matters for comparison of data across studies.

Given that cortisol did not increase robustly over the 3-yr study period, that changes in baseline cortisol were only related to changes in performance on three of the nine cognitive measures, and that group-level performance on some of the cognitive tasks seemed to improve with time (e.g., via inspection of descriptive statistics; RBANS Attention; RBANS Delayed Memory; Trails A; Trails B; FAS), these results should be interpreted cautiously. Although our study design allowed us to determine within person associations between cortisol and cognitive measures over time while controlling for age (see rationale in [64]), it should be noted that longitudinal studies of cognitive function can be influenced by practice effects [65], which could explain why we did not note group-level declines in some tasks. Additionally, the glucocorticoid cascade hypothesis was originally proposed to explain differences in groups of young vs. old male rats (e.g., 3–5 vs 24–28 months) [19]. Here, we analyzed cortisol among middle aged and older adults over a three-year span and, to isolate the impact of cortisol on cognitive function, included age in our model. Although, historically, the majority of studies have been completed using male subjects [66,67], sex can influence HPA axis function, at least partially due to the role of gonadal steroids [68–71]. In humans, women show greater cortisol response with aging compared to men (note: this meta-analysis reported gender, not sex) [23], and older females had higher cortisol levels (vs. younger females and young and older males) [72]; but this sex (gender) result is not always consistent [26]. Here, our sample population was majority female, and this composition could have influenced our results. It should also be noted that the age range of participants was large (40–70 years old), potentially masking group differences between middle-aged and elderly populations. Importantly, our study included participant age as a covariate in all models, spanning both within and between-person analyses. As a result, we believe this concern is mitigated. These factors may explain the lack of robust cortisol change over time in our study.

Prediction 2 – *CRFR1* genotype, cortisol, and cognitive function

Corticotropin-releasing factor (CRF) plays a major role in initiating the HPA axis response, and is involved in working memory [73], declarative memory [34,74], and other executive functions [74]. We predicted that individuals homozygous for the rare 'protective' alleles (rs110402, AA vs. AG or GG; rs242924 TT vs. TG or GG; rs7209436 TT vs. CT or CC) would be buffered from declines in cognitive performance, and we predicted this would be particularly pronounced in individuals with low baseline cortisol. For *CRFR1* rare, 'protective' allele haplotypes, carriers performed *worse* than those with the common 'risky', haplotype on several measures of cognitive function at both Time 1 and Time 2. Specifically, the 'risky', common haplotype outperformed the 'protective', rare haplotype on RBANS Total (Time 1 and Time 2), RBANS Immediate Memory (Time 1 and Time 2), RBANS Language (Time 1 and Time 2), RBANS Attention (Time 1 and Time 2), FAS Grand Total (Time 1 and Time 2), and Trails A (Time 1). When analyzing data at Time 1 and Time 2 separately, there were only two instances where baseline cortisol significantly predicted cognitive function. At Time 1, higher baseline cortisol was related to better performance on the Trails B test; at Time 2, higher baseline cortisol was related to increased performance on the FAS test. We found no instances where cortisol interacted with haplotype to predict cognitive function at either time point.

When data were analyzed to determine if Time 1 baseline cortisol predicted change in cognitive performance over time, we found similar results. Specifically, in those with the 'risky', common haplotype, increased baseline cortisol related to *better* performance over time in the RBANS Total, RBANS Immediate Memory, and RBANS Language. Again, we found no significant differences it the strength or directionality of relationship between baseline cortisol and cognitive performance between haplotypes.

Overall, our results suggest that *CRFR1* haplotype does impact performance on various aspects of cognitive function, namely that 'risky', common allele carriers perform better, both at single time points and change over time, but this effect does not appear to be strongly driven by differences in baseline cortisol levels. These three *CRFR1* SNPs (rs110402, rs7209436, rs242924) were chosen as previous data suggesting homozygous for the rare alleles (AA, TT, and TT, respectively) are protective. The rare allele combination, also referred to as the T-A-T haplotype (rs7209436, rs110402, rs242924, respectively), was first found to be protective for onset of adult depression following childhood abuse [75,76]. Additionally, individuals homozygous for the rare alleles (AA, TT, and TT) had lower peak cortisol levels in response to the Trier Social Stress Test, a result seemingly driven by being homozygous, vs. heterozygous, for the rare alleles [44]; in that study, however, SNP did not have an association with baseline cortisol. In another study, being homozygous for the common ('risky') allele at rs110402 (GG) and rs242924 (GG) was associated with increased cortisol response to Dex/CRF challenge, but only in those who experienced childhood maltreatment [77]; baseline cortisol was not assessed.

Previous studies on healthy adults found individuals who were homozygotes or carriers of the 'protective' alleles vs. 'risky' homozygotes (rs110402: AA and AG vs. GG; rs242924: TT and GT vs. GG), had higher accuracy on a word recall test of working memory [41,42]. This outcome was also impacted by exposure to, and severity of, early life stress: among individuals homozygous for the common 'risky' alleles (GG), those who experienced moderate to severe early life stress had lower test accuracy; among individuals with 'protective' A or T alleles, task accuracy was only lower in those who experienced severe early life stress [41]. When comparing genotype and age impacts on working memory, those with the common 'risky' homozygous GG genotype performed worse than A & T carriers overall, and this was driven by a

difference between groups in working memory accuracy at middle age (~ 40 yrs), but not at young (~25 yrs) or old (~70 yrs) ages [42]. In contrast, in depressed patients without early life trauma, three *CRFR1* SNPs (rs110402, rs7209436, rs242924) rare alleles (AA, TT, and TT, respectively) were associated with poorer performance on the California Verbal Learning Test [78], an outcome similar to our findings; baseline cortisol was measured but did not significantly predict cognitive performance in that study. Thus, genotype and age, as well as (early life) environmental interactions and type of cortisol measure (e.g., baseline or post-stress) can influence the relationship between *CRFR1* genotype and cognitive outcomes. Collectively, these data, including our findings, suggest the rare, 'protective' haplotype may only be protective in instances where previous (early life) trauma has occurred.

Our results suggest that in community dwelling adults, without data on any known early life trauma, the rare allele haplotype (AA, TT, TT) is associated with poorer performance on certain cognitive tasks and this relationship does not appear to be related to morning baseline cortisol. Disparate findings across the literatures show the relationship among *CRFR1*, aspects of cognitive function, and cortisol is complex and can be influenced by early life experience and affective state. We unfortunately do not have data on early life stressor exposure or HPA axis response to challenge in this dataset and could not probe that relationship here. Additionally, although the presence of various *CRFR1* SNPs is widely documented and likely relevant [79], the functional implications of these SNPs are not known [80], nor is it clear if/how SNPs additively impact CRF function across different brain regions (e.g., increase CRF in the prefrontal cortex decreases working memory whereas increased CRF in the hippocampus can increase long term potentiation, and there is likely an inverted-U shaped relationship between CRF and various hippocampal processes; [34,73,74,81]). Thus, it is difficult to draw conclusions about the mechanism underlying these relationships; more research is needed.

Prediction 3 – *FKBP5* genotype, cortisol, and cognitive function

Lastly, we predicted *FKBP5* T carriers (TT or CT vs. CC homozygotes) would have decreased cognitive performance and that this outcome would be particularly pronounced in individuals with higher baseline cortisol. This hypothesis was largely unsupported. *FKPB5* SNP 'protective' allele homozygotes (CC) and 'risk' allele carriers (CT, TT) did not differ in any measure of cognitive function at Time 1 or at Time 2. When analyzing data at Time 1 and Time 2 separately, there were only two instances where baseline cortisol significantly predicted cognitive function (not surprisingly mirroring findings of *CRFR1* analyses). We found no instances where cortisol interacted with haplotype to predict cognitive function at either time point.

When data were analyzed to determine if Time 1 baseline cortisol predicted change in cognitive performance over time, we found several significant relations. Specifically, higher baseline cortisol related to *higher* RBANS Total and RBANS Language scores overtime within both genotype groups (CC; CT/TT). Within CT/TT individuals only, increased baseline cortisol related to increased performance on the RBANS Immediate Memory over time. Within CC 'protective' individuals, higher baseline cortisol was related to decreased performance on the Trails B test over time. There was one significant difference in the relationship between baseline cortisol and cognitive performance between genotypes: for individuals with the 'protective' CC genotype the relationship of baseline cortisol and Trails B was significant and positive (higher cortisol = worse performance on Trails B), whereas for 'risky' genotypes (CT, TT) the relationship was negative (higher cortisol = better performance on Trails B), although for the 'risky' genotype this was not significant, the slopes for the genotype did significantly differ. Overall, these results suggest *FKBP5* genotype does not independently relate to multiple measures of cognitive performance at a given time, but that genotype does interact with baseline cortisol to predict change in cognitive function over time. And, in instances where baseline cortisol is predictive, data largely go against our initial hypotheses and suggest that higher baseline cortisol is related to better cognitive performance.

Cortisol exerts downstream effects by binding mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). GRs are important for regulation of negative feedback [82] and GR ligand-binding activity and translocation to the nucleus is regulated, in part, by FK506 binding protein 51 (FKBP5; [83,84]). The *FKBP5* SNP (rs1360780; C to T) results in enhanced expression of FKBP5 protein, which decreases GR affinity for cortisol and alters negative feedback, leading to prolonged elevation of cortisol; this rare TT genotype is associated with several clinical outcomes [46,85,86]. Within the glucocorticoid cascade hypothesis framework, this alteration in GR binding regulation and prolonged elevation of cortisol would be predicted to have negative impacts on cognitive function. Correspondingly, in a previous study, healthy aged individuals (over 50 yrs) who were T carriers (those that produce more FKBP5) performed worse on tests of working memory compared to CC individuals [87] . We did not replicate this finding in our dataset. In another recent study of healthy, young adults (~20 yrs), 'risky' allele carriers (CT or TT) had higher post-stressor (cold pressor test) salivary cortisol than CC homozygotes (baseline cortisol did not differ [88]). In CT and TT 'risky' individuals, pre-learning stressor exposure impaired immediate recall memory, and in CC 'protective' homozygotes, stressor exposure enhanced long-term recall memory, but this was not seen in CT or TT individuals [88]. Interestingly, the correlation between salivary cortisol change and recall memory performance was positive (and marginally significant) for CC individuals and negative (but not significant) for CT and TT individuals [88]. Thus, these findings suggest the CC genotype relates to blunted cortisol response to stress, and this lower increase in post-stress cortisol facilitates memory function. In our study we did not have ability to collect post-stressor cortisol, but we did find that for Trails B test, relationship directionality between baseline cortisol and change in performance over time differed between genotypes. However, contrary to the Zoladz and colleagues [88] finding, in our dataset CC individuals with higher baseline cortisol performed worse (positive relationship) whereas individuals with CT/TT with higher baseline cortisol performed better (negative relationship); although baseline cortisol was only significantly related to Trails B over time for the 'protective' CC genotype.

The FKBP5 protein is also proposed as a marker of resilience or flexibility in the HPA axis, with higher levels of FKBP5 (as seen in 'risky' genotype, CT or TT) relating to decreased ability to quickly modulate or alter HPA axis response and termination in the face of challenges [45]. Data from Zoladz and our current study suggest that FKBP5 genotype matters for the relationship between baseline or post-stress HPA axis function and cognitive outcomes, but more research in needed to determine if these genotype differences relate to flexibility in HPA axis function and beneficial changes in cognitive performance. Lastly, as with studies of *CRFR1* SNPs, there is ample evidence that *FKBP5* genotype interacts with early life stressor experience to impact multiple aspects of organismal function, but data on the main impacts of *FKBP5* genotype alone are not always clear (see [45]). In this study we had access to morning blood samples for analysis of baseline cortisol; we did not have the ability to commission stressor testing or Dex/CRF challenge. Therefore, our results are difficult to compare directly to other studies that found pronounced effects of post-stressor values, and it is unknown if our results would be different if post-stress data were available.

5. Conclusion

Overall, we found individual differences in genotype impact cognitive performance in a community dwelling, aging population of humans. However, the discrete category of cognitive performance associated with each genotype differed, and no measure of cognitive performance was universally significant across analyses. *CRFR1* genotype rare allele haplotype individuals performed worse on several measures of cognitive function, contrary to our predictions. *FKBP5* genotypes did not differ on any measure of cognitive function at Time 1 or Time 2. Collective data

from our analyses largely do not support the glucocorticoid cascade hypothesis as cortisol did not increase with age, and in the majority of instances where baseline cortisol was significantly related to cognitive measures, higher cortisol related to better performance.

Although our data do not robustly support the glucocorticoid cascade hypothesis, they do align with previously published work and other stress hypotheses [39]. Namely, our baseline cortisol data are more in line with an inverted-U hypothesis, in that small increases in baseline cortisol may be beneficial for cognitive function, arousal, learning, and memory [9,89,90]. For example, the administration of a cortisol synthesis inhibitor (metyrapone) impaired long-term (1 week) but not short-term (5 min) memory of an emotionally arousing story [91]. Conversely, when the cortisol synthesis blocker (metyrapone) was given before a psychosocial stressor (the Trier Social Stress Test), the treated participants showed impaired short-term memory of a neutral story [92], suggesting that elevation of cortisol is important for memory of not just emotional stories, but for neutral information as well. In line with this idea, an elegant within-person study showed that metyrapone significantly decreased delayed (20 min) recall ability on a neuropsychological tests of declarative memory (no emotional component), and replacement of cortisol, via hydrocortisone, restored recall function [93]. Taken together, these results and those from our current study show that physiological levels of cortisol are important for various aspects of learning and memory. In summary, our study adds important information on which aspects of HPA axis function and regulation are related to cognitive performance during aging.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

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