

## Pupillary dilation responses as a midlife indicator of risk for Alzheimer's disease: association with Alzheimer's disease polygenic risk



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### ABSTRACT

Locus coeruleus (LC) tau accumulation begins early. Targeting LC (dys)function might improve early identification for Alzheimer's disease (AD) risk. Pupillary responses during cognitive tasks are driven by the LC and index cognitive effort. Despite equivalent task performance, adults with mild cognitive impairment have greater pupil dilation/effort during digit span than cognitively normal (CN) individuals. We hypothesized that AD polygenic risk scores (AD-PRSs) would be associated with pupillary responses in middle-aged CN adults. Pupillary responses during digit span tasks were heritable ( $h^2 = 0.30–0.36$ ) in 1119 men aged 56–66 years. In a CN subset—all with comparable span capacities ( $n = 539$ )—higher AD-PRSs were associated with greater pupil dilation/effort in a high (9-digit) cognitive load condition (Cohen's  $d = 0.36$  for upper vs. lower quartile of AD-PRS distribution). Results held up after controlling for *APOE* genotype. Results support pupillary response—and by inference, LC dysfunction—as a genetically mediated biomarker of early mild cognitive impairment/AD risk. In combination with other biomarkers, task-evoked pupillary responses may provide additional information for early screening of genetically at-risk individuals even before cognitive declines.

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### 1. Introduction

Alzheimer's disease (AD) is a worldwide public health problem and the most costly disease in the United States (Alzheimer's Association, 2018). Pathological changes begin decades before onset of dementia, making early identification of AD risk of

paramount importance for slowing down disease progression (Golde et al., 2011; Sperling et al., 2011). Toward that end, we suggest that a focus on tau or tau-associated processes may be quite useful.

Postmortem data indicate that tau pathology is the earliest occurring AD biomarker, first appearing in the locus coeruleus (Braak and Del Tredici, 2011, 2012; Duyckaerts et al., 2015; Ehrenberg et al., 2017). Thus, focusing on tau or tau-associated processes may be quite useful. There is also cerebrospinal fluid—based evidence indicating that tau pathology can precede A $\beta$

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in people who progress to AD (de Leon et al., 2018). Tau is more strongly associated with cognition than A $\beta$  (Maass et al., 2018), and lower locus coeruleus (LC) neuronal density has been associated with faster cognitive decline in cognitively normal (CN) adults, and individuals with mild cognitive impairment (MCI) and autopsy-confirmed AD (Wilson et al., 2013). Finally, an animal model has now suggested the plausibility of Braak's model of the AD pathological process beginning in the LC by showing pretangle spread via abnormal LC tau production and progression associated with cognitive decline, with these changes occurring in the absence of amyloid (Ghosh et al., 2019).

There are established positron emission tomography and cerebrospinal fluid beta-amyloid (A $\beta$ ) and tau biomarkers, but both are costly and invasive and the tau assays do not adequately detect tau in the LC. Thus, there is good reason to search for additional, noninvasive markers of risk that could serve as indicators of early LC function. Our prior work suggests that pupillary dilation during performance of cognitive tasks—which has been linked to LC function—is one such early marker of AD risk (Granholtm et al., 2017). Here we sought to determine if this pupillary response is also a genetically mediated biomarker. An abnormal light reflex—another pupillary response—has been linked to AD and Parkinson's disease (Fotiou et al., 2000, 2009; Prettyman et al., 1997), but our focus here is on pupil dilation associated with cognitive effort rather than reflexive pupil constriction.

Increased pupillary dilation during performance of cognitive tasks is a validated, objective psychophysiological index of the brain's cognitive resource allocation, that is, cognitive effort (Ahern and Beatty, 1979; Beatty, 1982; Granholtm et al., 1996; Kahneman and Beatty, 1966; van der Meer et al., 2010). Ability level is inversely related to amount of effort—indexed by amount of pupil dilation—needed to perform a task. Pupil size increases with increasing cognitive effort as task demand, that is, cognitive load, increases (Ahern and Beatty, 1979; Beatty, 1982; Granholtm et al., 1996, 2017; Kahneman and Beatty, 1966; van der Meer et al., 2010). However, when task demands substantially exceed abilities and compensatory capacity, there is disengagement from the cognitive processing system; at that point, dilation drops off and performance declines (Ahern and Beatty, 1979; Beatty, 1982; Granholtm et al., 1996, 2017; van der Meer et al., 2010). These pupillary responses reflect activation in the LC (Alnaes et al., 2014; Aston-Jones and Cohen, 2005; Gilzenrat et al., 2010; Joshi et al., 2016; Koss, 1986; Murphy et al., 2014; Phillips et al., 2000; Raizada and Poldrack, 2007; Samuels and Szabadi, 2008). Although the LC has been viewed historically as important only in terms of broad arousal responses, Aston-Jones and Cohen's (2005) adaptive gain model supports a complex role of the LC-noradrenergic (LC-NE) system involving phasic activation with adaptive gain to optimize task performance and tonic activation associated with gain to optimize appropriate disengagement and a shift of focus to different stimuli or tasks. Thus, the LC-NE system is an important modulator of cognitive function and management of cognitive load (Aston-Jones and Cohen, 2005; Coull et al., 1999; Samuels and Szabadi, 2008; Sara, 2009; Wilhelm et al., 1999).

Several lines of research are consistent with the modulatory role of the LC-NE system with respect to cognition. There is evidence of age differences in LC function (Lee et al., 2018) and structural MRI measures of the LC have been associated with cognitive function in older adults (Clewett et al., 2016; Hämmerer et al., 2018). We showed associations between fMRI resting-state BOLD variance and pupillary dilation responses in cognitively normal and MCI participants in a subset of those in the present study (Elman et al., 2017). Other evidence is consistent with activation of the LC-NE system playing a role in promoting cognitive reserve in older adults (Clewett et al., 2016; Mather and Harley, 2016).

In previous work, based on the modulatory role of the LC-NE system, we hypothesized that if 2 individuals had the same cognitive test score, the one needing more effort is at higher risk for decline because they would be closer to their maximum capacity for compensation (cf. Riediger et al., 2006; Stern et al., 2018). On the other hand, someone who has already experienced substantial declines and has surpassed their compensatory threshold is likely to have both poor performance and reduced pupillary dilation responses. Pupillary dilation responses should thus be most useful as a very early marker of risk while there is still little or no observable cognitive decline. Our prior work with participants in the present sample supports these ideas (Granholtm et al., 2017). Individuals with single-domain amnesic MCI had elevated pupillary dilation responses at low or moderate processing loads during digit span tasks, despite equivalent performance to CN participants. Those with multiple-domain MCI—having more depleted resources—had both impaired performance and reduced pupillary responses. Similarly, patients with spatial neglect have very depleted attentional resources and also have reduced pupillary dilation responses during an attentional task (Walle et al., 2019).

Previously, we also showed that an AD polygenic risk score, which had been validated against both living and autopsy-confirmed AD cases (AD-PRS; Escott-Price et al., 2017a,b, 2015), was associated with increased odds of MCI in participants from the present sample, 89% of whom were <60 years old (Logue et al., 2018). The odds ratio for MCI was 3.2 for the upper versus the lower quartile of the PRS distribution. Results changed little after accounting for the effects of APOE (Logue et al., 2018), the largest single genetic determinant of AD risk (Corder et al., 1993; Lambert et al., 2013). If the pupillary dilation responses were also associated with the AD-PRS, it would provide proof of concept supporting the validity and potential utility of pupillary dilation responses.

The literature supports the idea that pupillary dilation responses are associated with cognitive resource allocation and are linked to LC structure and function. LC tau deposition has also been shown to be present in early adulthood, and the modulatory role of the LC-NE system appears to be less efficient in older adults. Pupillary responses during digit span can differentiate cognitively normal midlife adults from those with amnesic MCI even before there are observable task performance differences. Higher AD-PRSs have also been associated with increased odds of having MCI. Taken together, these findings suggest that pupillary dilation responses themselves—and by inference, LC dysfunction—in midlife and later life may be AD related.

Here we hypothesized that pupillary dilation responses during a cognitive task are a genetically mediated AD risk indicator in late middle-aged adults. We used the classical twin design to estimate the heritability of pupillary responses, thereby demonstrating that they are genetically influenced (Eaves et al., 1978; Neale and Cardon, 1992). Next, we tested our primary hypothesis that a higher AD-PRS would be associated with greater pupil dilation during a cognitive task even in cognitively normal middle-aged individuals. Having previously shown that pupillary dilation responses can differentiate cognitively normal midlife adults from those with MCI (Granholtm et al., 2017), their association with the AD-PRS would contribute additional proof of concept supporting the validity of pupillary dilation responses as an early and genetically mediated marker of AD-related risk.

## 2. Materials and methods

### 2.1. Participants

Participants were men in wave 2 of the Vietnam Era Twin Study of Aging (VETSA), a national, community-dwelling sample similar

to American men in their age range with respect to health and lifestyle characteristics based on Center for Disease Control and Prevention data (Kremen et al., 2013; Schoeneborn and Heyman, 2009). All served in the military sometime between 1965 and 1975, but ~80% reported no combat exposure. The flow of participant selection is shown in Fig. 1. Sample characteristics are shown in Table 1 for twin/heritability analyses of all participants with pupillometry data ( $n = 1119$ ) and those with maximum digit spans of 5–7 for the AD-PRS analyses ( $n = 539$ ) (explained below). The average general cognitive ability percentile scores of 61.7 and 63.3 correspond to IQ of 104–105 (Lyons et al., 2009, 2017). The threshold of 16 on the Center for Epidemiologic Studies Depression Scale (Radloff, 1977) was used to estimate the number of individuals with clinical depression. Participants were also asked if they ever had a head injury with loss of consciousness or confusion; almost all were defined as mild and occurred at an average of 35 years earlier (Kaup et al., 2019). Participants traveled to the University of California, San Diego or Boston University where identical protocols were implemented. Written informed consent was obtained from all participants, and the study was approved by Institutional Review Boards at participating institutions.

The present study began with 1207 participants (Kremen et al., 2006, 2013). Exclusions were as follows: self-reported history of glaucoma in either eye, penetrating eye wounds to both eyes, surgery to both eyes involving the muscle, or use of cholinesterase inhibitors or prescribed ocular medications ( $n = 57$ ); or equipment failures or excessive blinking ( $n = 34$ ). Depression and head injury were not exclusions because they are risk factors for dementia. This left 1119 individuals with valid pupillometry data (Granholtm et al., 2017) and 1085 with genotyping data who were of European ancestry. There were too few individuals of non-European ancestry to include in the AD-PRS analyses. There were 828 individuals who were both CN and had valid pupillometry data. Because we were primarily interested in examining whether pupil dilation can inform risk for AD when performance is comparable among individuals, we selected 539 of these 828 individuals with relatively similar maximum span capacities of 5–7 digits (see Discussion for further examination of this issue). Because our digit span task included 3-, 6-, and 9-digit conditions, max span for this subgroup was thus only  $\pm 1$  digit from the moderate 6-digit load. These included 87 monozygotic (MZ) twin pairs, 62 dizygotic twin pairs, and 241 unpaired twins.

## 2.2. Cognition

### 2.2.1. Definition of cognitively normal status

As described in detail elsewhere (Granholtm et al., 2017; Kremen et al., 2014; Logue et al., 2018), cognitive status was determined on the basis of 18 neuropsychological tests covering 6 cognitive domains. Using the Jak-Bondi approach (Jak et al., 2009), MCI was defined as having  $\geq 2$  tests in a domain that were each  $> 1.5$  SDs below normative means. To ensure that MCI reflected a decline in function rather than lifelong low ability, these values were determined after adjusting for general cognitive ability which was assessed at an average age of 20 years (Lyons et al., 2009, 2017). Individuals with no impaired domains (85%) were considered CN.

### 2.2.2. Digit span capacity

We used digit span tasks during pupillometry (see Section 2.3). Maximum span capacity was defined as the longest string of digits correctly recalled during standard testing with the Wechsler Memory Scale-III digit span subtest without the pupillometer (Wechsler, 1997).

## 2.3. Pupillometry

We used handheld NeurOptics PLR-2000 pupillometers to record pupil diameter from one eye at 30 Hz for up to 15 seconds while participants viewed a set of lights around a dark interior (~200 lux) inside in a viewing tube. The pupillometer contains recording optics and has a 1.5-inch viewing tube that surrounds the eye and blocks ambient light. To block the other eye, participants closed and held their hand over it. The pupillometer has excellent resolution (mean error = 0.052 mm; 99% CI = 0.048–0.056; NeurOptics data,  $N = 655$ ).

Pupillary responses were recorded during blocks of trials of 3 (low load), 6 (moderate/near capacity load), and 9 (high/overload) digits presented aurally at the rate of 1 per second. Stimuli were presented on a laptop computer at ~85 decibels. Participants heard “Ready” 1 second before the first digit and “Repeat” 1 second after the last digit. Experimenters initiated pupillary response recording when the word “Ready” was presented. We recorded from the right eye (72.4% of the time) unless the participant had had an injury or medical problem in that eye. Each trial was inspected for artifacts in a graphic display on the device. Trials were administered until 2 clean trials were recorded or 4 trials were attempted per digit span condition. We averaged trials within each condition and averaged pupil diameter samples for each second of recording (30 per second), corresponding to the presentation of digits at 1-second intervals. The primary dependent variable was the pupillary response score: average pupil size during the 1-second window immediately after presentation of the last digit minus average pupil size at baseline (i.e., the first 167 ms of each trial, which is before stimulus presentation). These difference scores remove individual differences in tonic pupil size. Fig. 2 shows a sample pupil response waveform.

## 2.4. Genotyping methods

These methods are described in detail elsewhere (Logue et al., 2018). Genome-wide genotyping was conducted on individual twins, with one randomly selected twin from each MZ pair at deCODE (Reykjavik, Iceland) with Illumina HumanOmniExpress-24 v1.0 A beadchips. GenomeStudio software indicated that the average call rate was 0.996. We performed cleaning and quality control with PLINK v1.9 (Chang et al., 2015). Single-nucleotide polymorphisms (SNPs) with  $> 5\%$  missing data or Hardy-Weinberg equilibrium  $p$ -values  $< 10^{-6}$  were excluded. Relationships and zygosity were confirmed by PLINK’s-genome procedure.

Ancestry was confirmed by SNP weights (Chen et al., 2013) and a principal components (PCs) analysis performed in PLINK v1.9 in conjunction with 1000 Genomes Phase 3 reference data (1000 Genomes Project Consortium et al., 2015). Weights for PCs were computed from 100,000 randomly chosen common (minor allele frequency  $> 5\%$ ) markers based on 1000 Genomes data and then

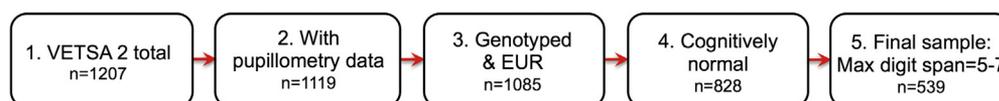


Fig. 1. Flowchart of participant selection. Abbreviation: EUR, participants of European-American ancestry.

**Table 1**  
Participant characteristics

Characteristic	Full sample for twin analyses (N = 1119)			Subsample for AD-PRS analyses (N = 539)		
	Mean/N	SD/%	Range	Mean/N	SD/%	Range
Age (y)	61.7	2.4	56.0–66.9	61.7	2.4	56.5–66.9
Education (y)	13.8	2.1	5–50	13.8	2.1	6–20
GCA	61.7	21.8	2–99	63.3	20.7	9–99
CES-D (no. of individuals above clinical depression threshold [ $\geq 16$ ])	149	13.4%	–	62	11.5%	–
History of head injury with loss of consciousness or confusion	312	27.9%	–	147	27.3%	–
APOE- $\epsilon 4$ carriers	325	29.7%	–	155	29.4%	–

Mean, standard deviation (SD), and range are presented for age, education, and GCA. N and percent are presented for CES-D and history of head injury. Subsample for AD-PRS analyses was cognitively normal individuals with maximum digit span of 5–7. Ns vary slightly for some measures because of missing data; percentages shown are the percent of participants with nonmissing data.

Key: AD-PRS, Alzheimer's disease polygenic risk score; CES-D, Center for Epidemiologic Studies Depression Scale (Radloff, 1977); GCA, general cognitive ability percentile score.

applied to the VETSA sample. Outliers from the EUR population (1000 Genomes European-ancestry super population) cluster were excluded from the genetically identified VETSA white non-Hispanic cohort. The remaining white non-Hispanic participants had >89% European ancestry as estimated by SNP weights. PCs for use as covariates to control for potential population substructure within white non-Hispanic participants were recomputed based on 100,000 randomly chosen common markers.

Imputation was performed using MiniMac (Fuchsberger et al., 2015; Howie et al., 2012) at the Michigan Imputation Server (<https://imputationserver.sph.umich.edu>). The 1000 genomes phase 3 EUR data were used as a haplotype reference panel. Only one randomly chosen individual in each genotyped MZ pair was submitted for imputation. The resulting imputed genotypes were then applied to the co-twin. The final sample with available imputation data included 1329 individuals.

#### 2.4.1. AD-PRS calculation and APOE genotyping

The AD-PRS was computed from summary data of an AD GWAS meta-analysis (Lambert et al., 2013). It is a weighted average of VETSA sample additive imputed SNP dosages with log-odds ratios for each SNP estimated in the GWAS used as the weights. We excluded rare SNPs (minor allele frequency <1%) and SNPs with poor imputation quality ( $R^2 < 0.5$ ) from the calculation. We trimmed the remaining SNPs for linkage disequilibrium using PLINK's clumping procedure ( $r^2$  threshold of 0.2 in a 500 kb window) based on linkage disequilibrium patterns in the 1000 Genomes EUR cohort. AD-PRSs were computed by PLINK v1.9 using 6  $p$ -value thresholds:  $p < 0.05, 0.10, 0.20, 0.30, 0.40, 0.50$ . In addition, we directly genotyped APOE as described previously (Schultz et al., 2008). The number of SNPs included at different thresholds has been documented in a prior publication (Logue et al., 2018). In our study of MCI and in studies of AD, the  $p < 0.50$  threshold provided the best case-control discrimination (Escott-Price et al., 2015, 2017a; Logue et al., 2018). We, therefore, used the  $p < 0.50$  threshold in the present study.

## 2.5. Statistical analysis

### 2.5.1. Heritability and estimation of genetic and environmental influences

In the classical twin design, variance of a phenotype is separated into proportions attributed to additive genetic (A), common environmental (C), and unique environmental (E) influences. C influences are environmental factors that make twins in a pair similar to one another; E influences are environmental factors that make twins in a pair different from one another, including measurement error (Eaves et al., 1978; Neale and Cardon, 1992). Additive genetic influences are assumed to correlate 1.0 between MZ twins, and 0.50 between dizygotic twins who on average share 50% of their

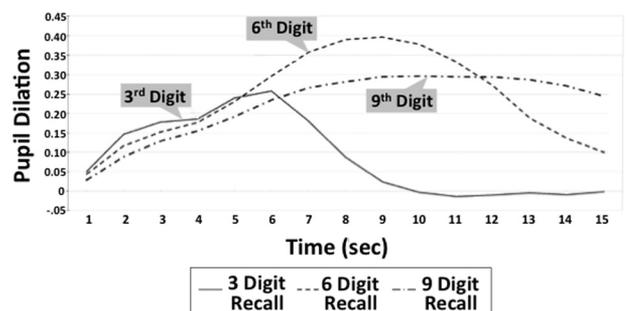
segregating genes. C influences are assumed to correlate 1.0 between members of a pair regardless of zygosity. E influences are, by definition, uncorrelated between members of a pair. Heritability is the proportion of total variance attributed to additive genetic influences.

Extending to the multivariate case, we examined the relative contribution of the genetic and environmental influences on pupil dilation responses at the 3 cognitive loads and the covariance between these measures by fitting a Cholesky decomposition to the data. The purpose was to determine the degree to which covariance between individual differences at the 3 cognitive loads can be explained by common versus distinct continua of liability. We began by fitting a Cholesky that included the A, C, and E effects, then tested if the A or C components could be removed without any change in model fit. We tested model fit using the likelihood-ratio chi-square test (LRT), which is the difference in the  $-2 \log$  likelihood ( $-2LL$ ) of the model in question relative to the full saturated model. Nonsignificant LRT values ( $p > 0.05$ ) indicate that a reduced model does not have a significantly worse fit relative to the comparison. In addition, we used the Akaike information criterion (AIC) as an indicator of goodness-of-fit; smaller values represent a better balance between goodness-of-fit and parsimony (Akaike, 1987). Analyses were conducted using the raw data option of the maximum likelihood-based structural equation modeling software OpenMx (Boker et al., 2011; Neale et al., 2015).

Residual pupillary response scores were used in the biometrical models, after adjustment for age, pupillometry device (4 of the same devices were used), and medications with anticholinergic properties. Relevant medications and their rankings for degree of anticholinergic properties have been documented previously (Granholm et al., 2017).

### 2.5.2. AD-PRS

These analyses were conducted using linear mixed-effects models (SAS Proc Mixed, version 9.4; SAS Institute Inc., 2013)



**Fig. 2.** Sample pupillary response waveform during digit span at 3 cognitive loads. Pupil dilation is calculated as mm change from baseline.

**Table 2**  
Variance components of pupillary dilation response measures

Standardized variance components			
Measure	A (95% CI)	C (95% CI)	E (95% CI)
Full Cholesky			
Dilation at 3 digits	0.36 (0.10; 0.52)	0.00 (0.00; 0.20)	0.64 (0.48; 0.83)
Dilation at 6 digits	0.33 (0.05; 0.59)	0.14 (0.00; 0.38)	0.53 (0.38; 0.74)
Dilation at 9 digits	0.36 (0.06; 0.54)	0.01 (0.00; 0.23)	0.63 (0.46; 0.84)
Reduced Cholesky			
Dilation at 3 digits	0.36 (0.17; 0.52)	–	0.64 (0.48; 0.83)
Dilation at 6 digits	0.30 (0.10; 0.60)	0.17 (0.00; 0.32)	0.53 (0.38; 0.73)
Dilation at 9 digits	0.37 (0.17; 0.54)	–	0.63 (0.46; 0.83)
Unstandardized variance components			
Measure	A	C	E
Reduced Cholesky			
Dilation at 3 digits	0.36	–	0.65
Dilation at 6 digits	0.57	0.32	1.01
Dilation at 9 digits	0.76	–	1.26

Key: A, additive genetic influences; C, common/shared environmental influences; CI, confidence interval; E, unique environmental influences.

accounting for the correlated nature of the twin data by including family (i.e., twin pair) as a random effect. The AD-PRS was standardized before analysis. We included the first 3 PCs, age, pupillometry device, and medications with anticholinergic properties as covariates. We also compared the upper versus lower quartile of the AD-PRS distribution. To determine effects of the AD-PRS after accounting for *APOE*, we performed additional analyses including directly genotyped *APOE-ε2* and *APOE-ε4*. Each was coded for presence/absence of at least one *ε2* or *ε4* allele, respectively. Results were based on type III tests of fixed effects.

### 3. Results

The full Cholesky provided a good fit to the data ( $-2LL = 4800.15$ ,  $df = 1570$ ,  $AIC = 1660.14$ ). Two C estimates accounted for  $\leq 1\%$  of variance. A reduced Cholesky with those parameters set to zero resulted in minimal change in fit ( $-2LL = 4800.43$ ,  $df = 1575$ ,  $AIC = 1650.43$ ,  $LRT = 0.29$ ,  $df = 5$ ,  $p > 0.999$ ). All 3 pupillary response measures were significantly heritable ( $h^2 = 0.30-0.36$ ); the remaining variance was primarily accounted for by unique environmental influences (Table 2). The unstandardized variance components for the reduced Cholesky also show that the genetic and the total variance in pupillary responses increased as cognitive load increased (Table 2). However, heritabilities changed little with increasing cognitive load because genetic and unique environmental variances were both increasing.

**Table 3**  
Phenotypic, genetic, and unique environmental correlations among pupillary dilation response measures

Measures	3 digits	6 digits	9 digits
Phenotypic correlations			
Dilation at 3 digits	1.00		
Dilation at 6 digits	0.42 (0.35; 0.49)	1.00	
Dilation at 9 digits	0.42 (0.35; 0.49)	0.60 (0.54; 0.65)	1.00
Genetic correlations			
Dilation at 3 digits	1.00		
Dilation at 6 digits	0.99 (0.58; 1.0)	1.00	
Dilation at 9 digits	0.73 (0.42; 0.96)	0.63 (0.18; 0.93)	1.00
Unique environmental correlations			
Dilation at 3 digits	1.00		
Dilation at 6 digits	0.17 (–0.26; 0.36)	1.00	
Dilation at 9 digits	0.24 (0.06; 0.42)	0.67 (0.53; 0.77)	1.00

Numbers in parentheses are the 95% confidence intervals. All estimates were derived from the reduced trivariate Cholesky decomposition.

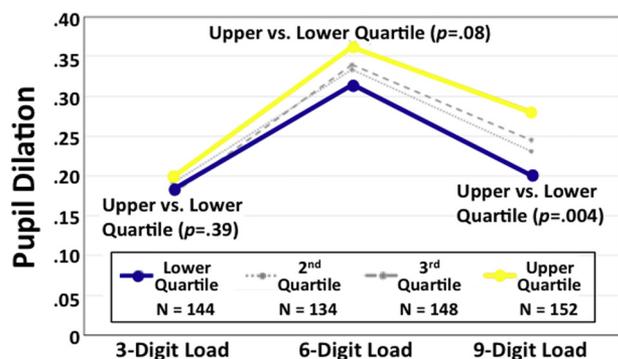
Table 3 shows the correlations among pupillary response measures derived from the reduced Cholesky. Phenotypic correlations, which represent the total shared variance between measures, were moderate ( $r_p = 0.42-0.60$ ). Genetic correlations, which represent only the shared genetic variance between measures (Neale and Cardon, 1992), were substantially higher ( $r_G = 0.73-0.99$ ). The high genetic correlations suggest that genetic influences affecting dilation at varying digit lengths are driven primarily by a single common factor. However, 2 genetic correlations were significantly different from 1.0, indicating that they are not entirely influenced by the same genes. Because unselected samples are thought to provide more unbiased heritability estimates, we also provide the full sample ( $n = 1119$ ) Cholesky and correlation results, which were very similar (Supplementary Tables 1 and 2). However, as already noted, we focus primarily on the subset of individuals with span capacities of 5–7 because of the very different meaning of the task for people at the extremes of span capacity.

The AD-PRS was significantly correlated with pupil dilation response during the 9-digit recall condition ( $r = 0.10$ ,  $p < 0.03$ ; Table 4). As expected, results for the cognitively normal sample including participants with the full range of span capacities were weaker, although in the same direction (Supplementary Table 3). The difference between the upper and lower quartiles of the AD-PRS distribution increased as the cognitive load increased (Fig. 3). The upper quartile had significantly larger pupil responses during 9-digit recall (Cohen's  $d = 0.36$ ,  $p < 0.005$ ; Table 5), and this comparison was at a trend level for the 6-digit recall ( $d = 0.22$ ,  $p < 0.08$ ). These sets of results held up after including maximum span capacity as a covariate ( $r = 0.09$ ,  $p < 0.03$ ; Supplementary Table 4) and after controlling for depression and history of head injury ( $r = 0.09$ ,  $p < 0.04$ ; Supplementary Table 5). After controlling for presence/absence of directly genotyped *APOE-ε2* and *APOE-ε4*, the AD-PRS was still significantly correlated with pupil dilation responses

**Table 4**  
Association of Alzheimer's disease polygenic risk score with pupillary dilation response

Digit span load	Estimate	SE	DF	<i>t</i>	<i>p</i>	<i>r</i>
3 Digits ( $n = 537$ )	0.003	0.007	139	0.42	0.67	0.02
6 Digits ( $n = 530$ )	0.014	0.010	135	1.42	0.16	0.06
9 Digits ( $n = 521$ )	<b>0.023</b>	<b>0.010</b>	<b>130</b>	<b>2.18</b>	<b>0.03</b>	<b>0.10</b>

Covariates include age, the first 3 principal components from the genome-wide genotyping data, pupillometry device, and total number of medications with anticholinergic properties. Data were restricted to cognitively normal individuals with a maximum digit span of 5–7 digits. Ns vary due to missing data for particular variables. Significant results ( $p < 0.05$ ) are shown in bold.



**Fig. 3.** Pupillary dilation response during digit span tasks: Upper versus lower quartiles of the AD-PRS distribution. Pupil dilation is calculated as mm change from baseline. Abbreviation: AD-PRS, Alzheimer's disease polygenic risk score.

during the high cognitive load condition ( $r = 0.11$ ,  $p < 0.02$ ; [Supplementary Table 6](#)). Neither *APOE* variant was associated with pupil dilation responses.

We compared pupillary dilation responses that were recorded from the left versus right eye. There was no difference at the 6-digit load ( $p = 0.12$ ) or the 9-digit load ( $p = 0.46$ ), but there was significantly greater dilation in the right eye at the 3-digit load ( $F(1,365) = 7.06$ ,  $p = 0.008$ ). As a check, we re-ran all the analyses with the eye from which pupillary responses were recorded as a covariate. These results are not reported here as there were no meaningful changes in the findings, and all significant results remained significant.

#### 4. Discussion

To our knowledge, this is the first evidence of the heritability of task-relevant pupillary dilation responses. High genetic correlations suggest that individual differences in dilation during different cognitive loads are driven primarily by a single common factor or underlying continuum of liability. We then showed that CN individuals at greater genetic risk for AD—based on the AD-PRS—had significantly greater pupil dilation when cognitive demand was high. The effect size comparing the upper and lower quartiles of the AD-PRS distribution was  $d = 0.36$ . Consistent with an underlying continuum of liability, there was an increasing effect size with increasing cognitive load.

We previously observed a wide distribution of pupillary responses in CN individuals and hypothesized that those with the highest pupil dilation would be at highest risk for progressing to MCI and AD ([Granholt et al., 2017](#)). Although we do not yet know who will develop these disorders, our results support this hypothesis because those who required the greatest effort as cognitive

**Table 5**  
Association of Alzheimer's disease polygenic risk score (upper vs. lower quartile) with pupillary dilation response

Digit span load	Estimate	SE	DF	<i>t</i>	<i>p</i>	<i>d</i>
3 Digits (n = 272)	−0.017	0.020	137	−0.86	0.39	0.10
6 Digits (n = 267)	−0.048	0.027	133	−1.79	0.08	0.22
9 Digits (n = 264)	<b>−0.080</b>	<b>0.028</b>	<b>128</b>	<b>−2.88</b>	<b>0.005</b>	<b>0.36</b>

Covariates include age, the first 3 principal components from the genome-wide genotyping data; pupillometry device, and total number of medications with anticholinergic properties. Data were restricted to cognitively normal individuals with a maximum digit span of 5–7 digits. Ns vary due to missing data for particular variables. Results presented represent the difference between the upper and lower quartiles of the AD-PRS distribution.

Significant results ( $p < 0.05$ ) are shown in bold.

Key: AD-PRS, Alzheimer's disease polygenic risk score.

load increased also tend to be those at highest genetic risk based on the AD-PRS. The minimal variation in actual performance in this sample and additional analyses controlling for maximum span show that risk was associated with effort needed rather than task performance. Thus, these results provide proof of concept that pupillary dilation responses during a cognitive task—a brief, low-cost, low-invasive assessment—might be a useful additional risk indicator for identifying participants for clinical trials or other research on determinants of onset and progression of AD. The present results and our previous finding that pupillary responses during digit span differentiated CN individuals from those with amnesic MCI suggest that this measure has potential as an adjunctive screening tool, probably in combination with other biomarkers. However, more work needs to be done before its utility in improving screening for AD risk can be determined.

To ensure relatively comparable difficulty level and performance across participants, we only included participants with max spans of 5–7 digits. For individuals with max spans  $>7$ , 9 digits is not as much of an overload, and for individuals with a max span of  $<5$  digits, 6 digits is closer to overload. These distinctions are important because, relative to individuals with lower ability, individuals with greater ability dilate less at low loads but more in higher load conditions ([Ahern and Beatty, 1979](#); [Granholt et al., 2017](#); [van der Meer et al., 2010](#)). It is therefore important to examine dilation relative to individual ability level. Put another way, at a given cognitive load, pupil responses for people with very high or very low span capacities probably reflect different processes. Thus, it was not unexpected that we observed weaker results for the full sample of cognitively normal individuals.

Here we used preset cognitive loads because it was important in our initial work ([Granholt et al., 2017](#)) to show that pupil responses differed in a systematic way as a function of capacity and processing load. Having demonstrated proof of concept, it will be necessary to implement idiographic approaches for meaningful future comparison of individuals across the full range of span capacities in which cognitive loads are tailored to each individual's capacity (e.g., defining high load as 2 digits above each individual's maximum span). Finally, we chose digit span, in part, due to practical constraints of the pupillometry device. However, we have successfully piloted pupil response on a new device with which we can assess episodic memory. Thus, proof of concept demonstrated here will be fully applicable to future studies using idiographic approaches with more AD-relevant episodic memory tests.

Here we acknowledge some limitations. Although this was a community-based sample, it was all male and largely white, non-Hispanic. All had past military service, but the large majority was non-combat-related. Generalization to women or racial/ethnic minorities remains to be determined. We would expect pupillary dilation responses to be heritable at younger ages, given that studies of younger individuals have shown substantial variability of pupillary responses and inverse associations between pupillary dilation and cognitive capacity ([Ahern and Beatty, 1979](#); [Beatty, 1982](#); [Granholt et al., 1996](#); [Kahneman and Beatty, 1966](#); [van der Meer et al., 2010](#)). However, we do not know the extent to which pupillary responses at younger ages may be influenced by the same or different genes. It is possible, for example, that more AD risk genes are associated with pupillary responses during midlife and later life compared with earlier life. We also do not know if the highest cognitive load would best predict risk in other age groups. However, if one's interest is in biomarkers of early risk for cognitive decline or AD, it is middle-aged adults that may be most appropriate. It will be of interest to determine how AD biomarkers (currently being assessed in this sample) are related to pupillary responses, and if pupillary responses might in some cases detect risk before currently defined A and tau thresholds are reached.

## 5. Conclusion

Pupillary dilation responses are largely driven by the LC-NE system (Samuels and Szabadi, 2008; Wilhelm et al., 1999), an important modulator of cognitive function (Aston-Jones and Cohen, 2005; Sara, 2009). The LC is also an early site of tau deposition. This led to our previous work comparing CN and MCI groups, which supports pupillary response as a potential psychophysiological biomarker of risk for MCI and AD (Granholm et al., 2017). Here we showed that pupillary dilation responses are associated with AD risk genes. Given evidence linking pupillary responses, LC, and tau, the association between the AD-PRS and pupillary response provides additional evidence that is consistent with pupillary responses as a genetically mediated MCI/AD biomarker. Although the utility of pupillary responses recorded during cognitive tasks remains to be determined, the results provide proof of concept that this brief, low-cost, low-invasive test may, in combination with other measures, aid in first-line screening to identify adults at increased genetic risk for AD while they are still cognitively normal. Identifying the specific genes associated with the pupillary response factors may improve understanding of the functioning of the LC-NE system and of genetically mediated factors affecting risk for MCI and AD.

## Disclosure

Dr Dale is a Founder of and holds equity in CorTechs Labs, Inc and serves on its Scientific Advisory Board. He is a member of the Scientific Advisory Board of Human Longevity, Inc and receives funding through research agreements with General Electric Healthcare and Medtronic, Inc. The terms of these arrangements have been reviewed and approved by UCSD in accordance with its conflict of interest policies. The remaining authors have no declarations of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neurobiolaging.2019.09.001>.

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