Development of thalamus mediates paternal age effect on offspring reading: A preliminary investigation

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Abstract

The importance of (inherited) genetic impact in reading development is well-established. *De novo* mutation is another important contributor that is recently gathering interest as a major liability of neurodevelopmental disorders, but has been neglected in reading research to date. Paternal age at childbirth (PatAGE) is known as the most prominent risk factor for *de novo* mutation, which has been shown repeatedly by molecular genetic studies. As one of the first effort, we performed a preliminary investigation of the relationship between PatAGE, offspring's reading, and brain structure in a longitudinal neuroimaging study following 51 children from kindergarten through third grade. The results showed that greater PatAGE was associated significantly with worse reading, explaining an additional 9.5% of the variations after controlling for a number of confounds — including familial factors and cognitive-linguistic reading precursors. Moreover, this effect was mediated by volumetric maturation of the left posterior thalamus from ages 5 to 8. Complementary analyses indicated the PatAGE-related thalamic region was most likely located in the pulvinar nuclei and related to the dorsal attention network, by using offspring's diffusion MRI data, brain atlases, and public datasets. Altogether, these findings provide novel insights into neurocognitive mechanisms underlying the PatAGE effect on reading acquisition during its earliest phase and suggest promising areas of future research.

Keywords

Paternal age, dorsal attention network, longitudinal design, pulvinar nuclei, reading, dyslexia

Highlights

- Paternal age at childbirth (PatAGE) is negatively correlated with reading in offspring.
- PatAGE is related to volumetric maturation of the thalamus.
- Brain maturation mediates the PatAGE effect on reading.
- PatAGE-related thalamic area is connected to the dorsal attention network.

Abbreviations

ARHQ, Adult Reading History Questionnaire; DAN, dorsal attention network; DNA, deoxyribonucleic acid; FDR, false discovery rate; FWE, family wise error; MatAGE, maternal age at childbirth; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; PA, phonological awareness; PatAGE, paternal age at childbirth; pIQ, performance intelligence quotient; RAN, rapid automatic naming; RD, reading disorder; READ, reading composite score; ROI, region-of-interest; RSFC, resting-state functional connectivity; SES, socioeconomical status; *t*1, time-point 1; *t*2, time-point 2; TIV, total intracranial volume; V5/MT, middle temporal visual area; VAN, ventral attention network; VBM, voxel-based morphometry

Introduction

There has been a global trend of postponed childbearing, especially in developed countries (Kohler, Billari, & Ortega, 2002). This so-called "postponement transition" is primarily owing to changing patterns of education, employment and marriage (Khandwala, Zhang, Lu, & Eisenberg, 2017; Sobotka, 2010). Although the research field is still in its infancy, increasing evidence reveals that advanced paternal age at childbirth (PatAGE) increases risk for a wide range of neuropsychiatric conditions, such as schizophrenia and autism spectrum disorders (D'Onofrio et al., 2014).

In contrast to the area of mental health, few studies investigated effects of PatAGE on offspring's cognition such as reading, which is essential to success in the modern society. The pioneering study in 1978 reported a negatively skewed distribution of PatAGE in 48 boys with reading disorders (RD; a.k.a. and referred here to dyslexia) (Jayasekara & Street, 1978). Four decades later, the topic remains controversial. In a broader sample of 7-year-old children, Saha and colleagues revealed a significantly negative effect of PatAGE on offspring's reading after controlling for maternal age at childbirth (MatAGE), gestational age, sex and race (Saha et al., 2009). However, when parental education and number of siblings were added to the same dataset, the effect of PatAGE on reading was no longer significant (Edwards & Roff, 2010). Such inconsistency underlies the importance of

more research that controls for possible confounds examining the PatAGE effect on reading.

In addition to the controversy over the PatAGE effect on reading, no studies have yet examined its underlying mechanisms. Nascent research in molecular genetics however, show that PatAGE explains nearly all the variance in the amount of *de novo* mutation, which is an alteration in a gene as the result of a mutation in a germ cell (egg or sperm) that increases by cell divisions of the gametes (approximately 38-fold in males at the age of 50 compared to females) (Breuss et al., 2019; Jónsson et al., 2017; Kong et al., 2012). Hence, *de novo* mutation is the most likely molecular mechanism underlying the PatAGE effect. In a separate line of research, *de novo* mutations is known to increase risk by up to 20-fold in neurodevelopmental disorders (De Rubeis et al., 2014; Deciphering Developmental Disorders Study, 2017). Taken together, it is conceivable that *de novo* mutations are at least partially responsible for the negative effect of PatAGE on offspring's mental health, offering a plausible explanation of the PatAGE effect on children's reading abilities.

At the neurocognitive level, whether and how cognitive-linguistic factors mediate the PatAGE effect on reading development is unknown. Studies to date have focused on genetic and environmental factors that contribute to the multifactorial liability of dyslexia (Pennington, 2006; Petrill, Deater-Deckard, Thompson, DeThorne, & Schatschneider, 2006). One such example, phonological processing, is thought to exert its effect more dominantly through inherited genetic

124 impact, often estimated by family reading history (van Bergen, Bishop, van Zuijen,
125 & de Jong, 2015). Under the same framework, whether PatAGE serves as a
126 contributor to the multifactorial liability, and if so, what is the neural and cognitive
127 mediators (that would likely be heritable but not inherited traits if caused by *de*
128 *novo* mutation), have not been examined. Brain measures derived from
129 neuroimaging techniques including magnetic resonance imaging (MRI) are
130 particularly informative as they have been suggested as useful mediators between
131 genetic etiology and behavioral outcome, acting as endophenotypes (Grasby et al.,
132 2020; Shaw et al., 2012). Further, longitudinal investigation, in combination with
133 cross-sectional analysis, can provide comprehensive insights into the neural basis
134 underlying typical reading acquisition and its impairment (Clark et al., 2014;
135 Yeatman, Dougherty, Ben-Shachar, & Wandell, 2012).

136 Therefore, we conducted a preliminary study examining behavioral and
137 multimodal neuroimaging data both cross-sectionally and longitudinally in a cohort
138 of 51 children followed from kindergarten through third grade in conjunction with
139 analyses of publicly available datasets. The aim of the study was threefold: (1) to
140 examine the relationship between PatAGE and offspring's reading while
141 systematically controlling for potential contributing/confounding factors; (2) to
142 examine the role of previously known cognitive-linguistic precursors,
143 neuroanatomy, and its maturational process in the relation to PatAGE and reading;
144 and (3) to understand the functional role of the neuroanatomical findings in this

study by identifying convergent evidence through the use of brain atlases, public datasets and our multimodal imaging data.

Materials and Methods

Participants

Participants in this study were drawn from a longitudinal NIH-funded project (K23HD054720) focusing on children's reading development and followed from kindergarten (time-point 1 [*t*1], mean age = 5.58 years, *SD* = 0.43) to third grade (time-point 2 [*t*2], mean age = 8.30 years, *SD* = 0.46). All children were healthy native English speakers without neurological/psychiatric disorders (e.g., attention deficit/hyperactivity disorder) or contraindications to MRI based on parental report. Among the participating children, 76% were White, 6% were Asian, and 18% were of multiradical heritage. In addition, 8% identified as Hispanic or Latino. Based on the household annual income, parental educational levels and occupation, the participants in this study was of relatively high socioeconomic status (also see Black et al., 2012). The initial sample consisted of 51 children recruited from local newspapers, school mailings, flyers, and mothers' clubs. In the behavior analyses, eight children were excluded because of attrition (n = 5), no record of PatAGE (n = 1), or being siblings (n = 2). In the latter case, the child with poorer T1 image quality was excluded. The final sample for behavioral analyses included 43 unrelated children (17 girls). In the neuroanatomical analysis, another 7 children (2 girls) were excluded because of incomplete T1 data collection or poor

image quality at either time-point. In the diffusion imaging analysis, a sub-group of 23 children (8 girls) with the same acquisition sequence were included. The differences in either familial or behavioral measures between the entire cohort and sub-groups were non-significant (all p's > 0.1). The Institutional Review Boards of Stanford University where data were collected and principal investigator was at the time of the study, and the University of California San Francisco where data were analyzed due to transition of the principal investigator, approved the present study. Both informed assent and consent were obtained from children and their guardians.

Behavioral measurements

Demographics, family information and behavioral measures are summarized in Table 1. Family information collected at $t1$ include: PatAGE; MatAGE; Adult Reading History Questionnaire from both parents (PatARHQ, MatARHQ) that were used to estimate familial history of reading difficulty (Lefly & Pennington, 2000); numbers of older and younger siblings; parental education (PatEDU, MatEDU); socioeconomic status (SES), a composite index computed from family annual income, parental education and occupation with principal component analysis (Noble, Wolmetz, Ochs, Farah, & McCandliss, 2006); Home Observation Measurement of the Environment (HOME), an index for home environment including home literacy environment (Segers, Damhuis, van de Sande, & Verhoeven, 2016). A battery of behavioral tests measuring intelligence, language and reading related skills was administrated. Verbal Comprehension, Concept Formation, and Visual Matching sub-tests of the Woodcock-Johnson III Tests of

188 Cognitive Abilities (McGrew & Schrank, 2007) were used to estimate general
189 cognitive abilities. These tests have reliabilities of at least 0.80 and have been used
190 as a proxy for intelligence quotient (IQ) (Shaw, 2010). Vocabulary was measured
191 with Peabody Picture Vocabulary Test (4th edition) (Dunn & Dunn, 2007). Blending,
192 Elision, Memory for Digit, Nonword Repetition sub-tests from the Comprehensive
193 Test of Phonological Processing (CTOPP 1st Edition) (Wagner, Torgesen, &
194 Rashotte, 1999) were used to measure phonological skills. Rapid Automatized
195 Naming (RAN; Objects and Colors sub-tests) (Wolf & Denkla, 2005) and Letter
196 Identification sub-test of Woodcock Reading Mastery Test R/NU (WRMT-R/NU)
197 (Mather, 1998) were also administered.

198 The same set of tests were used at *t*2 (Table 1). Numbers and Letters sub-
199 tests of RAN were further included to measure print-sound mapping efficiency.
200 Additionally, we administered tests measuring different aspects of reading,
201 including Sight Word Efficiency and Phonemic Decoding Efficiency sub-tests from
202 the Test of Word Reading Efficiency (TOWRE 1st Edition) (Torgesen, Wagner, &
203 Rashotte, 1999), Word Identification, Word Attack, and Passage Comprehension
204 sub-tests from WRMT-R/NU, and Reading Fluency and Spelling sub-tests from WJ-
205 III Tests of Achievement.

206 **Image acquisition**

207 High-resolution T1-weighted images were collected at both time-points with
208 the following parameters: 128 slices; thickness = 1.2 mm; NEX = 1; repetition time =

8.5 ms; echo time = 3.4 ms; inversion time = 400 ms; in-plane resolution = 256 × 256; voxel size = 0.9 × 0.9 × 1.2 mm^3; flip angle = 15 °; field of view = 22 cm. High-angular resolution diffusion-imaging data were collected at *t*2 with the following parameters: 46 axial slices; slice thickness = 3 mm; repetition time = 5000 ms; echo time = 81.7 ms; in-plane resolution = 128 × 128; voxel size = 2.0 × 2.0 × 3.0 mm^3; 150 directions with b = 2500 s/mm^2; 6 volumes with b = 0 s/mm^2. All images were acquired using a GE Healthcare 3.0 T 750 scanner with eight-channel phased-array head coil at Richard M. Lucas Center for Imaging at Stanford University. The quality of images was qualitatively evaluated by an investigator who was blinded to the behavioral and demographic information prior to any analyses.

Behavior analyses

To reduce dimensionality of behavioral metrics, factor analyses were conducted on reading-related tests for each time-point; *t*1: Blending, Elision, Memory for Digits, Nonword Repetition sub-tests of CTOPP, Objects and Colors sub-tests of RAN, Letter Identification sub-test of WRMT; *t*2: Blending, Elision, Memory for Digits, Nonword Repetition sub-tests of CTOPP, Numbers, Letters, Objects and Colors sub-tests of RAN, Sight Word Efficiency and Phonemic Decoding Efficiency sub-tests of TOWRE, Word Identification, Word Attack, Passage Comprehension sub-tests of WRMT-R/NU, Reading Fluency and Spelling sub-tests of WJ-III Tests of Achievement. Maximum Likelihood, Varimax, and Bartlett methods were used for extraction, rotation, and factor score calculation, respectively. Criteria of eigenvalue greater than 1 was used to identify factors.

From *t*1 behavioral metrics, we obtained two factors, explaining 53.8% of the total variance. Since phonological awareness (PA) and RAN loaded heavily on each factor, we named these factors as *t*1PA and *t*1RAN (Fig. 1A). Given PA and RAN are the most reliable predictors for reading development in alphabetic languages (Caravolas et al., 2012), we used these two composite scores as cognitive-linguistic precursors of reading in subsequent analyses. Using the same approach, we extracted three factors from *t*2 behavioral metrics, explaining 67.2% of the total variance. The scores were labeled as *t*2READ, *t*2PA, and *t*2RAN according to the corresponding factor loading (Fig. 1B).

Since a consensus on the definition of advanced paternal age remained lacking (Couture, Delisle, Mercier, & Pennings, 2020), in this study we treated PatAGE as a continuous variable rather than splitting children into different groups. To examine the relationship between PatAGE and reading, we first calculated zero-order correlation. Once the correlation was significant, hierarchical linear regressions were conducted to answer four questions in the following order: (1) whether the PatAGE effect on reading remains significant after controlling for demographic variables; (2) whether the PatAGE effect on reading exists after regressing out MatAGE, which is known to correlate highly with PatAGE and is a possible confound; (3) whether the PatAGE effect on reading is present above and beyond familial risk (representing inherited risk; Swagerman et al., 2017), and environmental factors to address the issue of multifactorial liability; (4) whether the

PatAGE effect on reading is explained by *t*1 cognitive-linguistic precursors of reading to examine whether the most common predictors were the mediating factor.

Specifically, in the first model we entered *t*2 age, sex, handedness and average performance IQ (pIQ) across *t*1 and *t*2 in the first step and PatAGE in the second step (Table 2). In the second model, besides the aforementioned nuisance variables, we regressed out MatAGE, which was correlated with both PatAGE and *t*2READ. In the third model, familial risk measured by ARHQ of both parents (van Bergen et al., 2015), and environmental factors including educational level of both parents (Edwards & Roff, 2010), number of older and younger siblings (Price, 2008), SES (Pan et al., 2016), HOME (Segers et al., 2016), which are known to be associated with reading were additionally controlled. In the final model, *t*1PA and *t*1RAN were entered in the fourth step, just before PatAGE, to examine whether the PatAGE effect was present beyond *t*1 cognitive-linguistic skills. Since *t*1RAN and *t*1PA did not correlate with PatAGE, these factors were not examined further in the mediation analyses. All statistics were done with SPSS 24.0 (IBM, Inc.), and *p*-values were two-tailed while statistical significance was set at 0.05.

Structural image preprocessing

Both cross-sectional and longitudinal analyses were conducted by using voxel-based morphometry toolbox (v435; http://www.neuro.uni-jena.de/vbm/) with SPM8 (v4667; http://www.fil.ion.ucl.ac.uk/spm8/) implemented in Matlab R2014a. In the cross-sectional data preprocessing for *t*1 and *t*2, individual T1 volumes were

segmented into gray matter, white matter and cerebrospinal fluid with a resampling at 1.5 mm^3. Then, the gray matter segments were registered to a T1 template in Montreal Neurological Institute (MNI) space by using both affine normalization and Diffeomorphic Anatomical Registration Through Expoentiated Lie Algebra (Ashburner, 2007), and subsequently modulated by the 'affine and non-linear' modulation (http://www.neuro.uni-jena.de/vbm/segmentation/modulation/). The modulated images containing regional tissue volume of gray matter for each voxel were smoothed with an 8-mm full-with half-maximum isotropic Gaussian kernel. Voxels with gray matter values < 0.1 were excluded to avoid edge effects.

In the longitudinal analysis, 'Preprocessing of Longitudinal Data' module that contains specific preprocessing steps for processing longitudinal structural MRI data was used. Intra-subject realignment, bias correction, segmentation, and normalization were conducted sequentially as described elsewhere (Ridgway et al., 2007). After applying spatial smoothing with an 8-mm full-width half-maximum Gaussian kernel, we obtained maps of gray matter volume for both time-points. We generated GMV maps reflecting change from *t*1 to *t*2 for further analyses (such that a positive value indicates enlarging from *t*1 to *t*2).

Whole-brain analyses

Prior to voxel-wise analyses, we examined relationships between PatAGE and global measurement (i.e., the total intracranial volume that defined as the sum of total gray matter, white matter and cerebrospinal fluid) at each time-point (*t*1TIV

294 and $t2$TIV). Then, we examined whether PatAGE correlated with the change of TIV
295 (ΔTIV) from $t1$ to $t2$ while controlling for the baseline measure ($t1$TIV). To explore
296 relationships between regional gray matter volume (GMV) at each time-point (i.e.,
297 cross-sectional analyses), as well as the change of regional GMV (ΔGMV) across
298 time-points ($t2$GMV-$t1$GMV) with PatAGE (i.e., longitudinal analysis), voxel-wise
299 whole brain regressions were conducted while controlling for global measurements.
300 Specifically, $t1$TIV or $t2$TIV was controlled in cross-sectional analyses for $t1$ and $t2$,
301 respectively. In the longitudinal analysis, $t1$TIV and ΔTIV were controlled to
302 exclude the effects from initial gross volume and its development. Since correlations
303 between $t1$TIV, ΔTIV, and PatAGE were not significant (all p's > 0.1), the models
304 were free from multicollinearity. Topological Family Wise Error (FWE) correction
305 was used to determine the corrected thresholds of statistical significance. All
306 clusters significant at a threshold of corrected $p < 0.05$ corrected for the whole brain
307 (p-voxel < 0.005 for height) were reported in MNI space. Since no significant
308 clusters were found for cross-sectional analyses at either time-point, the subsequent
309 analyses focused on longitudinal data. In particular, region-of-interest (ROI)
310 analyses were conducted in the significant clusters to examine the robustness and
311 specificity of the effect. For this, values of each voxel in the cluster were extracted
312 and averaged, then included in hierarchical multiple regression analyses as the
313 dependent variable. Demographic variables ($t1$ age, time interval between $t1$ and $t2$,
314 sex, handedness, average pIQ across $t1$ and $t2$), $t1$TIV and ΔTIV were entered in the
315 first step, while PatAGE was entered in the second step. Then, we further

controlled for MatAGE and MatARHQ since they showed significant correlation with PatAGE as in previous analyses of this study.

Next, we examined the relationship between ΔGMV and children's *t*2READ in the cluster that was significantly associated with PatAGE (hereafter PatAGE-cluster) by using small volume correction (p-voxel < 0.005, p-cluster < 0.05, topological FWE correction) while *t*1TIV and ΔTIV were statistically controlled. Average ΔGMV of the significant cluster was extracted for subsequent ROI analyses. Hierarchical multiple regression analyses were conducted to test for the robustness and specificity of the effect. In the first model, *t*2READ was the dependent variable and demographic variables (*t*2 age, sex, handedness, average pIQ across *t*1 and *t*2), *t*1TIV and ΔTIV were entered in the first step. In the second model, *t*1PA and *t*1RAN were additionally entered in the second step since they were significantly associated with *t*2READ in previous analysis of this study. The average ΔGMV was entered in the final step.

Mediation

In the region where the volumetric change significantly correlated with PatAGE and *t*2READ, we examined whether the negative PatAGE effect on reading was mediated by brain maturation. We first examined a model without controlling for covariates. Bootstrapping (10,000 samples) was used to obtain 95% confidence intervals of the indirect effect. If a significant indirect effect was observed, we further adjusted the model for demographic variables (*t*2 age, sex, handedness,

average pIQ across *t*1 and *t*2), *t*1TIV, ΔTIV, and *t*1 cognitive-linguistic precursors (*t*1RAN and *t*1PA; i.e., significant predictors on *t*2READ in addition to PatAGE as shown in the regression analysis) to confirm the uniqueness and robustness of the effect.

Existing evidence suggests that dyslexia is largely genetically transmitted from parent (often assayed by parental-report of reading difficulty) to offspring (Soden et al., 2015; Swagerman et al., 2017). Further, Twin studies find a dissociation between sources of variance in phonological and orthographic processes, with variance in phonological skills being primarily genetic compared to orthographic skills (Olson, Wise, Conners, Rack, & Fulker, 1989; Olson et al., 2011). These findings are consistent with the idea that PA mediates the negative effect of parental reading difficulty (a proxy for inherited genetic transmission) on reading in offspring (van Bergen et al., 2015). In line with the previous literature, we observed significant correlations between MatARHQ and *t*1PA, MatARHQ and *t*2READ, *t*1PA and *t*2READ. We therefore examined the role of poor PA on the relationship between history of maternal reading difficulty and lower reading performance in offspring. Age at *t*2, sex, handedness, average pIQ across *t*1 and *t*2, and *t*1RAN were controlled statistically.

If both a PatAGE effect (a proxy for non-inherited genetic risk) and MatARHQ effect via PA processing (a proxy for inherited genetic risk) are to be observed in the current sample, the result will provide support of the multifactorial liability model.

PROCESS procedure (release 2.16.1) implemented in SPSS was used to conduct these mediation analyses (Hayes, 2013).

Complementary analyses

We adopted multiple complementary analytical approaches to depict fine-grained spatial localization and connectivity patterns of the PatAGE-cluster, capitalizing on the fact that these have been shown to inform possible functional roles of a particular brain region (in this case, the left posterior thalamus) in the absence of a comprehensive set of cognitive measures. First, we spatially localized the PatAGE-cluster with two brain atlases. (1) Since the thalamus consists of multiple nuclei that have different functions, we calculated the percentage of overlapped voxels between the PatAGE-cluster and each thalamic nucleus from the Morel Atlas, a histological atlas that is optimal for thalamic targets in MNI space (Jakab, Blanc, Berényi, & Székely, 2012; Krauth et al., 2010); (2) Since the structural connectivity provides information about the function of a given brain region (Barron, Eickhoff, Clos, & Fox, 2015), we used Oxford Thalamic Connectivity Probability Atlas with the atlasquery tool implemented in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) to obtain the probability that the PatAGE-cluster is structurally connected to different cortical areas (de Moura et al., 2016).

To further identify the functional role of the PatAGE-cluster and complementary to the results from analyses using the histological and connectivity

probability atlases, we examined PatAGE-cluster-associated cortical patterns by using an online database, Neurosynth v0.5 (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011). In particular, we generated a co-activation map by including all fMRI studies in the database (N > 10,900), with the PatAGE-cluster as ROI. False Discovery Rate (FDR) corrected $q < 0.01$ was used as the threshold to obtain significant regions reported in fMRI studies when the PatAGE-cluster is also reported (i.e., forward inference). In addition, we generated a map of whole-brain resting-state functional connectivity (RSFC) by using the 1,000 Functional Connectome dataset (Biswal et al., 2010). The center of gravity of the PatAGE-cluster (MNI: x = -19, y = -28, z = 6) was used as the seed, and its connectivity to the rest of the brain was calculated. The resultant brain map was thresholded with a liberal cutoff value of $r = 0.01$, same as that in the previous literature (Yang, Rosenblau, Keifer, & Pelphrey, 2015). To be conservative, we only considered the overlapping regions between the co-activation map and the RSFC map. Dice coefficients between the conjunction map and the 7 large-scale intrinsic connectivity networks (i.e., visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default networks) from Yeo et al. (2011) were then calculated to examine which functional network most overlapped with the PatAGE-cluster-associated cortical pattern.

In the final step, we analyzed white matter connectivity, where fibers passing the PatAGE-cluster were reconstructed using deterministic tractography. Diffusion-weighted imaging preprocessing was performed by using ExploreDTI (Leemans,

Jeurissen, Sijbers, & Jones, 2009). Next, to visually inspect for possible artefacts, rigorous motion correction with CATNAP and eddy current correction were conducted by using the required reorientation of the b-matrix (Leemans & Jones, 2009). The diffusion tensors were calculated using a non-linear regression procedure (Pierpaoli & Basser, 1996). The individual datasets were non-rigidly normalized to MNI space. Next, whole brain tractography was performed for each individual dataset using a deterministic approach (Basser, Pajevic, Pierpaoli, Duda, & Aldroubi, 2000). Fibers (streamlines) were reconstructed by defining seed points distributed uniformly throughout the data at $2.0 \times 2.0 \times 2.0$ mm^3 resolution, following the main direction with step size set at 1.0 mm. Fiber tracking was discontinued when the fiber entered a voxel with fractional anisotropy < 0.2 or made a high angular turn (angle $> 40°$), or when the fiber was outside the fiber length range of 50-500 mm. Two analyses were then conducted: (1) To localize fibers and get a general view, the cluster related to PatAGE was used as ROI and all fibers passing through this cluster were delineated. The delineated fibers and its projection points were visually inspected, after which individual maps were binarized and summed to acquire probabilistic map across participants. (2) To complement the Neurosynth analysis above and to identify the functional network most relevant to the PatAGE-cluster, the numbers of streamlines passing through the PatAGE-cluster and each of the 7 functional networks (Yeo et al., 2011) were calculated and normalized by dividing this number by size of the target network. The results were treated as the connectivity strength and compared between

networks with one-way ANOVA (as well as post-hoc analyses). Furthermore, we examined the correlations between the connectivity strength with PatAGE and *t*2READ.

Results

PatAGE is negatively associated with offspring's reading above and beyond commonly known predictors

PatAGE (*M* = 36.12 years, *SD* = 4.91, *Range* = 25-47; Table 1; distribution presented in Fig. S1A) was positively correlated with MatAGE ($r = 0.63$, $p = 5 \times 10^{-6}$) but not with other potentially confounding demographic variables reported in the past such as SES, number of siblings and parental education (all *p*'s > 0.1; Table S1). Importantly, greater PatAGE was significantly correlated with lower reading composite scores in offspring (*t*2READ; $r = -0.39$, $p = 0.011$). Similar to PatAGE and not surprisingly, MatAGE was negatively correlated with *t*2READ ($r = -0.33$, $p = 0.031$). No significant correlations were found between PatAGE and cognitive-linguistic skills typically found to be predictors of later reading ability at either time-point (*p*'s > 0.1, for *t*1PA, *t*1RAN, *t*2PA, and *t*2RAN). In accordance with prior literature on factors that predict reading outcomes (Segers et al., 2016; Thompson et al., 2015; van Bergen et al., 2015), lower *t*2READ was predicted by poorer reading reported by mothers (MatARHQ; $r = -0.46$, $p = 0.002$), poorer home literacy environment measured by HOME ($r = 0.31$, $p = 0.047$), and poorer cognitive-

linguistic skills at time-point 1 (*t*1PA: $r = 0.46$, $p = 0.002$; *t*1RAN: $r = 0.31$; $p = 0.041$).

To examine whether the PatAGE effect on reading existed above and beyond commonly identified confounds and additional variables known to influence reading development, hierarchical linear regressions were conducted with *t*2READ as the dependent variable in a systematic and hypothesis-driven fashion. In the first model, before PatAGE was entered, confounds related to children were entered as predictors in the first step, including *t*2 age, sex, handedness and average pIQ across two time-points. The effect of PatAGE remained significant, explaining an additional 14.9% of the variance ($t = -3.12$, $p = 0.004$; Model 1 in Table 2; Fig. S1B). In the second model, MatAGE was further added in the second step, since it was significantly correlated with PatAGE and *t*2READ. As shown in Table 2 (Model 2), PatAGE explained an additional 9.7% of the variance in reading outcomes ($t = -2.48$, $p = 0.018$). Then, in the third model, familial risk (PatARHQ, MatARHQ) and environmental factors (number of siblings, parental education, SES and HOME) in relation to reading development were added. We still observed a significant PatAGE effect, explaining an additional 10.7% of the variance ($t = -2.45$, $p = 0.023$; Model 3 in Table 2). Thus far, we demonstrated that the PatAGE effect on reading was not accounted for by factors that predict child's reading outcome and known to be either inherited or environmental. In the final model, we investigated its relationship with early cognitive-linguistic predictors of reading outcomes by entering *t*1PA and *t*1RAN in the fourth step. The PatAGE effect on offspring's reading was above and

beyond that of cognitive-linguistic variables, explaining an additional 9.5% of the variance (t = -2.71, p = 0.014; Model 4 in Table 2). In accord with the prior literature, t1PA and t1RAN also significantly predicted t2READ in the final model and accounted for 13.8% of the variation (t1PA: t = 2.87, p = 0.010; t1RAN: t = 2.19, p = 0.042). That is, contributions from PatAGE and cognitive-linguistic precursors were relatively independent and jointly predicted children's reading outcomes.

PatAGE is associated with thalamic maturation

In the brain analyses, we found no significant correlations between PatAGE and TIV at t1, t2, or ΔTIV from t1 to t2 (all p's > 0.1). Second, whole brain analyses on regional GMV at each time-point did not show any significant clusters at the FWE corrected threshold of p < 0.05. Finally, we examined the PatAGE effect on regional ΔGMV while controlling for t1TIV and ΔTIV. Results revealed a significantly positive correlation between PatAGE and ΔGMV in a cluster covering the left posterior thalamus (i.e., PatAGE-cluster; p = 0.017, FWE corrected, 819 voxels, 2,764 mm^3, peak MNI coordinate [-27, -30, 6]; Fig. 2A). Specifically, greater PatAGE was associated with less GMV decrease (Fig. 2B). To verify that this effect was not due to confounds, hierarchical linear regression analyses were performed. In the first model, after regressing out nuisance variables commonly controlled in longitudinal VBM analysis (t1 age, time interval between t1 and t2, sex, handedness, average pIQ across t1 and t2, t1TIVand ΔTIV), PatAGE explained 34.6% of the ΔGMV variance of the PatAGE-cluster (t = 4.59, p < 0.001). Since MatARHQ and MatAGE were significantly correlated with PatAGE, we additionally

included them as covariates in the second model, and found PatAGE still explained 19.0% of the ΔGMV variance of the PatAGE-cluster (t = 3.28, p = 0.003).

The PatAGE effect on offspring's reading is mediated by ΔGMV in the left posterior thalamus

To examine whether ΔGMV in the PatAGE-cluster was correlated with reading, we performed voxel-wise regression analysis within the cluster while controlling for t1TIV and ΔTIV. As shown in Fig. 2A, there was a negative correlation between ΔGMV and t2READ in a sub-region of the PatAGE-cluster (p-cluster = 0.037, small volume FWE corrected, 86 voxels, 290 mm^3, peak MNI coordinate [-27, -31, 10]), indicating the less the thalamic GMV decrease between the two time-points, the poorer the reading (Fig. 2C). Hierarchical linear regression analyses were performed to verify this effect. ΔGMV of the significant cluster explained 19.7% of variance in t2READ after regressing out nuisance variables (t2 age, sex, handedness, average pIQ across t1 and t2, t1TIV and ΔTIV) in the first step (t = -3.20, p = 0.003). Since t2READ was significantly correlated with t1PA and t1RAN, we further examined whether the observed effect was above and beyond these two cognitive-linguistic skills by entering them in the second step. The results showed that ΔGMV additionally explained 19.9% of the variance in t2READ (t = -3.66, p = 0.001).

Given that ΔGMV in the left thalamic sub-region was associated with both PatAGE and t2READ, we conducted mediation analyses and observed ΔGMV

significantly mediated the PatAGE effect on offspring's reading; 95% confidence interval was [-0.522 -0.041] when age at *t*2, sex, handedness, average pIQ across *t*1 and *t*2, TIV at *t*1, ΔTIV, *t*1PA, and *t*1RAN were not controlled for, and [-0.552 -0.043] when these covariates were controlled (Fig. 2D). These results are in contrast to the commonly found results in the literature that we also find in the present study, i.e., *t*1PA mediates the negative effect of family history on offspring's reading (95% confidential interval was [-0.303 -0.022] when not controlling for age at *t*2, sex, handedness, average pIQ across *t*1 and *t*2, and *t*1RAN, and changed to [-0.249 -0.001] when all these covariates were controlled; Fig. S2).

PatAGE-cluster is localized in the pulvinar nuclei and linked to the dorsal attention network

To understand the neurostructural profile of the PatAGE-cluster in the left thalamus, we compared it with a histological atlas and a connectivity atlas. 548 out of 819 voxels in the cluster overlapped with the human thalamus of the Morel histological atlas (Jakab et al., 2012; Krauth et al., 2010), while the remaining 271 voxels were unlabeled, possibly because the cluster also contained white matter. As presented in Fig. 3A, within the overlapping region, 380 voxels (69.5%) were in the subdivision labeled as pulvinar nuclei, especially the medial portion, which is known to have widespread connections with the inferior parietal lobule (Arcaro, Pinsk, & Kastner, 2015). These results were corroborated by examining the Thalamic Connectivity Probability Atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslview/atlas.html): the PatAGE-cluster was most likely

localized in the subdivision that was connected to the posterior parietal cortex, with a probability of 38.9% (Fig. 3B).

Next, we examined functional connectivity of the PatAGE-cluster by utilizing two approaches available in Neurosynth v0.5 (Yarkoni et al., 2011). These included generation of a meta-analytic map of regions that co-activate with the PatAGE-cluster across more than 10,900 fMRI studies, as well as a RSFC map from the PatAGE-cluster to the rest of the brain by using the 1000 Functional Connectome dataset (Biswal et al., 2010). The co-activated areas included subcortical structures and cortical regions such as bilateral intraparietal sulci, inferior temporal gyrus, and frontal eye fields in the frontal cortex (Fig. S3A). The RSFC map showed similar but more widespread pattern than the co-activation map (Fig. S3B). A conjunction analysis revealed that bilateral frontal eye fields, intraparietal sulci, middle temporal visual area (V5/MT), and cerebellum were among the overlapped regions across the two approaches, in addition to subcortical structures.

Sørensen-Dice coefficients (s) between the overlapping areas and the previously identified networks deriving from resting-state functional MRI data (Yeo et al., 2011) were calculated. The derived pattern of overlapping areas showed the greatest resemblance to the dorsal attention network (DAN; $s = 0.360$; Fig. 4B) and to the ventral attention network (VAN; $s = 0.261$), much higher than its resemblance to other networks (visual network: $s = 0.086$; somatomotor network: $s = 0.071$; limbic network: $s < 0.001$; frontoparietal network: $s = 0.041$; and default network: $s = 0.005$). Together with the aforementioned findings utilizing structural

atlases, these results using large-scale fMRI databases from functional neuroimaging studies point to the attention network, in particular the DAN, to be the candidate brain functional system associated with the PatAGE-cluster in the left thalamus.

Finally, we used diffusion imaging data available in a sub-group of 23 participants to confirm DAN was more likely the candidate system associated with the PatAGE effect on reading. Using deterministic tractography, we reconstructed white matter fibers through the PatAGE-cluster, covering inferior fronto-occipital fasciculus, corticospinal tract, forceps major, superior corona radiata, as well as anterior and posterior limbs of the internal capsule. Fig. 5A shows reconstructed fibers in a representative child and Fig. 5B shows intersection across participants, for demonstrative purposes. More importantly, the PatAGE-cluster showed significantly stronger connectivity (defined as the total number of streamlines divided by the size of the target network) with DAN than with VAN (t = 6.61, p < 0.001; Fig. 5C). Finally, consistent with the aforementioned results, significant positive correlation between PatAGE-cluster-DAN streamlines and PatAGE (r = 0.49, p = 0.018; Fig. 5D), and significant negative correlation between PatAGE-cluster-DAN streamlines and t2READ (r = -0.45, p = 0.030; Fig. 5E) were observed. No significant correlations were found between PatAGE-cluster-VAN streamlines and PatAGE or t2READ (both p's > 0.1).

Discussion

In this study, we observed a significant effect of PatAGE on offspring's reading at the earliest stages of formal schooling from ages 5 to 8, independent of confounds (e.g., maternal age) and factors that play key roles in learning to read (i.e., family reading history, environmental factors, and cognitive-linguistic precursors of reading), explaining an additional 9.5% of the variance. Furthermore, we revealed volumetric maturation of the left thalamus as a potential neural endophenotype mediating this effect. With multimodal neuroimaging and public datasets, we identified that this area is most relevant to the dorsal attention network. These findings are in contrast to and complement the current literature linking phonological and orthographic processing in reading to the left temporo-parietal and occipito-temporal regions. The mediation revealed here was distinct from the mediating role of phonological processing on the relationship between reading and familial risk, which has been attributed to hereditary effects. Taken together, this study provides novel and converging evidence suggesting PatAGE as a significant factor that associated with offspring's reading, independent of phonological processing, possibly through the maturational process of the left posterior thalamus.

The PatAGE effect on offspring's reading

Jayasekara and Street (1978) for the first time reported that advanced PatAGE was associated with greater incidence of dyslexia, independent of SES and birth order. While their analysis was restricted to dyslexic boys, Saha and colleagues extended the finding to a broader population of 7-year-old children with

varying reading abilities measured using Wide Range Achievement Test (Saha et al., 2009). Negative PatAGE effects on several cognitive measures including reading were observed after controlling for confounds that included MatAGE, SES, and parental psychiatric illness. A follow-up study re-analyzed the same dataset and found that the PatAGE effect was no longer significant after further adjusting parental education and the number of siblings (Edwards & Roff, 2010). Therefore, the PatAGE effect on reading was equivocal, and the inconsistency was related to covariates controlled in the model, especially parental characteristics such as educational level.

In the present study, with the range of PatAGE restricted to 25-47 years, we found PatAGE was negatively associated with reading performance measured using variety of tests, even after additionally controlling for strong predictors of reading that were not included in previous studies (Edwards & Roff, 2010; Saha et al., 2009). These predictors included familial risk and cognitive-linguistic skills (e.g., phonological processing) that shown to be more genetically than environmentally mediated, as well as home literacy environment (Hulme, Snowling, Caravolas, & Carroll, 2005; Swagerman et al., 2017; van Bergen et al., 2015). These findings support an adverse PatAGE effect on reading and suggest such effect may occur through a mechanism different to factors such as inherited genetic and environmental risks.

While the number of studies examining the PatAGE effect on reading is too few to infer potential mechanisms, studies on other PatAGE-linked

618 neurodevelopmental disorders offer insights. One predominant explanation is that
619 advanced PatAGE exerts its effect on the risk of disorders such as autism and
620 schizophrenia through accumulated *de novo* genetic mutations and epigenetic
621 modifications (e.g., DNA methylation and repressive histone modification) in
622 paternal gametes (Deciphering Developmental Disorders Study, 2017; Girard et al.,
623 2016; Saha et al., 2009).

624 At a more macroscopic scale, understanding of the mechanisms can be
625 deepened by identifying intermediate (endo)phenotypes through behavioral and
626 neuroimaging measures such as we did in the current study. That is, advanced
627 PatAGE may impact precursors of neurodevelopmental disorders, which in turn
628 leads to higher occurrence of such disorders (Cannon, 2009). For example, the
629 likelihood of having impaired social functioning in offspring, a core symptom of
630 psychiatric disorders, increases with PatAGE (Weiser et al., 2008). While the
631 underlying mechanisms are yet to be fully understood, multifactorial liability
632 confers risk for neurodevelopmental disorders and may involve liability such as *de*
633 *novo* mutations in addition to inherited and environmental risks. Adding to prior
634 research, the current study offers insights into potential mechanisms at the
635 macroscopic level.

636 **The intermediary role of the left posterior thalamus**

637 The thalamus is an important relay center in the human brain, receiving
638 information from sensory cortices and relaying it to higher-level association cortex.

Previous studies paint a mixed picture on thalamic development: gross volume relative to its brain size is smaller (Sussman, Leung, Chakravarty, Lerch, & Taylor, 2016) or larger (Brain Development Cooperative, 2012) in older compared to younger children of ages 4 to 18, and the pulvinar compared to other thalamic nuclei show no apparent change with age (Raznahan et al., 2014). Despite controversial evidence on typical thalamic maturation, its anomalous development undoubtedly affects the maturation of other cortical and subcortical brain regions (Ball et al., 2012), which in turn could impact higher level cognitive processes that underlie typical reading acquisition. In support of this, anomalies in thalamic structure (Giraldo-Chica, Hegarty, & Schneider, 2015), activation (Diaz, Hintz, Kiebel, & von Kriegstein, 2012; Koyama, Molfese, Milham, Mencl, & Pugh, 2020), and connectivity (Müller-Axt, Anwander, & von Kriegstein, 2017; Paz-Alonso et al., 2018; Tschentscher, Ruisinger, Blank, Diaz, & von Kriegstein, 2018) are associated with dyslexia. While most of these studies adopt a cross-sectional design with adult participants, here we conducted a longitudinal investigation and found volumetric change in the posterior thalamus from ages 5 to 8 was significantly associated with PatAGE; children with younger fathers showed GMV decrease, whereas those with older fathers showed less decrease or an increase. This pattern suggests that PatAGE is associated with the development of this subcortical structure. Noteworthy is that although no PatAGE effect were observed when examining a single time-point (cross-sectional), it does not indicate this effect cannot manifest at a specific age (i.e., this may be due to a particular developmental stage examined).

Rather, it underscores the importance of considering developmental dynamics when examining brain-behavior relationships. A similar pattern has been revealed in white matter development in dyslexia (Yeatman et al., 2012).

Moreover, examination of the cluster location with the Morel Atlas suggested the foci in the left pulvinar, an integral region supporting visuo-spatial attention (Amso & Scerif, 2015; Fischer & Whitney, 2012), and attentional control (Barron et al., 2015; Xuan et al., 2016). Analysis with connectivity-based thalamic atlas showed that this region was most likely to overlap with the subdivision connected with posterior parietal areas. Furthermore, RSFC and co-activation maps produced by Neurosynth revealed connectivity patterns were suggestive of the attention networks, especially the DAN. The diffusion imaging-based connectivity were also suggestive of the DAN. At the behavioral level, studies have repeatedly demonstrated the relationship between visuo-spatial attention and reading (Facoetti, Franceschini, & Gori, 2019; Vidyasagar & Pammer, 2010). For example, selective visual attention is associated with acquisition of orthographic knowledge (Stevens & Bavelier, 2012) and decoding skill (Matthews & Martin, 2015). Besides, both dyslexic adults and children manifest visuo-spatial attention deficits such as lower visuo-spatial span capacities, and slower response in a visuo-spatial attention-orienting task (Bosse, Tainturier, & Valdois, 2007; Buchholz & Davies, 2008). Longitudinal research also demonstrated impaired visuo-spatial processing in pre-reading kindergarteners as an important risk factor for developing RD (Gori & Facoetti, 2015). Together, these findings indicate that maturation of the pulvinar

683 and brain networks underlying visuo-spatial attention are parsimonious
684 neurocognitive mechanisms impacted by advanced PatAGE, impeding on reading
685 acquisition.

686 To date, research investigating the PatAGE effect on brain networks and
687 corresponding cognitive processes is scarce. As the first step, Shaw et al. (2012)
688 revealed PatAGE effects on cortical morphometry. However, the authors did not
689 examine the relationship with cognitive functions, making the study somewhat
690 inconclusive as to the role of PatAGE on neurocognitive processes. Taking one step
691 further, the current study revealed thalamic maturation as an intermediary
692 between PatAGE and reading – a specific behavioral phenotype, offering insights
693 into the complex mechanisms underlying PatAGE effects.

694 **Limitations and future directions**

695 In the present study, we observed a negative PatAGE effect on offspring's
696 reading and provided possible brain intermediator. Given the preliminary nature of
697 this investigation and the small sample size, the findings should be interpreted with
698 caution until they are replicated in large samples. Second, PatAGE in this study
699 was restricted to 25-47 years, with which we observed a negative linear
700 relationship. On the other hand, the findings do not necessarily extend to children
701 with extreme young and old fathers. For example, Saha et al. (2009) observed a non-
702 linear relationship with a range of PatAGE between 14 to 66 years, while the
703 correlation appears to be linear in the age range as ours. Of relevance, young

704 fatherhood is also associated with adverse outcomes in offspring but possibly due to
705 other factors including immature sperm and economic disadvantages (Chen et al.,
706 2008). Third, the complementary analyses implied DAN as the candidate functional
707 system associated with PatAGE. But it should be noted that the brain atlases and
708 public datasets implemented in Neurosynth are primarily from research on adults.
709 Since pediatric samples may have specific characteristics in terms of brain
710 organization, the neurostructural profile and function of the PatAGE-cluster need to
711 be re-visited as pediatric-specific atlases and tools become available. Fourth, while
712 we found that the left posterior thalamus mediated the PatAGE effect on reading, it
713 remains unknown why this subcortical structure is susceptible to advanced PatAGE
714 (and related to *de novo* mutations). Given that typical thalamus maturation is also
715 affected by prenatal and postnatal factors such as preterm birth (Ball et al., 2012),
716 questions including how PatAGE influences maturation of the thalamus and
717 relevant functional systems, together with other factors, require further
718 elaboration.

719 To advance understanding of the PatAGE effect on reading, future studies
720 are warranted in which a more comprehensive battery of behavioral tests (e.g.,
721 measuring visuo-spatial attention, executive function, etc.), neural measures (e.g.,
722 task-driven activation), and molecular approaches measuring the number and
723 origins of *de novo* mutations (e.g., trio-based whole-genome/exome sequencing; Jin
724 et al., 2017) are included. Fusing such neural, cognitive and molecular genetic
725 approaches at multiple levels will provide the much-needed vertical and multi-level

explanatory models that will further our understanding of risk factors associated with poor reading. In particular, future research aiming at disentangling different sources of genetic variations associated with reading development and their interplays will greatly further our understanding. In addition, advanced research designs such as intergenerational neuroimaging approach can be adopted to gain in-depth knowledge on how multiple factors including PatAGE affect the development of offspring's reading and the corresponding networks interactively from preliteracy to mature stages of reading (Ho, Sanders, Gotlib, & Hoeft, 2016; Hoeft & Hancock, 2017).

Conclusion

The current study examined the PatAGE effect on offspring's reading at both behavioral and neurobiological levels. The results provide initial evidence that advanced PatAGE is an independent factor associated with poor reading in beginning readers, above and beyond previously identified familial and cognitive-linguistic precursors. This effect was mediated by maturation of the posterior thalamus, suggesting a neurobiological pathway to intergenerational influence on reading acquisition, complementing prior findings that offspring's reading is influenced by parental reading via offspring's phonological skills (van Bergen et al., 2015). Based on these evidences we argue that PatAGE should be regarded as an important factor influencing literacy development, and included in a cumulative risk (and protection) model (Hayiou-Thomas, Smith-Woolley, & Dale, 2020;

Menghini et al., 2010; Pennington, 2006; van Bergen, van der Leij, & de Jong, 2014).

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Table Legend

Table 1 Demographic profiles, familial variables and performance on reading-related tests (n = 43). *Acronyms: ARHQ, Adult Reading History Questionnaire; CS, composite score; CTOPP BW, Comprehensive Test of Phonological Processing, Blending sub-test; CTOPP EL, Comprehensive Test of Phonological Processing, Elision sub-test; CTOPP MD, Comprehensive Test of Phonological Processing, Memory for Digit sub-test; CTOPP NR, Comprehensive Test of Phonological Processing, Nonword Repetition sub-test; HOME, Home Observation Measurement of the Environment; Mat, maternal; Pat, paternal; PPVT, Peabody Picture Vocabulary Test; RAN COL, Rapid Naming, Colors sub-test; RAN LTR, Rapid Naming, Letters sub-test; RAN NUM, Rapid Naming, Numbers sub-test; RAN OBJ, Rapid Naming, Objects sub-test; RS, raw score; SES, socioeconomic status; SS, standard score; TOWRE PDE, Test of Word Reading, Phonemic Decoding Efficiency sub-test; TOWRE SWE, Test of Word Reading, Sight Word Efficiency sub-test; WJA RF, Woodcock-Johnson III Tests of Achievement, Reading Fluency sub-test; WJA SP, Woodcock-Johnson III Tests of Achievement, Spelling sub-test; WJC CF, Woodcock-Johnson III Tests of Cognitive Abilities, Concept Formation sub-test; WJC NR, Woodcock-Johnson III Tests of Cognitive Abilities, Numbers Reversed sub-test; WJC VC, Woodcock-Johnson III Tests of Cognitive Abilities, Verbal Comprehension sub-test; WJC VM, Woodcock-Johnson III Tests of Cognitive Abilities, Visual Matching sub-test; WRMT LID, Woodcock Reading Mastery Test, Letter Identification sub-test; WRMT PC, Woodcock Reading Mastery Test, Passage Comprehension sub-test;*

WRMT WA, Woodcock Reading Mastery Test, Word Attack sub-test; WRMT WID, Woodcock Reading Mastery Test, Word Identification sub-test.

Table 2 Results of multiple linear regression analyses examining the unique contribution of paternal age on offspring's reading at time-point 2. *Acronyms: ARHQ, Adult Reading History Questionnaire; EDU, educational level; HOME, Home Observation Measurement of the Environment; Mat, maternal; PA, phonological awareness; Pat, paternal; pIQ, performance intelligence quotient; RAN, rapid naming; SES, socioeconomic status; t1, time-point 1; t2, time-point 2.*

Figure Legends

Fig. 1 Principal components extracted from reading-related tests. **A**. Component loadings for each factor at time-point 1. **B**. Component loadings for each factor at time-point 2. *Acronyms: CTOPP BW, Comprehensive Test of Phonological Processing, Blending sub-test; CTOPP EL, Comprehensive Test of Phonological Processing, Elision sub-test; CTOPP MD, Comprehensive Test of Phonological Processing, Memory for Digit sub-test; CTOPP NR, Comprehensive Test of Phonological Processing, Nonword Repetition sub-test; RAN COL, Rapid Naming, Colors sub-test; RAN LTR, Rapid Naming, Letters sub-test; RAN NUM, Rapid Naming, Numbers sub-test; RAN OBJ, Rapid Naming, Objects sub-test; t1, time-point 1; t2, time-point 2; TOWRE PDE, Test of Word Reading, Phonemic Decoding Efficiency sub-test; TOWRE SWE, Test of Word Reading, Sight Word Efficiency sub-test; WJA RF, Woodcock-Johnson III Tests of Achievement, Reading Fluency sub-test; WJA SP, Woodcock-Johnson III Tests of Achievement, Spelling sub-test; WRMT LID, Woodcock Reading Mastery Test, Letter Identification sub-test; WRMT PC, Woodcock Reading Mastery Test, Passage Comprehension sub-test; WRMT WA, Woodcock Reading Mastery Test, Word Attack sub-test; WRMT WID, Woodcock Reading Mastery Test, Word Identification sub-test.*

Fig. 2 Results of whole-brain longitudinal voxel-based morphometry and mediation analysis. **A.** Brain regions significantly correlated with paternal age (yellow) and composite score of reading at time-point 2 (cyan). **B**. Scatter plot of the relationship

between gray matter volume change in the yellow cluster and paternal age. The linear regression line is presented. **C**. Scatter plot of the relationship between gray matter volume change in the cyan cluster and reading composite score at time-point 2. The linear regression line is presented. **D.** The effect of paternal age on offspring's reading is mediated by gray matter volume change in the thalamus. Confounds were controlled statistically. The bias corrected 95% confidence interval for indirect effect was [-0.052 -0.043], indicating a significant mediation relationship.

Fig. 3 Localization and structural connectivity pattern of the PatAGE-cluster (i.e., the left posterior thalamus) according to the Morel and Thalamic Connectivity atlases. **A.** Bar plot displaying the percentage of total voxels in the PatAGE-cluster overlaps with divisions of the Morel atlas. **B.** Bar plot showing the probability of the cluster belonging to different subdivisions of the Thalamic connectivity atlas, calculated by 'autoaq' function implemented in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). *Acronyms: CL, central lateral nucleus; CM, central median nucleus; LP, lateral posterior nucleus; VLpv, ventral lateral posterior nucleus, ventral; VPI, ventral posterior inferior nucleus; VPL, ventral posterior lateral nucleus; VPM, ventral posterior medial nucleus.*

Fig. 4 Functional co-activation and connectivity of the PatAGE-cluster produced by Neurosynth. **A.** Brain map representing overlapping regions between co-activation and resting-state functional connectivity maps of the PatAGE-cluster. **B.** Bar plot showing the degree of overlap between the overlapping areas and Yeo's 7 functional

networks represented by Dice coefficients. Dice coefficient measures the similarity between the overlapping areas and a given function network, ranging from 0 to 1. While 0 indicates the two networks are disjoint, 1 indicates the two networks are identical. *Acronyms: LH, left hemisphere; RH, right hemisphere.*

Fig. 5 White matter tractography of the PatAGE-related thalamic region using subject-specific diffusion imaging data. **A.** Example of reconstructed fibers in a representative child with the seed being the PatAGE-cluster. **B.** Intersection across 23 children with diffusion imaging data is shown for demonstrative purposes. In particular, only fibers (streamlines) observed in more than 25% of the subjects (6 children) are displayed. The color bar represents the number of subjects where the streamline is observed in a given voxel. **C.** The DAN compared to the VAN derived from Yeo's 7 networks showed significantly greater (normalized) number of streamlines to go through the PatAGE-cluster. **D.** Scatter plot of the positive correlation between paternal age and number of streamlines labeled as DAN passing through the cluster. The linear regression line and R^2 are shown. **E.** Scatter plot of the negative correlation between reading composite scores at time-point 2 and the number of streamlines passing through the PatAGE-cluster and DAN. The linear regression line and R^2 are presented. *Acronyms: DAN, dorsal attention network; LH, left hemisphere; RH, right hemisphere; VAN, ventral attention network.*

1096 **Tables**

Table 1 Demographic profiles, familial variables and performance on reading-related tests (n = 43).

	Mean	Std.Dev	Min	Max
Time-point 1				
Age (years)	5.58	0.43	5.03	6.99
Gender, Male (%)	60.50	--	--	--
Handedness, Right (%)	88.40	--	--	--
WJC VC (SS)	121.53	13.43	86	145
WJC CF (SS)	118.16	11.43	94	137
WJC VM (SS)	105.65	11.83	72	127
WJC NR (SS)	112.05	12.06	83	138
PPVT (SS)	121.23	9.94	97	148
# Older Siblings (RS)	0.65	0.81	0	3
# Younger Siblings (RS)	0.63	0.62	0	2
PatAGE (years)	36.12	4.91	24.78	46.71
MatAGE (years)	33.01	4.09	23.04	41.08
PatARHQ (RS)	0.35	0.14	0.09	0.66
MatARHQ (RS)	0.31	0.15	0.07	0.67
PatEDU (years)	16.95	2.05	13	22
MatEDU (years)	16.97	2.04	12	22
SES (CS) [a]	0.04	1.00	-2.87	2.38
HOME (RS) [b]	51.39	2.25	44	55
CTOPP BW (SS) [c]	12.28	1.80	8	17
CTOPP EL (SS) [c]	11.93	2.80	7	19
CTOPP MD (SS) [c]	10.79	2.22	7	16
CTOPP NR (SS) [c]	11.23	2.85	6	19
RAN OBJ (SS)	100.56	17.57	55	135

RAN COL (SS)	97.98	16.51	55	137
WRMT LID (SS)	109.84	10.84	80	138
Time-point 2				
Age (years)	8.30	0.46	7.51	9.76
WJC VC (SS)	116.07	10.18	92	144
WJC CF (SS)	118.21	12.29	97	150
WJC VM (SS)	99.23	15.04	77	138
WJC NR (SS)	109.26	15.13	80	140
PPVT (SS)	120.02	14.56	81	160
TOWRE SWE (SS)	111.49	12.29	86	138
TOWRE PDE (SS)	106.35	14.85	77	144
WRMT WID (SS)	116.23	11.66	94	139
WRMT WA (SS)	113.84	14.40	90	146
WRMT PC (SS)	114.91	9.95	99	141
WJA RF (SS)	112.49	16.31	84	162
WJA SP (SS)	105.28	18.33	74	148
CTOPP BW (SS) [c]	12.67	2.24	6	16
CTOPP EL (SS) [c]	13.00	3.11	4	17
CTOPP MD (SS) [c]	10.47	2.60	5	15
CTOPP NR (SS) [c]	10.09	2.26	6	16
RAN NUM (SS)	100.35	12.47	76	129
RAN LTR (SS)	102.98	11.63	78	134
RAN OBJ (SS)	96.53	17.07	62	132
RAN COL (SS)	97.02	15.29	60	121

Notes: [a] SES: n = 38; [b] HOME: n = 41; [c] T Scores are presented for CTOPP sub-tests where mean is 10 and SD is 3. All other test scores are in standard scores where mean is 100 and SD is 15.

Table 2 Results of multiple linear regression analyses examining the unique contribution of paternal age on offspring's reading at time-point 2

Model	Step	Predictor	ΔR^2	β
1	1	Age (*t*2)	0.284 *	-0.351 *
		Sex		-0.022
		Handedness		0.081
		Average pIQ		0.302 *
	2	PatAGE	0.149 **	-0.393 **
2	1	Age (*t*2)	0.284 *	-0.351 *
		Sex		-0.022
		Handedness		0.079
		Average pIQ		0.309 *
	2	MatAGE	0.052 †	0.026
	3	PatAGE	0.097 *	-0.408 *
3	1	Age (*t*2)	0.279 *	-0.315 †
		Sex		-0.002
		Handedness		0.119
		Average pIQ		0.203
	2	MatAGE	0.071 †	0.269
	3	PatARHQ	0.170	-0.129
		MatARHQ		-0.162
		# Older Siblings		-0.298
		# Younger Siblings		-0.171
		PatEDU		0.031
		MatEDU		-0.075
		SES		-0.214
		HOME		0.233
	4	PatAGE	0.107 *	-0.592 *

4	1	Age (*t*2)	0.279 *	-0.261
		Sex		-0.051
		Handedness		0.136
		Average pIQ		-0.013
	2	MatAGE	0.071 †	0.200
	3	PatARHQ		-0.105
		MatARHQ		-0.039
		# Older Siblings		-0.174
		# Younger Siblings		-0.144
		PatEDU		0.086
		MatEDU		-0.211
		SES		-0.146
		HOME		0.201
	4	Time 1 PA	0.138 *	0.423 *
		Time 1 RAN		0.350 *
	5	PatAGE	0.095 *	-0.567 *

Note: β is value at the final step (all predictors included). ** $p < 0.01$; * $p < 0.05$, † $p < 0.1$

Figures

Fig. 1

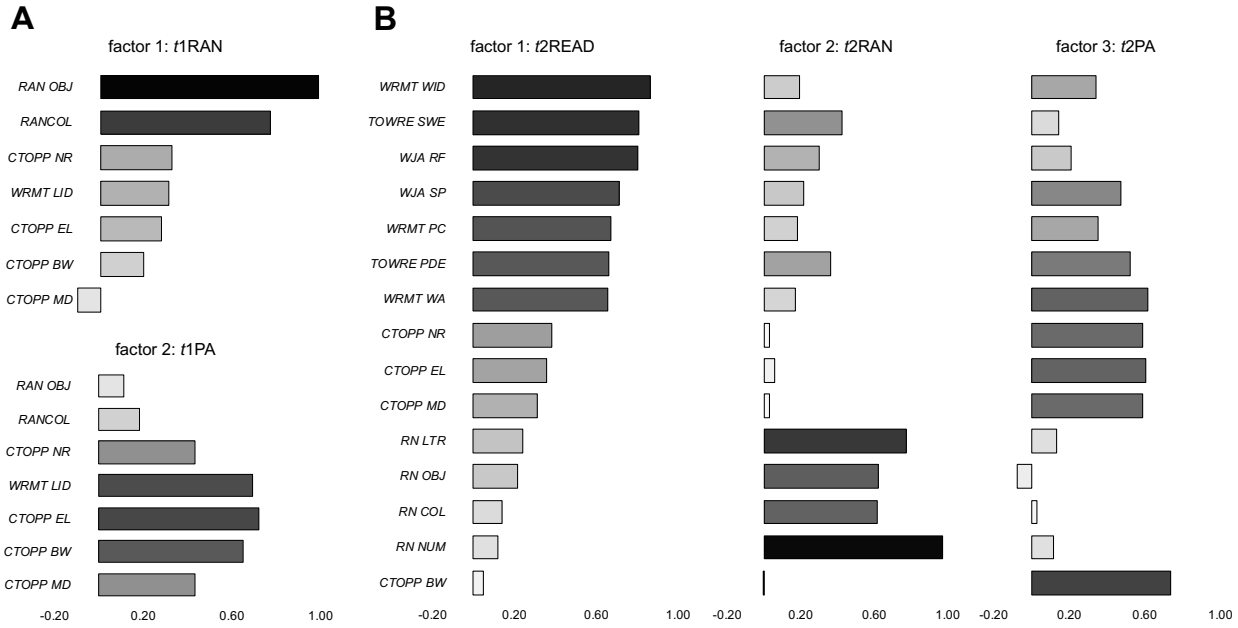


Fig. 2

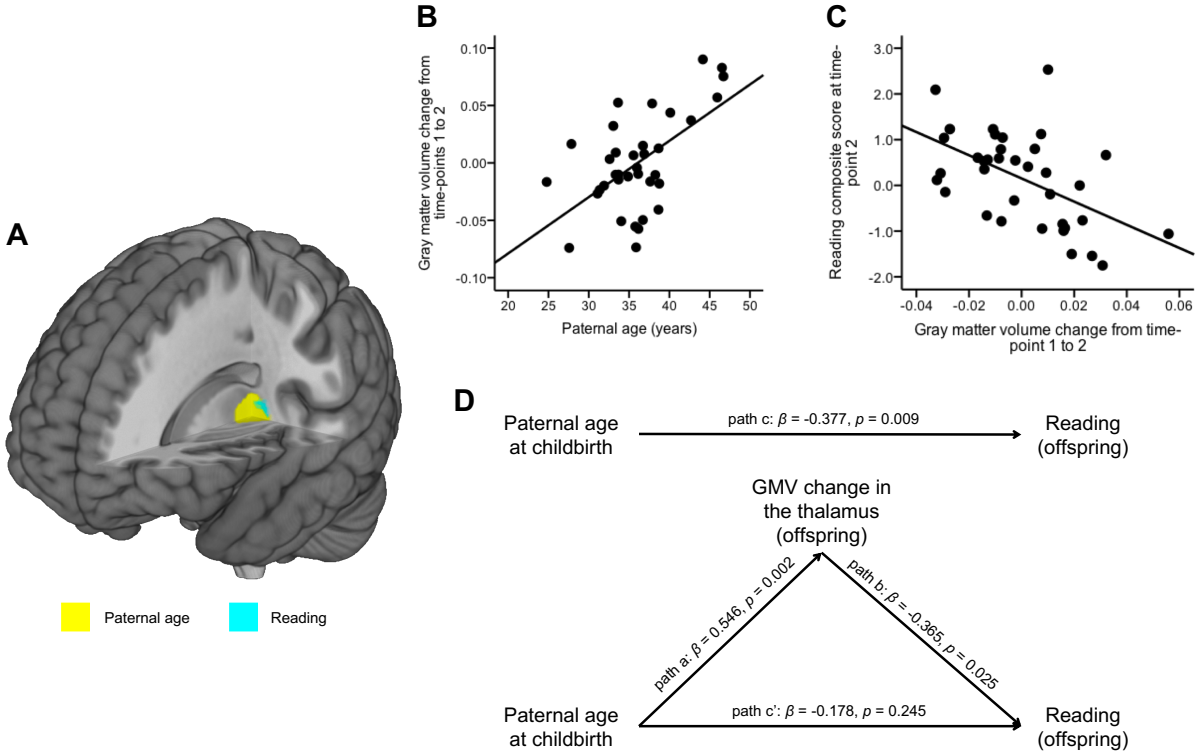

A
Paternal age
Reading
B
Gray matter volume change from time-points 1 to 2
0.10
0.05
0.00
-0.05
-0.10
20
25
30
35
40
45
50
Paternal age (years)
C
Reading composite score at time-point 2
3.0
2.0
1.0
0.0
-1.0
-2.0
-0.04
-0.02
0.00
0.02
0.04
0.06
Gray matter volume change from time-point 1 to 2
D
Paternal age at childbirth
path c: β = -0.377, p = 0.009
Reading (offspring)
GMV change in the thalamus (offspring)
path a: β = 0.546, p = 0.002
path b: β = -0.365, p = 0.025
Paternal age at childbirth
path c': β = -0.178, p = 0.245
Reading (offspring)

Fig. 3

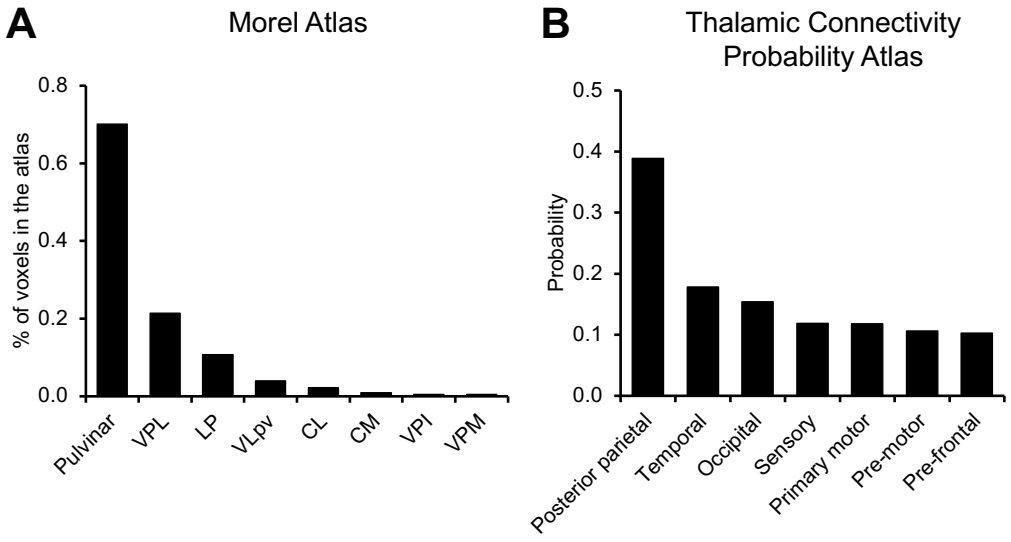

A
Morel Atlas
% of voxels in the atlas
0.8
0.6
0.4
0.2
0.0
Pulvinar
VPL
LP
VLpv
CL
CM
VPI
VPM
B
Thalamic Connectivity
Probability Atlas
Probability
0.5
0.4
0.3
0.2
0.1
0.0
Posterior parietal
Temporal
Occipital
Sensory
Primary motor
Pre-motor
Pre-frontal

Fig. 4

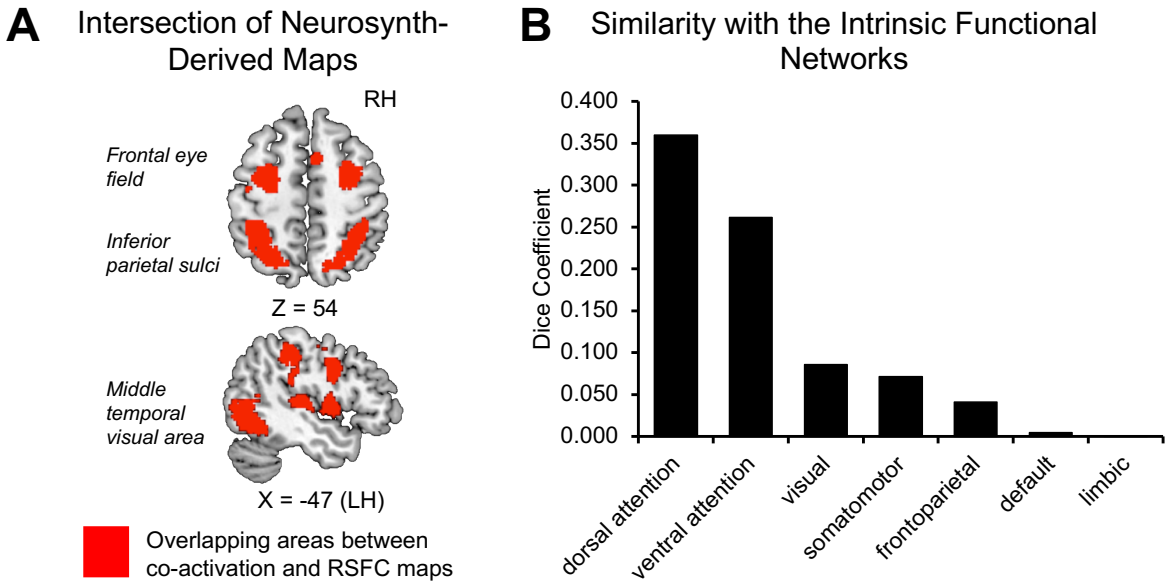
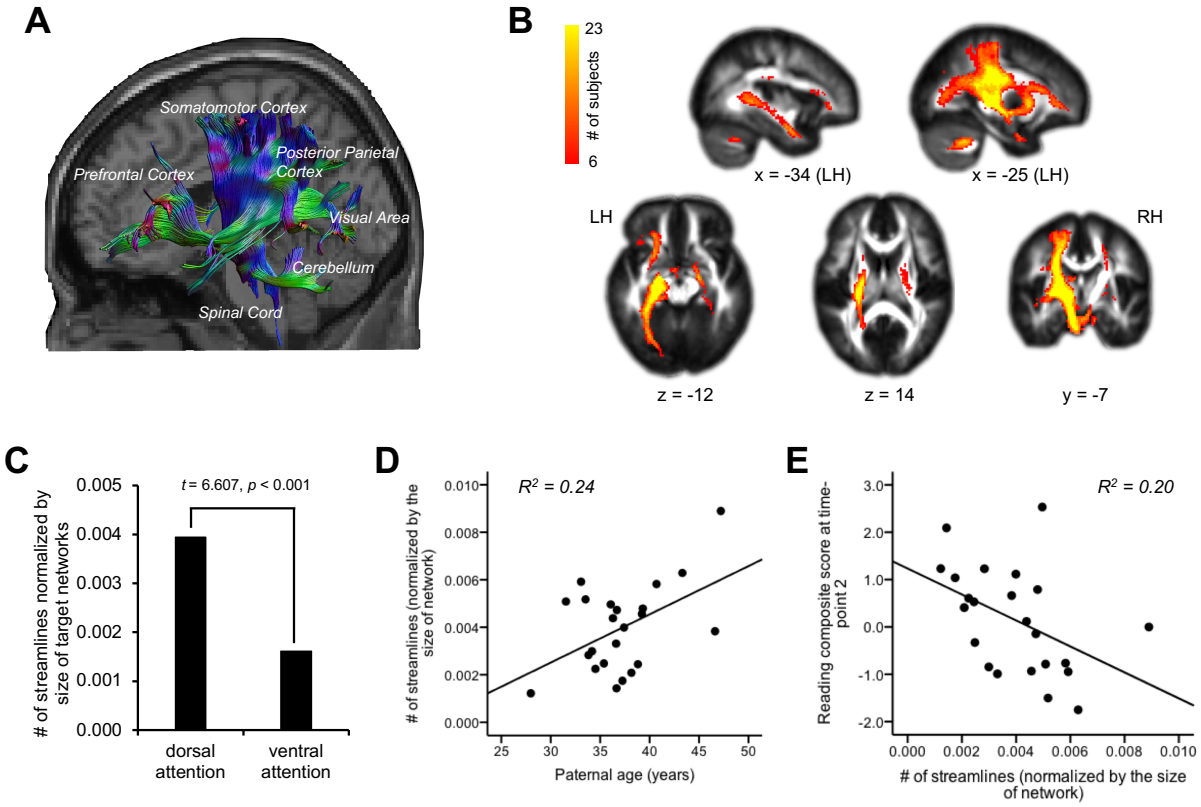

A
Intersection of Neurosynth-Derived Maps
RH
Frontal eye field
Inferior parietal sulci
Z = 54
Middle temporal visual area
X = -47 (LH)
Overlapping areas between co-activation and RSFC maps
B
Similarity with the Intrinsic Functional Networks
Dice Coefficient
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
dorsal attention
ventral attention
visual
somatomotor
frontoparietal
default
limbic

Fig. 5


A
Somatomotor Cortex
Posterior Parietal Cortex
Prefrontal Cortex
Visual Area
Cerebellum
Spinal Cord
B
23
# of subjects
6
x = -34 (LH)
x = -25 (LH)
LH
RH
z = -12
z = 14
y = -7
C
t = 6.607, p < 0.001
# of streamlines normalized by size of target networks
0.005
0.004
0.003
0.002
0.001
0.000
dorsal attention
ventral attention
D
R² = 0.24
# of streamlines (normalized by the size of network)
0.010
0.008
0.006
0.004
0.002
0.000
25
30
35
40
45
50
Paternal age (years)
E
R² = 0.20
Reading composite score at time-point 2
3.0
2.0
1.0
0.0
-1.0
-2.0
0.000
0.002
0.004
0.006
0.008
0.010
# of streamlines (normalized by the size of network)