Is There an Association between Advanced Paternal Age and Endophenotype Deficit Levels in Schizophrenia?

Debby Tsuang1,2, Michelle Esterberg2, David Braff3,4, Monica Calkins5, Kristin Cadenhead4, Dorcas Dobie2,6, Robert Freedman7, Michael F. Green8,9, Tiffany Greenwood4, Raquel Gur5, Ruben Gur5, William Horan8,9, Laura C. Lazzeroni10, Gregory A. Light3,4, Steven P. Millard6, Ann Olincy7, Keith Nuechterlein9, Larry Seidman11, Larry Siever12, Jeremy Silverman12, William Stone11, Joyce Sprock4, Catherine Sugar8,9, Neal Swerdlow4, Ming Tsuang4, Bruce Turetsky5, Allen Radant2

1 VISN-20 Geriatric Research, Education, and Clinical Center, VA Puget Sound Health Care System, Seattle, Washington, United States of America, 2 Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington, United States of America, 3 VISN-22 Mental Illness Research, Education, and Clinical Center, VA San Diego Healthcare System, San Diego, California, United States of America, 4 Department of Psychiatry, University of California San Diego, San Diego, California, United States of America, 5 Department of Psychiatry, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America, 6 VISN-20 Mental Illness Research, Education, and Clinical Center, VA Puget Sound Health Care System, Seattle, Washington, United States of America, 7 Department of Psychiatry, University of Colorado Health Sciences Center, Denver, Colorado, United States of America, 8 Department of Psychiatry and Biobehavioral Sciences, Geffen School of Medicine at University of California Los Angeles, Los Angeles, California, United States of America, 9 VA Greater Los Angeles Health Care System, Los Angeles, California, United States of America, 10 Department of Psychiatry and Behavioral Sciences, Stanford University, Palo Alto, California, United States of America, 11 Massachusetts Mental Health Center Public Psychiatry Division of the Beth Israel Deaconess Medical Center, Harvard Medical School Department of Psychiatry, Boston, Massachusetts, United States of America, 12 Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, United States of America; VISN-3 Mental Illness Research, Education, and Clinical Center, James J. Peters VA Medical Center, New York, New York, United States of America

Abstract

The children of older fathers have increased risks of developing schizophrenia spectrum disorders, and among those who develop these disorders, those with older fathers present with more severe clinical symptoms. However, the influence of advanced paternal age on other important domains related to schizophrenia, such as quantitative endophenotype deficit levels, remains unknown. This study investigated the associations between paternal age and level of endophenotypic impairment in a well-characterized family-based sample from the Consortium on the Genetics of Schizophrenia (COGS). All families included at least one affected subject and one unaffected sibling. Subjects met criteria for schizophrenia (probands; n = 293) or were unaffected first-degree siblings of those probands (n = 382). Paternal age at the time of subjects’ birth was documented. Subjects completed a comprehensive clinical assessment and a battery of tests that measured 16 endophenotypes. After controlling for covariates, potential paternal age–endophenotype associations were analyzed using one model that included probands alone and a second model that included both probands and unaffected siblings. Endophenotype deficits in the Identical Pairs version of the 4-digit Continuous Performance Test and in the Penn Computerized Neurocognitive Battery verbal memory test showed significant associations with paternal age. However, after correcting for multiple comparisons, no endophenotype was significantly associated with paternal age. These findings suggest that factors other than advanced paternal age at birth may account for endophenotypic deficit levels in schizophrenia.


Editor: James Bennett Potash, University of Iowa Hospitals & Clinics, United States of America
Received September 30, 2013; Accepted January 7, 2014; Published February 11, 2014

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This material is based upon work supported (or supported in part) by the Office of Research and Development Medical Research Service, Department of Veterans Affairs. This study was supported by NIMH grants R01 MH65571, R01 MH042228, R01 MH65588, R01 MH65562, R01 MH65707, R01 MH65554, R01 MH65578, R01 MH086135, and R01 MH65558. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: dwt1@uw.edu

Introduction

Paternal age and risk of schizophrenia

The children of older fathers have an increased risk of developing a number of psychiatric disorders that include deficits in behavioral and cognitive functioning. Studies have demonstrated associations between advanced paternal age and epilepsy [1], bipolar disorder [2,3], autism [4,5,6,7], dyslexia [8], neurocognitive impairments [9], and deficits in social functioning [10]. The literature also suggests a relationship between patients’ advanced paternal age at birth and the development of schizophrenia spectrum disorders. Malaspina and colleagues [11] found that after paternal age at birth exceeded 25, the relative risk of schizophrenia for offspring increased with each five-year increment in paternal age at birth and that the greatest risk occurred for the offspring of the oldest paternal group: fathers age 45 and older. In that study, older paternal age at birth remained a significant predictor of schizophrenia risk even after controlling for maternal...
Paternal Age and Schizophrenia Endophenotypes

Clinical ascertainment and assessment

Materials and Methods

Goals of the current study

Despite the evidence demonstrating paternal age effects on the development of schizophrenia-spectrum disorders in offspring, we do not know whether advanced paternal age is associated with the endophenotypic deficits observed in schizophrenia. By studying these associations, we have the opportunity to understand whether paternal age has differential effects on schizophrenia-related neurophysiological and neurocognitive endophenotypic deficits with known neurobiological functions. Endophenotypes, which were first described by Gottesman and Gould [22], are defined as stable traits that are more common in affected individuals but are not associated with the severity of the illness, that cosegregate with illness in families, and that are more common among family members of affected individuals.

Endophenotype assessment battery

The COGS endophenotype assessment battery and its use in the different COGS studies have been discussed in detail elsewhere. For the current study, endophenotypes included standard assessments of antisaccade performance (proportion correct) [32]; prepulse inhibition (PPI) [33], which was measured as 100 × (1 − (magnitude of startle to pulse preceded by prepulse/magnitude of startle to pulse without a preceding prepulse)) using 60 msec pulses; the Degraded Stimulus (DS), 3-digit Identical Pairs (IP), and 4-digit IP versions of the Continuous Performance Test (CPT), which were measured using the signal/noise discrimination index (d’) [34]; the Forward and Reordered condition of the Letter-Number Span (LNS) [35], measured as the total number of correctly recalled sequences; the California Verbal Learning Test (CVLT), specifically the total and total semantic clustering scores on trials 1–5 (CVLT total and CVLT semantic) [36]; and the abstraction and mental flexibility, verbal memory, face memory, spatial memory, spatial processing, sensorimotor dexterity, and emotion processing cognitive domains of the Penn Computerized Neurocognitive Battery (CNS) [34,37], which were measured using the number of correct responses standardized to a z-score based on the COGS community control subjects.

Data analysis

Demographic differences between schizophrenia probands and their unaffected siblings were tested using linear mixed-effects models [38] for continuous variables and generalized linear mixed-effects models [39] for categorical variables. In these analyses, family membership served as a random effect to account for the relatedness of observations among family members. Families in
which at least one parent or sibling of the proband also had a

diagnosis of schizophrenia or schizoaffective disorder were

considered multiplex; all other families were considered simplex.

Paternal age at birth was calculated by subtracting offspring age

from parental age; it was treated as continuous.

To assess a possible effect of paternal age on the severity of

symptoms, or on the age of onset of symptoms, separate linear

models were used to model SANS, SAPS, and age of onset as a

function of paternal age, gender, and a paternal age–by-gender

interaction.

We conducted two separate types of analyses to test the

association of each endophenotype with paternal age: proband-

only analyses and simultaneous analyses of both the probands and

their unaffected siblings. Our proband-only analyses consisted of

linear regression models in which an endophenotype was modeled

as a function of paternal age along with the covariates test site,

subject age, subject gender, multiplex status, and parental

education (maximum grade level of mother or father), as well as

paternal age–by-gender and paternal age–by-multiplex status

interaction terms. Simultaneous analyses of probands and their

unaffected siblings consisted of linear mixed-effects models that

mirrored those used in the proband-only analyses but with family

as a random effect, with group (proband versus sibling) as a fixed

effect, and with additional interaction terms: all second-order

interaction terms involving group. For all analyses of the CVLT

endophenotype, WRAT-3 standardized reading scores were

included as a covariate in place of parental education as suggested

by Stone and colleagues [36]. Missing values for parental

education and WRAT-3 scores were imputed as described previously [36,40].

For any term involving paternal age at subject birth, nominal

significance was defined as a $P$ value less than 5%. Adjustment for

multiple comparisons was performed using the method of Holm

[41]. One set of adjustments accounted for the 16 endophenotypes,

and another set of adjustments accounted for the 16

endophenotypes and two models (proband only versus probands

plus unaffected siblings). Neither set of adjustments accounted for

the presence of multiple terms that involved paternal age at birth.

We performed three sets of sensitivity analyses: one in which

interaction terms were omitted, one in which paternal age was

treated as categorical (<40 years old versus $\geq 40$), and one in which

interactions terms were omitted and paternal age was treated as
categorical.

All analyses were performed using R version 2.12.2 [42]. Linear

mixed-effects models were fit using the R package nlme [43], and
generalized linear mixed-effects models were fit using the R

package lme4 [44].

**Results**

**Demographic and clinical variables**

For subjects with at least one valid endophenotype, paternal age

at birth was obtained from 293 of 345 (83%) schizophrenia

probands and from 392 of 456 (84%) unaffected siblings. The 392

unaffected siblings represent 279 families, of which 17 (6%) were

multiplex; 18 of the 293 probands (6%) were from multiplex

families. As expected, probands and siblings were similar in age

and paternal age at birth, but probands had a significantly higher

proportion of males and smokers, had significantly lower average

education levels, and performed worse on the WRAT-3 (Table 1).

Of the 293 probands, 23 (8%) were born to fathers 40 years old or

older.

**Association of paternal age with symptom levels and age

of onset in probands**

Seven (2%) of the probands were missing values for SANS and

SAPS, and 5 (2%) were missing values for age at onset; these

probands were all born to fathers less than 40 years old. None of

the linear models for SANS, SAPS, or age of onset in probands

showed a significant effect for paternal age or a paternal age–by-

gender interaction.

**Association of paternal age with endophenotypic deficits**

Table 2 presents descriptive statistics for the endophenotypes by

subject group. Table 3 displays the results of linear models that test

the association of paternal age at birth with endophenotypic

deficits in probands alone and the results of linear mixed-effects

models that test the association of paternal age at birth with

endophenotypic deficits in probands and unaffected siblings

combined.

Of the 16 endophenotypes considered, only the 4-digit CPT-IP

(CPT-IP 4-digit) and CNB verbal memory test showed nominally

significant associations with paternal age. CPT-IP 4-digit showed a

positive association with paternal age (i.e., subjects with older

fathers performed better), and the CNB verbal memory test also

showed an interaction effect between paternal age and multiplex

status (test scores were more negatively associated with paternal

age for multiplex subjects than simplex subjects). In the analysis of

both probands and unaffected siblings, the verbal memory test also

showed an interaction effect between paternal age and gender (test

scores were more negatively associated with paternal age for

females than males). After adjusting for multiple comparisons

accounting for the 16 endophenotypes, none of the results

remained statistically significant (Table 3 shows only results

involving paternal age that are nominally significant before

adjusting for multiple comparisons; Table S1 shows results for

all terms involving paternal age for all endophenotypes, regardless

of significance).

For the sensitivity analyses, when paternal age at birth was

treated as continuous and interaction terms were omitted from the

models, only the CPT-IP 4-digit and the California Verbal

Learning Test semantic clustering (CVLT semantic) showed

significant associations with paternal age. CPT-IP 4-digit again

showed a positive association with paternal age and CVLT

semantic showed a negative association. After adjusting for

multiple comparisons accounting for the 16 endophenotypes, only

the effect of paternal age on CPT-IP 4-digit in the probands-only

analysis remained statistically significant. After adjusting for

multiple comparisons accounting for the 16 endophenotypes and

two models (proband only versus probands plus unaffected

siblings), none of the results remained statistically significant.

When paternal age at birth was treated as categorical (<40

versus $\geq 40$) and interaction terms were omitted from the models,

CPT-IP 4-digit again showed a positive association with paternal

age, but this association was not significant after adjusting for

multiple comparisons accounting for the 16 endophenotypes.

When paternal age at birth was treated as categorical (<40

versus $\geq 40$) and interaction terms were included in the models,

CPT-IP 3-digit, CPT-IP 4-digit, and CNB spatial ability all showed positive

associations with paternal age. Also, similar to when paternal age

was treated as continuous, in the analysis of both probands and

unaffected siblings, CNB verbal memory showed an interaction

effect between paternal age and multiplex status (test scores were

more negatively associated with paternal age for multiplex subjects

than simplex subjects). CNB sensorimotor dexterity showed the

same kind of paternal age–by–multiplex status interaction effect,

and CNB abstraction/mental flexibility showed an interaction

between paternal age and multiplex status (test scores were

more negatively associated with paternal age for multiplex subjects

than simplex subjects).
Table 1. Demographic and clinical characteristics of schizophrenia subjects and unaffected siblings.

<table>
<thead>
<tr>
<th></th>
<th>Schizophrenia subjects (N = 293)</th>
<th>Unaffected siblings (N = 382)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Odds Ratio (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male)</td>
<td>222</td>
<td>173</td>
<td>&lt;0.0001</td>
<td>3.9 (2.8, 5.5)</td>
</tr>
<tr>
<td>Race (white)</td>
<td>216</td>
<td>305</td>
<td>0.0001</td>
<td>4.0 (2.7, 5.7)</td>
</tr>
<tr>
<td>Smoker&lt;sup&gt;d&lt;/sup&gt;</td>
<td>126</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiplex status&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.7</td>
<td>35.0</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Education (years)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.6</td>
<td>15.4</td>
<td>&lt;0.0001</td>
<td>−1.7 (−2.0, −1.4)</td>
</tr>
<tr>
<td>Parental education (years)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>15.9</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRAT-3&lt;sup&gt;h&lt;/sup&gt;</td>
<td>102.5</td>
<td>96.5</td>
<td>&lt;0.0001</td>
<td>−4.0 (−5.3, −2.7)</td>
</tr>
<tr>
<td>Age at onset of symptoms (years)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>20.9</td>
<td>20.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SANS&lt;sup&gt;j&lt;/sup&gt;</td>
<td>9.5</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAPS&lt;sup&gt;k&lt;/sup&gt;</td>
<td>6.2</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal age at birth (years)</td>
<td>30.7</td>
<td>30.7</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; SANS, Schedule for the Assessment of Negative Symptoms; SAPS, Schedule for the Assessment of Positive Symptoms; SD, standard deviation; WRAT-3, Wide Range Achievement Test, 3rd edition.
<sup>a</sup>Unaffected siblings represent 279 families, of which 17 (6%) were multiplex.
<sup>b</sup>Between-group P values are based on generalized linear mixed-effects models for categorical variables and linear mixed-effects models for continuous variables; P values were not computed for race, multiplex status, parental education, or age at onset of symptoms.
<sup>c</sup>For continuous variables, odds ratios and 95% CIs for odds ratios are based on generalized linear mixed-effects models. CIs are shown only for variables where the P value for the between-group test is <0.05.
<sup>d</sup>4 missing values for schizophrenia subjects; 2 missing values for unaffected siblings.
<sup>e</sup>Multiplex families were those for which at least one parent or sibling of the proband had a history of schizophrenia or schizoaffective disorder.
<sup>f</sup>2 missing values for schizophrenia subjects; 2 missing values for unaffected siblings.
<sup>g</sup>6 missing values for schizophrenia subjects; 3 missing values for unaffected siblings.
<sup>h</sup>6 missing values for schizophrenia subjects; 3 missing values for unaffected siblings.
<sup>i</sup>10 missing values for schizophrenia subjects; 12 missing values for unaffected siblings.
<sup>j</sup>5 missing values for schizophrenia subjects.
<sup>k</sup>7 missing values for schizophrenia subjects; 7 missing values for unaffected siblings.
<sup>l</sup>17 missing values for schizophrenia subjects; 74 missing values for unaffected siblings.

doi:10.1371/journal.pone.0088379.t001

Discussion

Our overall findings do not suggest that advanced paternal age at birth negatively affects endophenotypes associated with schizophrenia; rather, our findings imply that factors other than paternal age at birth are operating to influence performance on these neurocognitive and neurophysiologic measures.

The current study operated under the initial hypothesis that advanced paternal age may serve as a proxy for the presence of genetic variations that may increase the risk of developing schizophrenia and may also potentially increase schizophrenia-associated endophenotypic deficit levels. Some investigators have hypothesized that the association between the risk for sporadic schizophrenia and advanced paternal age can be attributed to de novo mutations in paternal germ lines [11]. Specifically, given the increased rate of mitotic cell division in the sperm cells relative to the oocyte, the possibility of error in gene replication greatly increases along with advanced paternal age. Indeed, advanced paternal age has been linked with human and rodent sex chromosomal aneuploidies as well as a fourfold increase in structural chromosomal abnormalities (i.e., breaks and/or rearrangements) and other DNA damage in human and mouse sperm [45]. Thus, molecular mechanisms associated with aging males could explain the relationship between advanced paternal age and the development of major psychiatric disorders such as schizophrenia.

However, in the current study, paternal age at birth played little to no role in influencing performance on endophenotypic measures. For example, although we found an association between paternal age at birth and performance on the CPT-IP 4-digit, this association ran counter to our hypothesis in that our subjects with paternal age at birth played little to no role in influencing performance on endophenotypic measures. For example, although we found an association between paternal age at birth and performance on the CPT-IP 4-digit, this association ran counter to our hypothesis in that our subjects with older fathers actually performed better on this measure. In the analyses that combined the probands and unaffected siblings, we found a significant paternal age-by-multiplex status interaction with the verbal memory component of the CNB such that test scores in multiplex subjects showed a more negative association with paternal age than in simplex subjects. However, after adjustment for multiple comparisons, none of these associations remained statistically significant, suggesting that these findings may be due primarily to Type I error.
Table 2. Unadjusted endophenotypes for schizophrenia subjects and unaffected siblings.

<table>
<thead>
<tr>
<th>Endophenotype</th>
<th>Schizophrenia subjects (N = 293)</th>
<th>Unaffected siblings (N = 382)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Antisaccade (proportion correct) 9</td>
<td>242</td>
<td>0.62</td>
</tr>
<tr>
<td>PPI (percent) 10</td>
<td>198</td>
<td>44</td>
</tr>
<tr>
<td>DS-CPT (d') 10</td>
<td>252</td>
<td>2.4</td>
</tr>
<tr>
<td>CPT-IP 4-digit (d') 10</td>
<td>242</td>
<td>2.2</td>
</tr>
<tr>
<td>CPT-IP 3-digit (d') 10</td>
<td>241</td>
<td>1.3</td>
</tr>
<tr>
<td>LNS Forward (number correct) 10</td>
<td>282</td>
<td>13</td>
</tr>
<tr>
<td>LNS Reordered (number correct) 10</td>
<td>281</td>
<td>9</td>
</tr>
<tr>
<td>CVLT total (number correct) 10</td>
<td>277</td>
<td>43</td>
</tr>
<tr>
<td>CVLT semantic (number correct) 10</td>
<td>277</td>
<td>0.5</td>
</tr>
<tr>
<td>CNB (z-transformed number correct) 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abstraction/mental flexibility 10</td>
<td>258</td>
<td>−0.56</td>
</tr>
<tr>
<td>Verbal memory 10</td>
<td>149</td>
<td>−0.71</td>
</tr>
<tr>
<td>Face memory 10</td>
<td>267</td>
<td>−0.83</td>
</tr>
<tr>
<td>Spatial memory 10</td>
<td>261</td>
<td>−0.61</td>
</tr>
<tr>
<td>Spatial processing 10</td>
<td>247</td>
<td>−0.06</td>
</tr>
<tr>
<td>Sensorimotor dexterity 10</td>
<td>263</td>
<td>−0.20</td>
</tr>
<tr>
<td>Emotion processing 10</td>
<td>261</td>
<td>−0.88</td>
</tr>
</tbody>
</table>

Abbreviations: CNB, Computerized Neurocognitive Battery; CPT-IP, Continuous Performance Test, Identical Pairs version; CVLT, California Verbal Learning Test; DS-CPT, Degraded Stimulus Continuous Performance Test; LNS, Letter-Number Span; PPI, prepulse inhibition.

1Three-digit d’.
2Four-digit d’.
3After each sequence, the participant is asked to recall the numbers and letters in the same exact order, with no reordering of the stimuli. The number of digits and letters increases by one on each trial, from one up to a maximum length of 8 stimuli. Three sequences of the same length are presented during each trial. The test is discontinued when the subject fails three consecutive sequences of the same length. The score is the total number of correctly recalled sequences.

4After each sequence, the participant is asked to repeat the numbers in ascending order first and then the letters in alphabetical order.
5Trials 1–5 Free Recall Correct.
6Total semantic clustering scores on trials 1–5.
7The number of correct responses standardized to a z-score based on the COGS community control subjects.

doi:10.1371/journal.pone.0088379.t002

Table 3. Significant terms involving paternal age at birth in linear and linear mixed-effects models for endophenotype performance.

<table>
<thead>
<tr>
<th>Endophenotype</th>
<th>Schizophrenia subjects only</th>
<th>Schizophrenia subjects and unaffected siblings combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Significant term* (slopeb, P valuec,d, 95% CI)</td>
<td>Significant term* (slopeb, P valued,e, 95% CI)</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>R²</td>
</tr>
<tr>
<td>CPT-IP 4-digit, by Multiplex status</td>
<td>Paternal age (slope = 0.19, P = 0.03, 95% CI = [0.01, 0.36])</td>
<td>Paternal age by Gender (Male slope = −0.05, 95% CI = [−0.34, 0.25]; Female slope = −0.43, 95% CI = [−0.83, −0.03]; P for difference in slopes = 0.049)</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.41</td>
</tr>
<tr>
<td>Verbal memory, by Multiplex status</td>
<td>Paternal age by Multiplex status (Simplex slope = −0.07, 95% CI = [−0.42, 0.29]; Multiplex slope = −1.0, 95% CI = [−1.9, −0.15]; P for difference in slopes = 0.03)</td>
<td>Paternal age by Multiplex status (Simplex slope = −0.05, 95% CI = [−0.34, 0.25]; Multiplex slope = −0.89, 95% CI = [−1.5, −0.32]; P for difference in slopes = 0.005)</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CPT-IP, Continuous Performance Test, Identical Pairs version.

*Only terms involving paternal age at subject birth with an associated P value <0.05 are reported, and only endophenotypes with such terms are reported.

1Slope and confidence intervals are in units of a 10-year increase in paternal age at birth. A positive slope indicates that subjects with older fathers perform better on the endophenotype.
2P values are based on linear models with effects for paternal age, paternal age-by-gender, and paternal age-by-multiplex status, with subject age, test site, subject gender, and paternal education as covariates.
3After adjusting for multiple comparisons accounting for the 16 endophenotypes, none of the results are significant at an overall Type I error level of 5%.
4P values are based on linear mixed-effects models with effects for paternal age, paternal age-by-gender, and paternal age-by-multiplex status, with group (proband versus sibling), subject age, test site, subject gender, parental education, and all second-order interactions involving group as covariates. Family membership served as a random effect to account for the relatedness of observations among family members.

doi:10.1371/journal.pone.0088379.t003
As for clinical symptoms, there was no difference in negative symptoms (as measured by the SANS) nor age of onset between the probands with younger fathers and the probands with older fathers. This finding contrasts with recent research demonstrating a relationship between advanced paternal age at birth and positive symptoms as measured by the PANSS [20]. It is possible that the differences between our findings and the findings in the study by Rosenfield and colleagues [20] could be explained by differences in our study design; for example, our study included probands who were on antipsychotic medications (their symptoms may have therefore been less severe), whereas the study by Rosenfield and colleagues only included unmedicated subjects. Furthermore, our study used a family-based design rather than a case-control design, and this difference in recruitment methodology may have contributed to our nonsignificant findings.

Our study has some limitations. First, as mentioned in our comparison with the study by Rosenfield and colleagues, the severity of schizophrenia symptoms might be more evident when subjects are not on antipsychotics, and thus our inclusion of such subjects may make our results difficult to compare to studies that excluded subjects on antipsychotics. Second, subjects who were missing data for paternal age at birth tended to display poorer clinical outcomes than subjects with paternal age data available. If these subjects tended to have higher (or lower) paternal age at birth than the subjects with paternal age data available, this could potentially bias our results. And third, our study had a relatively high number of subjects with missing endophenotype data, and if these missing values were not missing at random, there is a potential for bias.

In summary, findings from our study do not suggest that advanced paternal age has a substantial effect on the endophenotypic deficits in schizophrenia probands or their unaffected siblings, nor on the severity of clinical symptoms or the age of onset of schizophrenia. The strength of this study is that its large, clinically well-characterized family-based sample enabled a thorough investigation of these associations. Another strength of our study is that we collected endophenotypic data from unaffected siblings in families with schizophrenia (not unrelated controls), which allowed us to make direct comparisons between affected and unaffected individuals who had the same fathers. Although the mechanisms by which advanced paternal age increases the risk of developing schizophrenia remain unknown, they do not appear to play a substantial role in the presence of endophenotypic deficits in schizophrenia. Additional studies are necessary to increase our understanding of the ways in which factors associated with advanced paternal age may adversely affect the developing brain.

Supporting Information

Table S1 Terms involving paternal age at birth in linear and linear mixed-effects models for endophenotype performance. Abbreviations: CI, confidence interval; CPT-IP, Continuous Performance Test, Identical Pairs version; LNS, Letter-Number Span; CVLT, California Verbal Learning Test. Only terms involving paternal age at subject birth are reported. Slope and confidence intervals are in units of a 10-year increase in paternal age at birth. A positive slope indicates that subjects with older fathers perform better on the endophenotype. P values are based on linear models with effects for paternal age, paternal age–by–gender, and paternal age–by–multiplex status, with subject age, test site, subject gender, and parental education as covariates. After adjusting for multiple comparisons accounting for the 16 endophenotypes, none of the results are significant at an overall Type I error level of 5%. P values are based on linear mixed-effects models with effects for paternal age, paternal age–by–gender, and paternal age–by–multiplex status, with group (proband versus sibling), subject age, test site, subject gender, parental education, and all second-order interactions involving group as covariates. Family membership served as a random effect to account for the relatedness of observations among family members.

Acknowledgments

The authors wish to thank all of the subjects and their family members for participating in this study. We also thank Andrew David for his editorial assistance and all of the research staff for their work on this study.

Author Contributions

Conceived and designed the experiments: DT DB MC KC DD RF MFG TG Raquel Gur Ruben Gur WH LCL GAL AO KN L. Seidman L. Siever J. Silverman WS J. Sprock CS NS MT BT AR. Analyzed the data: LCL SPM CS. Wrote the paper: DT ME SPM. Contributed to and have approved the final manuscript: DT ME DB MC KC DD RF MFG TG Raquel Gur Ruben Gur WH LCL GAL AO KN L. Seidman L. Siever J. Silverman WS J. Sprock CS NS MT BT AR.

References


44. Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-39.