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

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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 (proband; 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.


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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
age. Dalman and Allebeck [12] showed that having a father age 45 or older at birth was associated with a doubled risk of developing schizophrenia compared to having a father age 20 to 24. Similarly, Byrne and colleagues [13] demonstrated a sex-specific relationship between advanced paternal age and schizophrenia, showing that the risk for schizophrenia increased for sons of fathers age 55 years and older and for daughters of fathers age 50 years and older. However, a recent meta-analysis did not find gender differences [4,14,15,16,17,18,19].

**Paternal age, severity of symptoms, and cognitive functioning in schizophrenia**

Among those offspring who develop schizophrenia, advanced paternal age has also been associated with severity of clinical symptoms. Rosenfield and colleagues [20] found that patients with no family history of schizophrenia and a paternal age at birth of ≥ 35 showed more severe symptoms (higher total scores and higher positive, activation, and autistic preoccupation scores on the Positive and Negative Syndrome Scale [PANSS]) during a medication-free phase than patients with younger fathers. Others have suggested that paternal age is also associated with cognitive functioning in schizophrenia. Using demographic and clinical data collected from subjects with schizophrenia or schizoaffective disorder, Lee and colleagues [21] defined paternal age-related schizophrenia (PARS) as having a father age 35 or older at the time of the subject’s birth and not having a family history of schizophrenia. They performed numerous k-means cluster analyses and found that 20 of 34 (59%) individuals with PARS clustered in a group which exhibited significantly greater discrepancy between verbal and performance intelligence on the WAIS-R subtests compared to subjects in six other clusters. These findings suggest that the cognitive performance deficits in patients with schizophrenia may be associated with advanced paternal age.

**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.

Studying the impact of paternal age at birth on both individuals with schizophrenia and their unaffected siblings may highlight important relationships between nonfamilial factors and endophenotypes for schizophrenia. Thus, in the current study, we hypothesized that advanced paternal age would be associated with greater deficits in neurophysiological and neurocognitive schizophrenia-related endophenotypes.

**Materials and Methods**

**Clinical ascertainment and assessment**

The current study utilizes data from the Consortium on the Genetics of Schizophrenia (COGS) [23,24,25], a multisite, family-based study on the genetics and heritability of neurocognitive and neurophysiologic schizophrenia endophenotypes. Participants in this study met COGS criteria as schizophrenia probands (n = 293) or unaffected siblings of those probands (n = 382). The probands and their unaffected siblings completed a structured clinical diagnostic interview (the Diagnostic Interview for Genetic Studies [26]) and a best-estimate consensus diagnostic procedure that included a comprehensive evaluation of psychotic, mood, and substance use disorders and related symptomology [23,27]. This information was used in concert with a standard medical record review to establish handedness, age at onset of psychosis (for probands), education level, parental education level, and parental age; the Family Interview for Genetics Studies (FIGS) [28] was used to assess subjects’ family history of schizophrenia or schizoaffective disorder; and the reading subtest of the Wide Range Achievement Test, 3rd edition (WRAT-3) [29], was used to estimate premorbid intellectual functioning. The probands were also assessed using the Schedule for the Assessment of Negative Symptoms (SANS) [30] and Schedule for the Assessment of Positive Symptoms (SAPS) [31]; all probands met Diagnostic and Statistics Manual of Mental Disorders, 4th edition (DSM-IV), diagnostic criteria for schizophrenia. Recruitment and ascertainment strategies, in-depth inclusion and exclusion criteria, and in-person training and quality assurance guidelines for the assessment team and endophenotype testers are detailed in an earlier manuscript by Calkins and colleagues [23]. The COGS encompasses study sites at the University of California, San Diego; University of California, Los Angeles; University of Colorado, Denver; Harvard University; Mount Sinai School of Medicine; University of Pennsylvania; and University of Washington; institutional review boards at these sites and/or their affiliated institutions approved the COGS procedures in adults age 18 and older. All subjects demonstrated an understanding of their involvement in the study and provided signed informed consent before commencing study procedures as well as ongoing verbal and physical assent throughout the study.

**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 (CNB) [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 (probands 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 ≥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 382 of 456 (84%) unaffected siblings. The 382
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
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 (probands-only versus probands plus unaffected
siblings), none of the results remained statistically significant.

When paternal age at birth was treated as categorical (<40
versus ≥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
≥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 abstraction/mental flexibility showed the
same kind of paternal age–by–multiplex status interaction effect,
and CNB abstraction/mental flexibility showed an interaction
the results remained statistically significant. accounting for the 16 endophenotypes and two models, none of their unaffected siblings. After adjusting for multiple comparisons accounting for the 16 associated with paternal age for unaffected siblings). After adjusting for multiple comparisons accounting for the 16 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.

**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 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)</td>
<td>242</td>
<td>0.62</td>
</tr>
<tr>
<td>PPI (percent)</td>
<td>198</td>
<td>44</td>
</tr>
<tr>
<td>DS-CPT (d')²</td>
<td>252</td>
<td>2.4</td>
</tr>
<tr>
<td>CPT-IP 3-digit (d')³</td>
<td>242</td>
<td>2.2</td>
</tr>
<tr>
<td>CPT-IP 4-digit (d')⁴</td>
<td>241</td>
<td>1.3</td>
</tr>
<tr>
<td>LNS Forward (number correct)</td>
<td>282</td>
<td>13</td>
</tr>
<tr>
<td>LNS Reordered (number correct)</td>
<td>281</td>
<td>9</td>
</tr>
<tr>
<td>CVLT total (number correct)</td>
<td>277</td>
<td>43</td>
</tr>
<tr>
<td>CVLT semantic (number correct)</td>
<td>277</td>
<td>0.5</td>
</tr>
<tr>
<td>CNB (z-transformed number correct)</td>
<td>258</td>
<td>−0.56</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>149</td>
<td>−0.71</td>
</tr>
<tr>
<td>Face memory</td>
<td>267</td>
<td>−0.83</td>
</tr>
<tr>
<td>Spatial memory</td>
<td>261</td>
<td>−0.61</td>
</tr>
<tr>
<td>Spatial processing</td>
<td>247</td>
<td>−0.06</td>
</tr>
<tr>
<td>Sensorimotor dexterity</td>
<td>263</td>
<td>−0.20</td>
</tr>
<tr>
<td>Emotion processing</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.

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>Significant term² (slope³, P value⁴, 95% CI)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT-IP 4-digit</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>0.13</td>
</tr>
<tr>
<td>Verbal memory</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>
<td>0.11</td>
</tr>
</tbody>
</table>

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

1Proportion correct out of a maximum of 60 trials.
2Prepulse inhibition using 60 msec pulses.
3Overall signal/noise discrimination (d').
4Slope 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.
5Values 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.
6After adjusting for multiple comparisons accounting for the 16 endophenotypes, none of the results are significant at an overall Type I error level of 5%.
7Values 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.

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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. a Only terms involving paternal age at subject birth are reported. b 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.

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Author Contributions

Conceived and designed the experiments: DT ME DB MC DD RF MFG TG Raquel Gur Rahen 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 MF NG TG Raquel Gur Rahen Gur WH LCL GAL AO KN L. Seidman L. Siever J. Silverman WS J. Sprock CS NS MT BT AR.

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