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Archival Report

Advanced Paternal Age and Early Onset of Schizophrenia in Sporadic Cases: Not Confounded by Parental Polygenic Risk for Schizophrenia

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ABSTRACT

BACKGROUND: Whether paternal age effect on schizophrenia is a causation or just an association due to confounding by selection into late parenthood is still debated. We investigated the association between paternal age and early onset of schizophrenia in offspring, controlling for both paternal and maternal predisposition to schizophrenia as empirically estimated using polygenic risk score (PRS) derived from the Psychiatric Genomics Consortium.

METHODS: Among 2923 sporadic schizophrenia cases selected from the Schizophrenia Trio Genomic Research in Taiwan project, 1649 had parents' genotyping data. The relationships of paternal schizophrenia PRS to paternal age at first birth (AFB) and of maternal schizophrenia PRS to maternal AFB were examined. A logistic regression model of patients' early onset of schizophrenia (≤18 years old) on paternal age was conducted.

RESULTS: Advanced paternal age over 20 years exhibited a trend of an increasing proportion of early onset of schizophrenia (odds ratio per 10-year increase in paternal age = 1.28, $p = .007$) after adjusting for maternal age, sex, and age. Older paternal AFB also exhibited an increasing trend of paternal schizophrenia PRS. Additionally, a U-shaped relationship between maternal AFB and maternal schizophrenia PRS was observed. After adjusting for both paternal and maternal schizophrenia PRS, the association of paternal age with patients' early onset of schizophrenia remained (odds ratio = 1.29, $p = .04$).

CONCLUSIONS: The association between paternal age and early onset of schizophrenia was not confounded by parental PRS for schizophrenia, which partially captures parental genetic vulnerability to schizophrenia. Our findings support an independent role of paternal age per se in increased risk of early onset of schizophrenia in offspring.

Keywords: De novo mutation, GWAS, Paternal age, Polygenic risk score, Schizophrenia, Selection into late fatherhood

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Evidence is accumulating that advanced paternal age is associated with a variety of psychiatric disorders (1–8). In particular, advanced paternal age has been associated with an increased risk of schizophrenia in a meta-analysis of 12 studies (4). Meanwhile, a three-generation study showed that not only paternal but also grandpaternal age was associated with an increased risk of schizophrenia in offspring, showing that the paternal age effect could accumulate across generations (9). Furthermore, older paternal age was associated with an earlier onset in a sample of consecutively collected patients with schizophrenia (10) as well as among co-affected siblings in multiplex schizophrenia families (11).

The de novo mutations hypothesis has been postulated to explain the paternal age effect (12). There are numerous divisions in spermatogonial stem cells over the life span, and delaying fatherhood may produce more mutations (13) and increase the occurrence of more severe biological sequelae (14). Recent whole-genome sequencing studies indeed have demonstrated a linear relationship between paternal age and the rate of de novo mutations in offspring, with approximately two de novo mutations per increased year of paternal age (15), in addition to providing evidence that the father transmits three to four times more de novo mutations than the mother does (15,16). However, empirical evidence quantifying the magnitude of offspring psychiatric risk conferred by paternal age–related de novo mutations is still lacking.

Alternatively, the association between advanced paternal age and an increased risk of schizophrenia may result from

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confounding by the selection into late fatherhood, which may reflect fathers' own increased predisposition to schizophrenia (17). This line of reasoning has been examined by analyzing the risk of schizophrenia within national data by excluding first-born children in each family (17–19). After adjusting for paternal age at first birth (AFB) of the child, the father's age at the conception of second-born or later-born children was not associated with an increased risk of schizophrenia, despite the father's de novo mutations having accumulated over time (17–19). However, the coefficient of paternal age at the birth of the second-born or later-born children in these analyses may diminish owing to its high collinearity with paternal AFB.

It remains challenging to examine the paternal age effect with an appropriate control for the potential confounding by selection into late fatherhood. With the advent of genome-wide association studies (GWASs) and polygenic risk score (PRS) approaches, it has become feasible to empirically estimate the parental predisposition to schizophrenia as a function of the schizophrenia risk alleles they carry (20,21). Under these circumstances, an empirically determined parental predisposition to schizophrenia, e.g., a PRS derived from a GWAS of large sample size, would be an ideal alternative to control for confounding. For example, recent studies have demonstrated that the PRS of a patient could predict the patient's age at onset of psychiatric disease, such as schizophrenia (22) and depression (23), and the PRS of a patient with schizophrenia from a multiplex family could predict an earlier age at onset in co-affected siblings (24). Using this approach, mothers with greater PRSs for schizophrenia were found to have older maternal AFB (20,21). That is, greater maternal predisposition to schizophrenia may also lead to late motherhood and an increased risk of schizophrenia in offspring. Thus, both paternal and maternal predisposition to schizophrenia need to be adjusted for in the evaluation of the effect of advanced paternal age.

In this study, we assessed a large sample of patients with schizophrenia and their parents, a portion of whom had been genotyped with PsychChip, a GWAS chip of single nucleotide polymorphisms enriched with other types of genetic variants. Using these trios, this study aimed to investigate whether the effect of paternal age on the age at onset of schizophrenia remained after controlling for the parental predisposition to schizophrenia as measured by PRS.

METHODS AND MATERIALS

Participants

Patients with schizophrenia and their first-degree relatives were recruited from the S-TOGET (Schizophrenia Trio Genomic Research in Taiwan) project funded by the National Institute of Mental Health (25). This study was approved by the institutional review boards of the participating hospitals (Supplemental Methods). Written informed consent was obtained from all subjects after complete descriptions were provided.

Using the Diagnostic Interview for Genetic Studies, 3093 patients with schizophrenia from 3008 families were interviewed (26,27). Detailed clinical evaluation is shown in Supplemental Methods. Among the participants, 2923 patients who did not have apparent family history on brief clinical screening, i.e., sporadic cases, were included in this study. Based on a previous family study that conducted direct interviews with first-degree relatives of patients with schizophrenia (28), our misclassification rate of sporadic cases was likely to be less than 2%. Among the 2923 families of sporadic cases, 354 families had the proband as singleton, and the remaining 2569 families had one or more unaffected siblings, of which 920 probands were first-born offspring.

Definition of Early Onset

The Diagnostic Interview for Genetic Studies includes a psychosis section that inquires about age at onset of the first psychotic episode. Adolescence-onset or childhood-onset schizophrenia is a severe form of the disorder that may disrupt the normal processes of brain maturation and may cause neurodevelopmental deviances (29,30). In addition, patients with childhood-onset schizophrenia have a higher rate of structural genomic variations than patients with adult-onset schizophrenia (31). We categorized the age at onset into a dichotomous variable by defining early onset of schizophrenia as the manifestation of psychotic symptoms before the age of 18 years (more details are provided in Results).

Genetic Analysis

Blood samples were taken from all participants for DNA analysis. Genotyping was performed using the PsychChip array, funded by the Psychiatric Genomics Consortium (PGC), the Stanley Center for Psychiatric Research, the Friedman Brain Institute, and the Icahn Institute for Genomics and Multiscale Biology at Mount Sinai. There were 1649 trios that passed quality control and were subjected to the PRS analysis. Quality control measures included removing individuals with more than 2% missing variants and deleting the variants for a call rate <2% deviation from Hardy-Weinberg equilibrium ($p < 10^{-6}$ in controls and $p < 10^{-10}$ in cases).

Data from PGC2, a PGC meta-analysis, were used as a discovery sample (32), consisting of 34,241 cases, 45,604 controls, and 1235 parent affected-offspring trios, to identify the schizophrenia risk variants. Variants genotyped in the S-TOGET sample were used for the generation of the PRS. To remove variants in linkage disequilibrium, variants were pruned with a pairwise R^2 threshold of .5 and a sliding window size of 50 single nucleotide polymorphisms. A set of variants with p value $< .05$ for the association test with schizophrenia was chosen, which is the threshold that reached good balance between true-associated and null signals to capture the heritability of schizophrenia (32). The schizophrenia PRS was calculated using the PLINK score procedure, weighted by the log of their association odds ratio (OR) estimated from the discovery sample (33). The PRS was then normalized to a Z score.

We derived paternal and maternal 10 multidimensional scaling components based on parents' genotype to account for ancestry. The multidimensional scaling components plot (Supplemental Figure S1) indicated that there is no extensive population structure after the top four dimensions in both parents. Among the paternal and maternal 10 population stratification dimensions, none was associated with patient's early onset of schizophrenia after multiple-testing correction (smallest $p > .01$). Hence, we chose to adjust for parental first

four population stratification dimensions in the PRS association analyses.

Statistical Analysis

The theoretical model for paternal age effect on patients' early onset of schizophrenia is illustrated using the directed acyclic graph in Figure 1. Potential confounders in this model include advanced maternal age, paternal and maternal PGSs for schizophrenia, population stratification dimensions, and other demographic characteristics. We adjusted for patients' age at recruitment because both delaying fatherhood and the quality of medical care may change with time. Although the analyses were conducted conditional on case dataset (i.e., schizophrenia proband in offspring), which opens up the collider, the backdoor paths from advanced paternal age to the outcome through the collider were blocked if we fully adjusted for the common causes of advanced paternal age and the outcome.

In the whole S-TOGET sample of patients without apparent family history, we first drew a box plot of age at onset of schizophrenia by paternal age to examine whether there was a pattern. The paternal age for each patient was calculated by the father's age minus the offspring's age at the time of recruitment. Then we drew scattergrams of paternal age against patient's onset age with the bubble size proportional to the number of participants, applying locally weighted regression smoothing. If there was a linear relationship between the two, we would estimate the Pearson correlation to indicate the linear extent. The association of paternal age (exposure variable) with patients' early onset of schizophrenia (outcome variable) was examined using multivariable logistic regression with adjustment for age at recruitment, sex, and maternal age.

For the subsample with GWAS data, we first examined the relationships of paternal AFB (exposure variable) to paternal PRS for schizophrenia (outcome variable) as well as that of maternal AFB to maternal PRS for schizophrenia using linear or curve linear models (e.g., squared AFB), based on the likelihood ratio test for model selection.

Next, we explored if the effect of paternal age may be due to confounding by parental genetic risk for schizophrenia. If advanced paternal age per se played an independent role in patients' early onset of schizophrenia, the association between paternal age and early onset of schizophrenia would remain after further adding both paternal and maternal PRSs for schizophrenia into the multivariable logistic regression model that already had covariates, including maternal age, population stratification dimensions, and demographics.

Because adjusting for parental predisposition to schizophrenia by means of PGC-derived PRS is of an exploratory nature in the association of paternal age with patient's early onset of schizophrenia, we set the significance level at $p = .05$. All statistical analyses were performed using SAS version 9.4 for Windows statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Sample Characteristics

The sample of S-TOGET patients with schizophrenia without apparent family history ($n = 2923$), i.e., sporadic cases, and the subsample with GWAS data of the proband and both parents ($n = 1649$) were comparable in distributions of demographic and clinical characteristics (Table 1).

Pattern of Paternal Age and Age at Onset of Schizophrenia

Figure 2A displays the box plot of age at onset of schizophrenia by paternal age group, in which the distributions are right-skewed. Comparing the age at onsets across different paternal age groups, there was an inflection around the paternal age group of 20 to 24 years, with this group having the oldest onset (in both mean and median), whereas the younger (<20 years) and older (≥25) groups had younger ages at onset. The box plot stratified by sex is shown in Supplemental Figure S2; no significant sex differences in the paternal age effect were evident.

Figure 2B displays the scattergrams of paternal age against patient's onset age and the corresponding locally weighted regression smoothing plot. It appeared to have an inflection point around onset age of 18 years. The correlation between paternal age and patient's onset age was .01 ($p = .72$) for the early-onset subgroup (≤18 years of age),

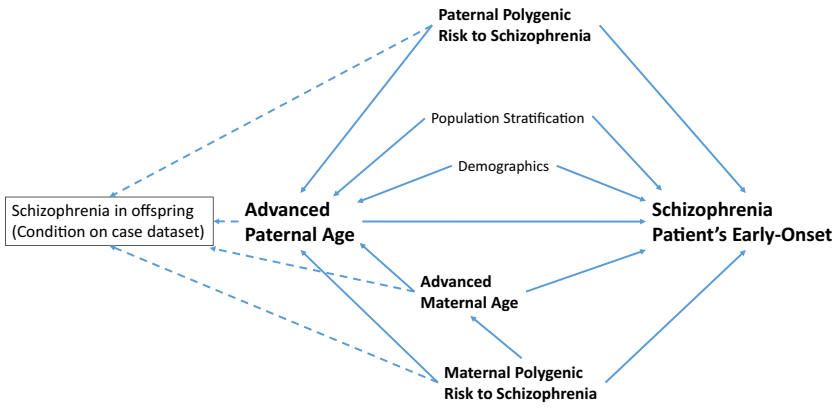

Figure 1. Directed acyclic graph of the theoretical model for paternal age effect on patients' early onset of schizophrenia. Advanced maternal age, paternal and maternal polygenic risk for schizophrenia, population stratification dimensions, and other demographic characteristics are potential confounders to the association between advanced paternal age and patients' early onset of schizophrenia. The analyses were performed within a schizophrenia case dataset. Schizophrenia in offspring is potentially caused by advanced paternal age, advanced maternal age, and paternal and maternal polygenic risk for schizophrenia. Although the analyses were conducted conditional on schizophrenia in offspring, which opens up the collider, the backdoor paths from advanced paternal age to the outcome through the collider were blocked if we fully adjusted for the common causes of advanced paternal age and outcome (i.e., parental polygenic risk for schizophrenia, advanced maternal age, population stratification, and other demographic characteristics).

Table 1. Distribution of Demographic and Clinical Characteristics in S-TOGET Families Without Apparent Family History and Subsample With PsychChip Data

Variables	Patients Without Apparent Family History	Subsample With PsychChip Data	*p* Value[a]
Total Participants	*n* = 2923	*n* = 1649	
Sex, male, *n* (%)	1782 (61.0)	1010 (±61.2)	.72
Age, years, mean (±SD)	35.7 (±8.3)	35.1 (±8.0)	< .0001
Paternal age, years, mean (±SD)	30.0 (±5.2)	30.7 (±5.2)	< .0001
Maternal age, years, mean (±SD)	25.9 (±4.1)	26.2 (±4.1)	< .0001
Onset age, years, mean (±SD)	21.9 (±6.1)	21.4 (±5.8)	< .0001
Early onset, ≤18 years old, *n* (%)	1017 (34.8)	606 (36.7)	.01
Male Participants	*n* = 1782	*n* = 1010	
Age, years, mean (±SD)	36.1 (±8.2)	35.6 (±7.8)	.004
Paternal age, years, mean (±SD)	29.8 (±5.0)	30.5 (±5.0)	< .0001
Maternal age, years, mean (±SD)	25.7 (±4.1)	26.0 (±4.2)	.0001
Onset age, years, mean (±SD)	21.9 (±6.0)	21.4 (±5.7)	< .0001
Early onset, ≤18 years old, *n* (%)	612 (34.3)	373 (36.9)	.009
Female Participants	*n* = 1141	*n* = 639	
Age, years, mean (±SD)	35.2 (±8.5)	34.3 (±8.2)	.0002
Paternal age, years, mean (±SD)	30.3 (±5.6)	31.0 (±5.5)	< .0001
Maternal age, years, mean (±SD)	26.1 (±4.1)	26.5 (±4.0)	.0001
Onset age, years, mean (±SD)	21.8 (±6.4)	21.5 (±6.1)	.03
Early onset, ≤18 years old, *n* (%)	405 (35.5)	233 (36.5)	.44

S-TOGET, Schizophrenia Trio Genomic Research in Taiwan.
[a]Difference in the distribution of demographic and clinical characteristics between subsample with PsychChip data and subsample without PsychChip data.

whereas the correlation was −.04 (p = .09) for the late-onset subgroup (>18 years of age). Patients in the early-onset subgroup had older paternal age than patients in the late-onset subgroup (30.6 years vs. 29.7 years, $p < .0001$). Because of this nonlinear pattern, patients' ages at onset were dichotomized using 18 years as the threshold for subsequent analyses. Sensitivity analyses for the definition of early onset were shown later.

Association of Paternal Age With Patient's Early Onset of Schizophrenia

Table 2 displays the distribution of early-onset schizophrenia and its relationships to paternal age and maternal age. The affected probands with a parental age of 20 to 24 years had the smallest proportion of early onset of schizophrenia (23.7% for paternal age and 30.4% for maternal age). Regarding paternal age groups, patients with paternal age <20 years had a higher proportion of early onset of schizophrenia. Paternal age groups older than 20 years exhibited a trend of an increasing proportion of early onset of schizophrenia (i.e., increasing adjusted OR [aOR] over age groups, p = .009 for the trend test) with adjustments for maternal age, sex, and age. The aOR increased 1.28-fold (95% confidence interval 1.07–1.54, p = .007) per 10-year increase in paternal age over 20 years.

Regarding maternal age groups, despite the fact that ORs for age groups 25 years or older in the crude model were significantly greater than 1, the corresponding aORs became nonsignificant after adjustments for paternal age, sex, and age. Similarly, a trend of increasing ORs over age groups for the crude model became nonsignificant for the adjusted model. The aOR per 10-year increase in maternal age over 20 years was also very small (1.04-fold) and not significant. If the age group of <20 years was chosen as the reference for both paternal age and maternal age, the results remained similar (Supplemental Table S1).

Additionally, we analyzed the age at onset of schizophrenia as a continuous variable via multiple linear regressions (Supplemental Table S2). The regression results remained similar to those of age at onset of schizophrenia as a binary variable, i.e., negative beta for continuous paternal age.

Sensitivity Analyses for Definition of Early Onset of Schizophrenia

Defining early onset of schizophrenia by different age cutoffs from 16 to 20 years, the results of the association of paternal age with patients' early onset of schizophrenia (Supplemental Table S3) remained similar (aOR ranging from 1.20 to 1.30; p value ranging from .13 to .007). The association signal was most enriched when defining early onset of schizophrenia as ≤18 years (smallest p value = .007).

Relationship Between PRS for Schizophrenia and AFB

To examine whether the PGC-derived PRS for schizophrenia was associated with late parenthood, standardized PRS scores against paternal (Figure 3A) and maternal (Figure 3B) AFB of child were plotted. Although older paternal AFB seemed to have greater paternal PRS for schizophrenia, the increasing trend did not reach statistical significance (p for trend test = .11) (Figure 3A). Regarding maternal AFB, a U-shaped relationship between maternal AFB and maternal PRS for schizophrenia (lowest maternal PRS for AFB of 25–29

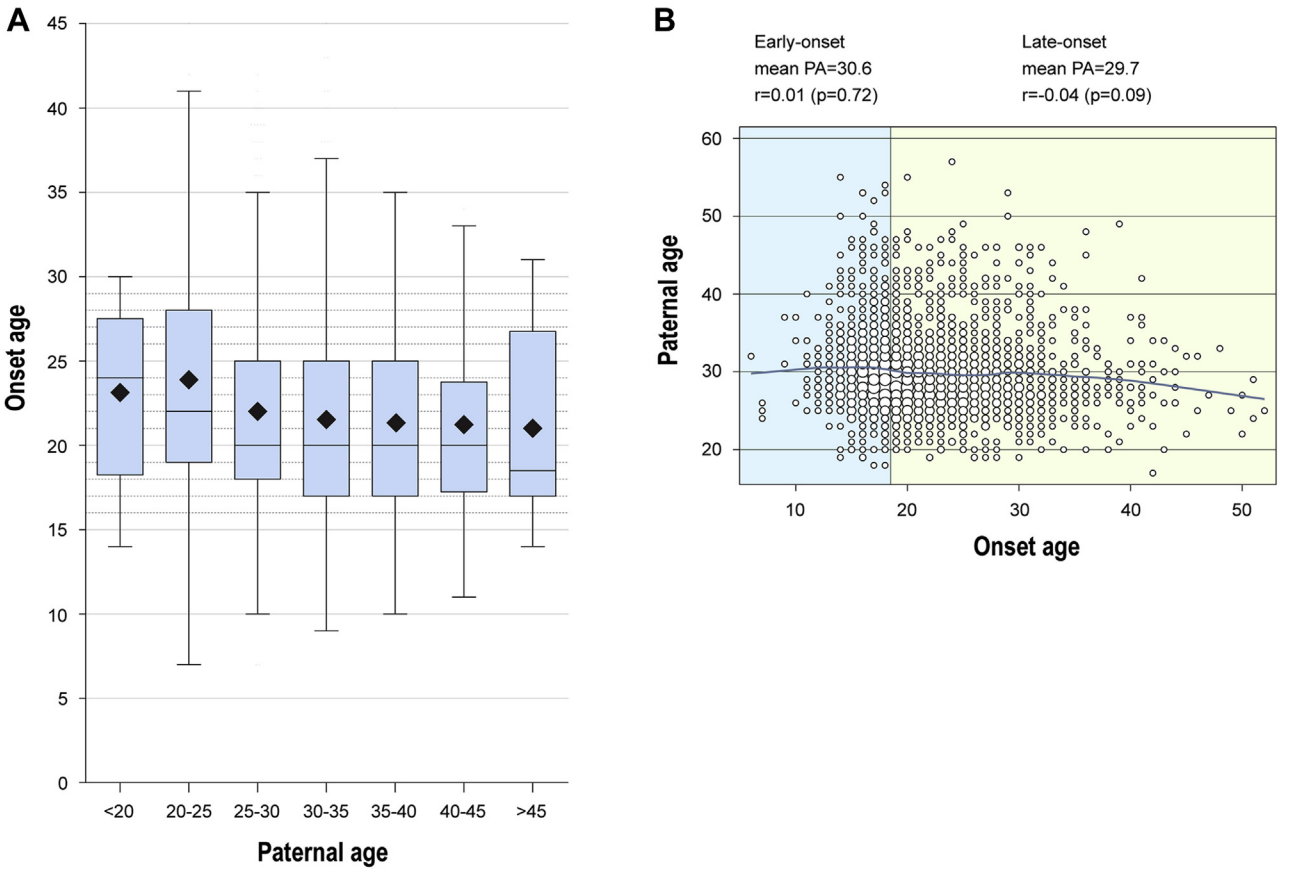

Figure 2. **(A)** Box plots of patients' age at onset of schizophrenia by paternal age among 2923 patients with schizophrenia without apparent family history. The bottom and top of the box represent the first and third quartiles; the band inside the box is the median; the diamond is the mean; the ends of the whiskers are the lowest (highest) datum still within 1.5 interquartile ranges of the lower (upper) quartile. **(B)** Locally weighted regression smoothing plot and scattergrams of paternal age against patient's onset age with the bubble size proportional to the number of participants. The distribution of paternal age and the Pearson correlation (*r*) between paternal age and patient's onset age were compared between the subgroups of early onset (≤18 years of age) and late onset (>18 years of age) of schizophrenia. PA, paternal age.

years) was observed, and the result of likelihood ratio test indicated that a polynomial model with quadratic terms for AFB led to a better fit of the curvilinear relationship (χ^2 = 3.91, p = .048).

Adjustment for Parental PRS for Schizophrenia

Using the subsample in which GWAS data of parents were available, we further evaluated whether the effect of paternal age on early onset of schizophrenia remained after adjusting for parental PRS for schizophrenia and parental population stratification dimensions. As shown in Table 3, the magnitude of the aOR (1.29, 95% confidence interval 1.01–1.65, p = .04) for per 10-year increase in paternal age remained similar to that of the whole sample shown in Table 2.

Sensitivity Analyses of Adjustment for Parental PRS for Schizophrenia

We conducted further sensitivity analysis by adjusting parental PRSs for schizophrenia that were derived at different thresholds from .5 to .000005 (Supplemental Table S4). After adjusting for the parental PRS to schizophrenia derived at the threshold with the greatest proportion explained in probands' early onset of schizophrenia, the effect of paternal age remained.

DISCUSSION

To the best of our knowledge, this study is the first to evaluate the paternal age association with patients' early onset of schizophrenia with adjustment for parental predispositions to schizophrenia using PGC-derived PRS. In a sample of patients with schizophrenia without apparent family history on brief screening, we first demonstrated an increasing trend in the proportion of early onset of schizophrenia per year increase in paternal age over 20 years after adjusting for maternal age, sex, and age. Second, older paternal AFB exhibited an increasing trend of paternal PRS for schizophrenia. Finally, we found that the association of paternal age with patients' early onset of schizophrenia remained after adjusting for both paternal and maternal PRSs for schizophrenia. Our findings provide support for an independent role of paternal age per se in the increased risk of early onset of schizophrenia in offspring.

Table 2. Logistic Regression Analysis of Early-Onset (≤18 Years Old) Schizophrenia Versus Late-Onset (>18 Years Old) Schizophrenia and Paternal Age Among 2923 Patients With Schizophrenia Without Apparent Family History in Taiwan

Variable	Sample Size, *n*	Early-Onset Cases, *n* (%)	Crude Model		Adjusted Model[a]	
			OR (95% CI)	*p* Value	aOR (95% CI)	*p* Value
Paternal Age Group, Years						
<20	14	6 (42.9)	2.41 (0.81–7.22)	.11	2.81 (0.86–9.25)	.09
20–24	270	64 (23.7)	Reference	Reference	Reference	Reference
25–29	1282	424 (33.1)	1.59 (1.17–2.16)	.002	1.42 (1.02–1.97)	.04
30–34	891	338 (37.9)	1.97 (1.44–2.69)	< .0001	1.57 (1.10–2.23)	.01
35–39	294	120 (40.8)	2.22 (1.54–3.20)	< .0001	1.63 (1.06–2.49)	.02
40–44	112	38 (33.9)	1.65 (1.02–2.68)	.04	1.52 (0.89–2.59)	.12
≥45	60	27 (45.0)	2.63 (1.47–4.71)	.001	2.18 (1.18–4.03)	.01
Trend Test for OR[b]				< .0001		.009
Paternal Age, per 10-Year, ≥20			1.39 (1.20–1.61)	< .0001	1.28 (1.07–1.54)	.007
Maternal Age Group, Years						
<20	125	39 (31.2)	1.04 (0.70–1.55)	.85	1.24 (0.80–1.92)	.34
20–24	1028	312 (30.4)	Reference	Reference	Reference	Reference
25–29	1255	443 (35.3)	1.25 (1.05–1.49)	.01	0.99 (0.82–1.21)	.95
30–34	427	183 (42.9)	1.72 (1.36–2.17)	< .0001	1.17 (0.88–1.56)	.29
≥35	88	40 (45.5)	1.91 (1.23–2.97)	.004	1.25 (0.75–2.07)	.40
Trend Test *I* for OR[c]				< .0001		.28
Maternal Age, per 10-Year, ≥20			1.60 (1.33–1.93)	< .0001	1.04 (0.82–1.32)	.72

aOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

[a]Estimated from a logistic regression model with adjustments for the age of the other parent (i.e., maternal age with regressions on paternal age and paternal age with regressions on maternal age), sex, and age.

[b]Testing for the trend of paternal age from the interval of 20–24 years to the interval of ≥45 years.

[c]Testing for the trend of maternal age from the interval of 20–24 years to the interval ≥35 years.

This study adopted a binary classification of patients' age at onset, i.e., early onset as of ≤18 years of age, because of a nonlinear pattern in the relationships of patients' onset age and paternal age. Expanding our previous finding that older paternal age was associated with an earlier onset in co-affected siblings (11), this study further demonstrated a trend of increasing proportions of early-onset cases among patients with schizophrenia without apparent family history. Intriguingly, the association was not seen for maternal age.

A major strength of this study was that we were able to adjust for not only paternal but also maternal predisposition to schizophrenia using PGC-derived PRSs in evaluating the relationship between early onset of schizophrenia and paternal age. Indeed, we found a linear trend in the relationship between increasing PRS and delaying fatherhood. Our further adjustment for maternal predisposition to schizophrenia came from two lines of evidence. We found a U-shaped relationship between maternal PRS for schizophrenia and maternal AFB, which is in accordance with previous findings in community cohorts of schizophrenia (20). Women with schizophrenia were more likely to have a child with an older man (34). Hence, this study was able to disentangle the paternal age effect from selection into late parenthood by means of the PGC-derived PRS. In contrast, previous registry-based studies (17,19) evaluated such potential confounding by means of adjusting for paternal AFB, which may suffer from statistical collinearity. Our findings for an independent role of paternal age per se in the increased risk of early-onset offspring is in line with a recent twin study, which showed that although there is a genetic component influencing late fatherhood, the paternal age association was not explained by a genetic liability for psychiatric disorders in the parents (35).

Another strength of this study was that the analyses were conducted in sporadic cases that explored the association of polygenic risk for schizophrenia, paternal age, and early onset of schizophrenia. Contrasted with familial cases that are assumed to arise mainly owing to genetic liability, sporadic cases have been assumed to arise mainly owing to higher paternal age–related de novo mutations or other environmental influences (36).

However, the current single nucleotide polymorphism–based PRS did not capture completely the genetic vulnerability to schizophrenia. In general, common susceptibility variants for schizophrenia explained a small part of the variance, 3% to 18%, of the disease in large-scale PGC studies (32,37). Furthermore, this study used cross-population GWAS results to calculate PRS, which may lower predictive variance (38). For example, the PRS derived from the PGC2, in which the majority of participants were Caucasian and the variance explained by the PRS was approximately 18% (32), explained 7% of the disease status in the S-TOGET sample of Han-Taiwanese that had PsychChip data (1649 trios in this study plus 50 trios that had family history, i.e., 1699 trios) (C.M. Liu, Ph.D., *et al.*, unpublished data, July 2018). For comparison, in a PGC meta-analysis for schizophrenia in East Asian populations (PGC Asia) that had a smaller sample size (22,778 cases and 35,362 controls) than PGC2, the PRSs derived from PGC Asia explained about 2% to 3% of the variance, whereas PRSs derived from PGC2 explained about 2% at thresholds of .05 to .5 (39). Similarly, applying the PRSs derived from PGC Asia in this study could explain approximately 4% to 6% of

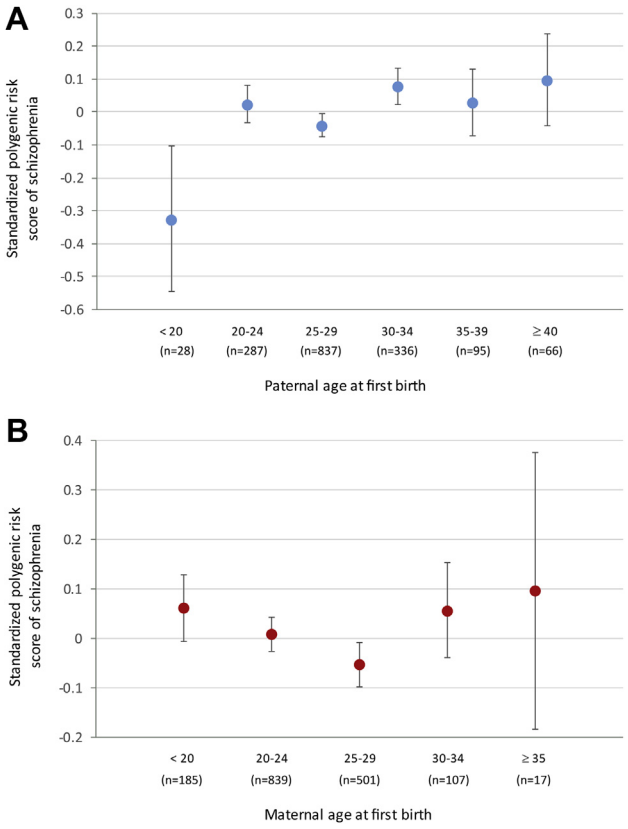

Figure 3. Mean polygenic risk score for schizophrenia grouped by age at birth of first child among 1649 patients with parental genotyping data. Error bars are SEs. **(A)** Paternal polygenic risk score of schizophrenia and paternal age at first birth, in which p for the trend test = .11. **(B)** Maternal polygenic risk score for schizophrenia and maternal age at first birth. Polynomial modeling was conducted by including either linear or quadratic terms for age at first birth, indicating a better fit of the curvilinear relationship (p = .04 for linear term and p = .05 for quadratic term).

Table 3. Association Between Paternal Age, Parental PGC-Derived PRS for Schizophrenia, and Early Onset of Schizophrenia Among 1642 Patients With Parental Genotyping Data, Excluding Those With Paternal Age <20 (n = 7)

Variable	OR (95% CI)	p Value
Model 1[a]		
Paternal age, per 10-year, ≥ 20	1.29 (1.01–1.65)	.04
Maternal age, per 10-year	0.99 (0.73–1.34)	.95
Model 2[b]		
Paternal age, per 10-year, ≥ 20	1.29 (1.01–1.65)	.04
Maternal age, per 10-year	0.99 (0.73–1.35)	.94
Paternal PRS for schizophrenia	0.99 (0.89–1.10)	.77
Maternal PRS for schizophrenia	0.96 (0.87–1.06)	.50

CI, confidence interval; OR, odds ratio; PGC, Psychiatric Genomics Consortium; PRS, polygenic risk score.

[a]Adjusted for sex, age, and father's and mother's four population stratification dimensions.

[b]Adjusted for sex, age, father's and mother's four population stratification dimensions, paternal PRS derived at the threshold of p value < .05, and maternal PRS derived at the threshold of p value < .05.

schizophrenia risk at thresholds of .05 to .5 (C.M. Liu, Ph.D., *et al.*, unpublished data, July 2018). Taken together, besides the issue of sample size for the training data, these cross-population PRS results indicate that the PGC-derived PRS does capture the genetic basis of schizophrenia shared across different populations.

In addition to the genetic vulnerability to schizophrenia, other psychosocial factors could be associated with selection into late parenthood, e.g., educational attainment, financial status, social culture, and personality. Nevertheless, a previous family study (11), which was free from such influences, compared age at onset of schizophrenia between co-affected sib-pairs with the same familial predisposition and found that the proportion of younger siblings with earlier onset was larger than that of older siblings with earlier onset, in accordance with the de novo mutation hypothesis.

Previous population genetic modeling has shown that the increased risk of psychiatric illness in children of older fathers is explained mostly by the genetic factors that are shared between parents and their offspring, and only 10% to 20% of the increased risk was explained by age-related mutations (40). Hence, the de novo mutation hypothesis and selection into late fatherhood hypothesis are not mutually exclusive (40), and both may contribute to the paternal age association.

In addition, age-related epigenetic modifications in regulating offspring's gene expression, e.g., aberrant methylation, may also contribute to the paternal age effect (8,41,42). As the epigenetic modifications are acquired over time and accumulate over a father's life span, this epigenetic process would lead to the same prediction as the paternal age–related de novo mutation. In addition, environmental factors are an alternative hypothesis proposed to explain the paternal age effect (8). Environmental characteristics, which change with the age of the father, could be associated with psychiatric development. Nevertheless, these mechanisms do not rule out each other and may work together (8,36).

Another tendency for paternal age was also noteworthy, i.e., a very young father was associated with an increased risk of early onset of schizophrenia in offspring. This finding echoed previous reports that having a younger father was associated with an increased risk of schizophrenia (4) and earlier onset of schizophrenia (11) in offspring, reflecting a bimodal influence of paternal age on psychiatric disorders. Animal research has shown that mice born from young postpubescent fathers exhibit poorer behavioral performances than mice born from mature fathers (43). The lower quality of progeny among very young fathers may be due to the immaturity of spermatids. Another possible explanation may be that an impulsive characteristic was associated with early fatherhood as well as the disease.

As paternal ages are increasing in the developed world (44), determining whether the link between advanced paternal age and increased risk and/or earlier onset of schizophrenia is just an association or a causation is becoming more important. There have been two lines of evidence: 1) the association between advanced paternal age and higher mutation rates in offspring and 2) the association between advanced paternal

age and higher risk of schizophrenia and earlier onset of schizophrenia. Further whole-genome sequencing studies are needed to directly explore the role of de novo mutations in relationships of paternal age and offspring disease risk and/or age at onset.

This study has other limitations. First, the sample size of this trios study with GWAS data is relatively small compared with case-control GWAS studies. Further studies including more trios are warranted to explore the mechanism behind the increased risk of early-onset schizophrenia in affected offspring associated with advanced paternal age. Second, the observed association between paternal polygenic risk for schizophrenia and delayed fatherhood, measured as AFB, in this case-only analysis could be spurious if advanced paternal age per se plays a role on offspring's risk of schizophrenia (see Supplemental Figure S3 for directed acyclic graph). Third, multiple hypothesis tests were conducted to assess various associations between parental age, schizophrenia PRS, parental AFB, and patients' early onset of schizophrenia; hence, chances of false-positive findings could be increased. These results were thus considered exploratory rather than confirmatory. Lastly, despite our efforts to recruit patients with schizophrenia throughout the country such that the ascertained samples largely represent the patient population of schizophrenia in Taiwan (C.M. Liu, Ph.D., *et al.*, unpublished data, July 2018), some ascertainment bias may still be present. For example, given the same age of patients, those with early onset of schizophrenia would have longer duration of illness and hence more available records for referral. Nevertheless, we have incorporated patients' age in our regression model such that the influence of duration of illness has been adjusted for. Another example of potential ascertainment bias is our requirement that a patient should have both parents alive for participation. Thus, patients with older current age may have a higher likelihood that their parents were not alive at ascertainment. Nevertheless, the interquartile range of our patients' age was 30 to 41 years. Given that the life expectancies of Taiwanese were approximately 75 years, this would not have much impact on our ascertainment.

In conclusion, using a large collection of trios, we showed that PRS for schizophrenia was associated with AFB in both parents. After adjusting for both paternal and maternal PRSs for schizophrenia, the association between paternal age and early onset of schizophrenia remained. Our findings support the independent role of advanced paternal age per se in the increased risk for early onset of schizophrenia in offspring.

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