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Paternal age of schizophrenia probands and endophenotypic differences from unaffected siblings

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ABSTRACT

We evaluated the discrepancy of endophenotypic performance between probands with schizophrenia and unaffected siblings by paternal age at proband birth, a possible marker for de novo mutations. Pairs of schizophrenia probands and unaffected siblings ($N=220$ pairs) were evaluated on 11 neuropsychological or neurophysiological endophenotypes previously identified as heritable. For each endophenotype, the *sibling-minus-proband* differences were transformed to standardized scores. Then for each pair, the average discrepancy was calculated from its standardized scores. We tested the hypothesis that the discrepancy is associated with paternal age, controlling for the number of endophenotypes shared between proband and his or her sibling, and proband age, which were both associated with paternal age. The non-significant association between the discrepancy and paternal age was in the opposite direction from the hypothesis. Of the 11 endophenotypes only sensori-motor dexterity was significant, but in the opposite direction. Eight other endophenotypes were also in the opposite direction, but not significant. The results did not support the hypothesized association of increased differences between sibling/proband pairs with greater paternal age. A possible explanation is that the identification of heritable endophenotypes was based on samples for which schizophrenia was attributable to inherited rather than de novo/non-inherited causes.

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1. Introduction

Genetic factors have long been implicated in schizophrenia, but identifying specific genetic causes has been difficult; it is highly likely that multiple genes of modest effect are involved, but rare de novo (i.e. non-familial) genetic variants also appear to have key roles in some cases (Gejman et al., 2011). For genetic studies seeking to isolate specific genes related to schizophrenia liability,

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it would be useful to identify characteristics that might separate cases with primarily familial variants from those with de novo genetic mutations.

Previous studies observed the following generalities: (a) Increased age is associated with a higher rate of de novo mutations and other genomic abnormalities in male germ cells (Crosnoe and Kim, 2013; Singh et al., 2003); (b) Such abnormalities are more common in schizophrenia than in normal controls (Walsh et al., 2008); and, (c) Older paternal age is associated with greater risk of schizophrenia (Malaspina, 2001). A model that explains how (a) and (b) could imply (c) is that de novo mutations and other genomic abnormalities are more frequently among the contributing factors leading to schizophrenia when the proband has an older father versus a younger one (Kong et al., 2012). It is further supported by a report that schizophrenia patients without a family history of schizophrenia have significantly older fathers than those with such a history (Malaspina et al., 2002). In this model, the proportion of schizophrenia attributable to de novo mutations is larger when the father is older. Conversely, inherited schizophrenia and its associated characteristics will be less common as the father's age increases. Although each piece of the model has been tested, the full model has not been directly tested. The present study was designed to test whether the age of the father at the birth of a schizophrenia proband – called "paternal age" – might be useful in this way for genetic studies of schizophrenia endophenotypes.

An endophenotype is a heritable characteristic associated with a disorder of interest that may be more strongly associated with some liability genes than others. Using endophenotypes may thus reduce genetic complexity, compared with the disorder itself (Gottesman and Gould, 2003), although some traits may possess their own complexity. A defining characteristic of an endophenotypic deficit is that – compared with the general population – not only will it be more prevalent in those with the disorder, but also in their relatives without the disorder, albeit perhaps smaller (Gottesman and Gould, 2003). Heritability implies that measures of endophenotypes will tend to be more similar between probands and their siblings than either would be with unrelated unaffected individuals. In contrast, when the genetic component of the disease is primarily caused by de novo genetic mutations, probands and their unaffected siblings, who are not monozygotic twins, would have more discrepant measures of endophenotypes.

We hypothesized that the endophenotypic discrepancy would increase as paternal age increased. To test this hypothesis, we used the Consortium on the Genetics of Schizophrenia (COGS) family database (Calkins et al., 2007) of schizophrenia proband families with one unaffected sibling (called here "sibling/proband pairs"). From a battery of psychophysiological and neuropsychological endophenotypes, we selected the 11 previously identified in a family-based study as heritable (Greenwood et al., 2007). For each sibling/proband pair, we summarized the differences in scores on those endophenotypes that were measured on both members of the pair, and tested whether in fact endophenotypic discrepancies increased as paternal age increased.

2. Methods

2.1. Subjects

Subjects were participants in the COGS-I, a multi-site family-based study of the genetics of neuro-cognitive and neurophysiological endophenotypes associated with schizophrenia. Full details of the COGS-I recruitment and assessment procedures are reported elsewhere (Calkins et al., 2007) and included standardized clinical assessment interviews of all subjects. All participants who are endophenotyped were between the ages of 18–65 and able to understand and provide informed consent. Consenting parents older than 65 had their blood drawn for genotyping purposes but did not undergo endophenotyping. The core family structure for COGS-I required at least one sibling/proband pair, i.e., the presence of a proband with schizophrenia and at least one unaffected full sibling. The probands were required to meet criteria for DSM-IV schizophrenia, following a standardized clinical assessment, medical record review, and best estimate diagnostic evaluation. Unaffected siblings never met criteria for any psychotic disorder, major affective disorder, or schizotypal personality disorder by the same clinical assessment. Subjects were screened for illicit drug or alcohol use at the time of testing and excluded if they tested positive. Also excluded were subjects who met criteria for a substance abuse disorder in the past month or a substance dependence disorder in the past 6 months. In the present study, a sibling/proband pair from each family was identified from those for whom the paternal and maternal ages at birth of the proband were known. Interviewers and endophenotypers were not blind to diagnostic group, but blind to the hypothesis of the study. An endophenotype that was tested in both the sibling and the proband from the same family was called a "shared endophenotype." If there was more than one unaffected sibling, we used the one with the largest number – at least two – of shared endophenotypes (if tied, the oldest sibling).

2.2. Endophenotype assessment

From the COGS endophenotype assessment battery, we selected those among the primary measures previously identified as heritable in a family-based study (Greenwood et al., 2007), and described in detail elsewhere:

1) *Antisaccade performance*. In this test of oculomotor inhibition, participants are asked to fixate on a central target and respond to a peripheral cue by looking in the opposite direction at the same distance. The ratio of correct antisaccades to total interpretable saccades is measured (Radant et al., 2007).
2) *Prepulse Inhibition of the startle response* (*PPI*). Prepulse inhibition refers to the dampening of a response to a strong startling stimulus (pulse) when the stimulus is preceded by a weaker prestimulus (prepulse).This was measured as the percentage of inhibition of the startle reflex in response to a weak pre-stimulus using a 60-ms interstimulus interval (Braff et al., 1978; Braff et al., 2001).
3) *Continuous Performance Test* (*CPT*). The Degraded Stimulus version of the CPT (DS-CPT) is a widely used measure of deficits in sustained, focused attention with a high perceptual load. Participants detect a blurred target digit in a series of blurred other digits. DS-CPT performance was measured using the signal/noise discrimination index (*d′*) (Nuechterlein and Asarnow, 1999; Nuechterlein et al., 1983).
4) *Letter-Number Span* (*LNS*). This is a measure of working memory information storage with manipulation. It is measured as the correct reordering of intermixed numbers and letters (Horan et al., 2008; Gold et al., 1997; Lenzenweger and Gold, 2000).
5) *California Verbal Learning Test* (*CVLT*). The CVLT is a list-learning test that assesses verbal learning and memory. The total recall score of a list of 16 verbally presented items summed across five trials is measured (Stone et al., 2011).
6) *Penn Computerized Neurocognitive Battery* (*CNB*). The Penn CNB is a computerized neuropsychological test battery used to assess a range of neuropsychological functions (Gur et al., 2001b; Gur et al., 2001a). Each test is measured as "efficiency," a combination of accuracy (percentage correct) and speed (median response time in milliseconds), which is calculated as accuracy/log 10 (speed) and is expressed as standard equivalents (*z* scores). In the COGS modification of the Penn CNB, the following six cognitive domains were found to be heritable in the earlier family-based study and thus included in the present study:

a) abstraction and mental flexibility – four objects are presented on a computer screen and the participant must choose the one that does not belong;
b) face memory – participants are asked to recognize 20 previously presented target faces among 20 distracter faces;
c) spatial memory – identical format as face memory except that Euclidean shapes, rather than faces, are used;
d) spatial processing – two lines are presented at an angle, and the corresponding lines must be identified on a simultaneously presented array;
e) sensorimotor dexterity – the participant is asked to click as quickly as possible using the computer mouse on a target that gets increasingly smaller; and
f) emotion processing – involves correctly identifying a variety of facial expressions of emotion.

2.3. Statistics

For each of the endophenotypes, it was hypothesized that the difference between the unaffected sibling and the proband would be larger for older paternal age. If we had complete data, the hypothesized relationships for each endophenotype could be tested by a repeated measures analysis of covariance, with the dependent variables being the sibling score minus the proband score in each