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The Relationship Between Advanced Paternal Age and Clinical Indicators Among Individuals at Clinical High Risk for Psychosis

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M.A., Emory University, 2012

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Abstract

The Relationship Between Advanced Paternal Age and Clinical Indicators Among Individuals at Clinical High Risk for Psychosis
By Arthur T. Ryan

Since the middle of the twentieth century, researchers have demonstrated that children born to fathers of advanced age are at increased risk for a variety of health conditions, including psychotic disorders such as schizophrenia. Modern research has shown that this effect may be due to accumulating de novo mutations in the germ line sperm cells of older men. During the last twenty years, researchers have begun to investigate the pre-morbid period before the development of psychotic illnesses. Much of this research has focused on individuals determined to be at increased risk of developing a psychotic illness on the basis of clinical signs and symptoms, i.e., at clinical high risk (CHR) for psychosis. This dissertation seeks to combine these two lines of research. The relationship between paternal and maternal age with attenuated positive symptoms, negative symptoms, social functioning, and family history of psychotic illness was examined within a sample of CHR individuals. No significant relationship between paternal age and these variables was found. Maternal age was shown to have a mixed relationship with positive symptoms, in that increased maternal age predicted the presence of attenuated positive symptoms, but was inversely correlated with their severity. The null results for paternal age are interpreted in the context of the established findings linking paternal age and offspring risk for schizophrenia. Ideas for future studies to further elucidate the relationship between parental ages and psychotic illnesses are discussed.

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Schizophrenia and other psychotic illnesses, e.g., bipolar disorder with psychotic features, rank among the top ten causes of disability in developed countries worldwide (Murray & Lopez, 1996). Modern treatments for schizophrenia do not reliably alleviate symptoms or restore functioning in a majority of afflicted individuals (Aggen & Johnson, 2004). Likewise, the etiology and biological underpinning of schizophrenia remain poorly understood. In order to understand this complex and debilitating disease, researchers are now focusing upon the premorbid period preceding the onset of schizophrenia symptoms, known as the clinical high risk (CHR) or "prodromal" period (Correll, Hauser, Auther, & Cornblatt, 2010). This dissertation seeks to contribute to this body of research by exploring the relationship between advanced paternal age at conception (i.e., the offspring of older fathers) and the symptoms and functioning of CHR individuals.

Background Literature Review

The Clinical High Risk Syndrome for Psychotic Illness

Clinicians have long noted that the onset of schizophrenia is often proceeded by a retrospectively identifiable period of non-specific symptoms, functional decline, and distress. Nearly one hundred years before the term "schizophrenia" itself was coined, British psychiatrist John Haslam described how "The attack [of schizophrenia] is almost imperceptible; some months usually elapse before it becomes the subject of particular notice, and fond relatives are frequently deceived by the hope that it is only an abatement of excessive vivacity…" (Haslam, 1809). Haslam's period of decline is now be referred to as the as the Clinical High Risk (CHR) state or, when retrospectively assessed in individuals who have gone on to develop schizophrenia, the schizophrenia prodrome. The

CHR period remained relatively unstudied throughout most of the following two centuries of research into schizophrenia and its etiology. In the past two decades, however, research into the CHR state has rapidly expanded (Correll et al., 2010). This precipitous increase was triggered by the development of clinical interviews which could prospectively identify a cohort of individuals, 20% to 40% of whom would go on to develop a full-blown psychotic illness within a few years (McFarlane, 2011). Studies have documented pathological changes within the brain that precede the development of full blown psychotic symptoms (Lodge & Grace, 2011). It is hoped that research on the CHR period may help to characterize, arrest, and ultimately reverse these pathological changes.

While schizophrenia is the psychotic illness most often associated with the CHR state, CHR individuals often go on to develop a variety of other psychotic disorders, e.g., bipolar disorder with psychotic features (Woods et al., 2009). This review will mostly focus on studies of individuals with schizophrenia, though many of the individual findings described below will apply to other psychotic illnesses and schizophrenia spectrum disorders more generally (e.g., schizotypal personality disorder). For clarity, key psychosis-related terms are defined in Table 1.

Researchers determine whether an individual meets criteria for the CHR state through the use of one of several structured interviews. Several "CHR syndromes" (i.e., sets of diagnostic criteria) have been proposed by researchers. Individuals meeting criteria for any one of these CHR syndromes are considered to be at CHR. Broadly speaking, these syndromes fall into 3 categories: (1) attenuated positive symptoms (i.e., attenuated manifestations of delusional thinking, hallucinations, or thought disorder) that

have begun or increased in intensity in the last year, (2) high genetic risk for psychosis (i.e., having a first or second degree relative with a psychotic disorder) and a recent decline in functioning, and/or (3) brief and self-limiting psychotic symptoms with a recent onset (Addington et al., 2007). While inclusion criteria vary somewhat between studies, the 20% to 40% two-year incidence rate of schizophrenia in CHR individuals demonstrated across studies is several hundred times greater than the corresponding incidence rate in the general population of 0.4 per 1,000 individuals, suggesting that CHR criteria are useful even while they remain under active revision and research (Eaton, 1999).

The CHR state is associated with a suite of clinical symptoms, cognitive deficits, and abnormal biological markers. CHR individuals usually experience attenuated positive symptoms. For example, a CHR individual might notice that voices sound oddly distorted without experiencing full-blown auditory hallucinations. Importantly, CHR individuals maintain some insight into their positive symptoms, e.g., believing that their auditory experiences might be a product of their brain, which differentiates their symptoms from the delusional conviction seen during psychotic illness. CHR individuals also have cognitive, social, and functioning deficits intermediate between those of healthy controls and individuals with psychotic illnesses. They evince abnormal scores on neuroanatomical (e.g., grey matter volume) and other biological (e.g., salivary cortisol) measures, again intermediate between those of healthy controls and individuals with psychotic illnesses (Correll et al., 2010). CHR individuals also often suffer from comorbid mood and anxiety disorders, including major depression and social anxiety (Rosen, Miller, D'Andrea, McGlashan, & Woods, 2006).

Advanced Paternal Age and Offspring Outcomes

For decades, researchers have known that individuals born to older fathers are at increased risk for certain genetic disorders (Penrose, 1955). However, research and public knowledge of this phenomenon has increased precipitously in the last two decades (e.g., Gupta, 2014). This growing body of research has linked father's age with risk for a range of disorders, including schizophrenia (McGrath et al., 2014).The section that follows will describe the changing demographics of fatherhood, its association with various offspring outcomes, and possible mechanisms that may underlie this association.

Operationalizing advanced paternal age. What constitutes advanced paternal age has varied across cultures, historical periods, and research programs. In Western countries, average maternal and paternal ages at conception have been climbing steadily since from the 1970s to the present (OECD, 2014). For example, in England and Wales between 1970 and 2002, the average parent's age at their child's birth rose from 26.4 to 29.3 in women and from 29.2 to 32.1 in men (Bray, Gunnell, & Smith, 2006).

While a variety of cutoffs for father's age have been employed, studies have generally found a linear negative effect of father's age on offspring outcomes above a lowest risk age range for fathers in their twenties (B. Miller et al., 2011). Before continuing, it should be noted that the offspring of fathers whose ages are below the lowest risk age range may also be at increased risk for poor health outcomes. Some studies have found increased risk for schizophrenia in the offspring of very young fathers, though these findings have been mixed (B. Miller et al., 2011). Offspring of very young fathers are at increased risk for congenital abnormalities and preterm births (Archer, Langlois, Suarez, Brender, & Shanmugam, 2007). Younger paternal age is associated

with lower offspring brain volumes (Shaw et al., 2012); this effect has been replicated in controlled studies of non-human animals (Ryzhavskii et al., 2004). Little is known about the mechanisms that underlie the young father effect, but evidence suggests that these mechanisms are likely to be different than those underlying the older father's age effect (Auroux, Nawar, Naguib, Baud, & Lapaquellerie, 1998; McGrath et al., 2014).

Older father's age and schizophrenia. There is now a consensus among researchers that the offspring of older fathers are at increased risk for developing schizophrenia. A 2011 meta-analysis found that offspring born to fathers who were 50-years-old or older were 1.66 times more likely to develop schizophrenia than were offspring born to fathers between the ages of 25 and 29: this effect was present in both male and female offspring and generally consistent across studies (B. Miller et al., 2011). This meta-analysis also contained an estimate of the population attributable risk percentage (PAR%) of father's age. PAR% is a statistic designed to provide a rough estimate of the percentage of affected individuals who would not have developed an illness if they had not been exposed to the risk factor. The averaged PAR% for male and female offspring of fathers 30-years old or older was about 10% across the studies included in their analysis. To put this in perspective, this analysis suggests that about 10,000 of the estimated 100,000 new cases of schizophrenia that develop in the United States each year (McGrath et al., 2004) could be prevented if all fathers had their children between the ages of 25 and 30. Again, this number can only be considered a rough estimate, as PAR% is influenced by a variety of factors (e.g., prevalence of other risk factors, proportion of older fathers); however, this result suggests that father's age is an important topic for research and a potential target for public health interventions. In

addition to the diagnosis of schizophrenia, a few studies have linked father's age with the severity of schizophrenia symptoms. For example, offspring of older fathers show more severe symptoms during medication-free periods than do patients with younger fathers (Rosenfield et al., 2010).

Father's age, autism spectrum disorders, and social functioning. Father's age is a well-established risk factor for autism spectrum disorders (Reichenberg et al., 2006) and social functioning deficits more generally (Weiser et al., 2008). A recent meta-analysis of population based studies suggested that offspring born to men 50-years-old or older are 2.2 times more likely to have autism than men aged 29 years or younger (Hultman, Sandin, Levine, Lichtenstein, & Reichenberg, 2011). The increasing age at which men have children has been suggested as one of the factors contributing to the increased rate of autism diagnoses within the United States over the course of the last 20 years. The link between father's age and autism is particularly relevant to the study of schizophrenia and the CHR state, as autism spectrum disorders and social impairment are relatively common among individuals with schizophrenia, schizophrenia-related personality disorders, and CHR syndromes (Esterberg, Trotman, Brasfield, Compton, & Walker, 2008). This suggests a potential overlap in the etiology, neuropathology, and symptomatology of autism spectrum disorders and schizophrenia spectrum disorders**.**

Father's age and other offspring outcomes. Father's age has been associated with increased risk for a wide range of neurological and psychiatric conditions. These include bipolar disorder (Frans et al., 2008) and ADHD (D'Onofrio et al., 2014), mental illnesses whose symptomatology overlaps partly with schizophrenia's (e.g., delusional thinking and attention deficits, respectively). Father's age is also associated with

intellectual and learning deficits, including dyslexia (Saha et al., 2009) and intellectual disability (McGrath et al., 2014). Offspring of older fathers are more likely to suffer from disorders reflecting abnormal neural development, such as epilepsy (Vestergaard, Mork, Madsen, & Olsen, 2005), hydrocephalus (Savitz, Schwingl, & Keels, 1991), and neural tube defects (McIntosh, Olshan, & Baird, 1995). They are also more likely to suffer from disorders of fetal development, including cleft palate, congenital heart defects (Archer et al., 2007), Apert syndrome (Moloney et al., 1996), and fetal death more generally (Andersen, Hansen, Andersen, & Smith, 2004). Father's age is also associated with some diseases which, like schizophrenia, do not have their onset until much later in the offspring's life. These include some adult onset cancers (Zhang et al., 1999) and Alzheimer's disease (Bertram et al., 1998).

In addition to diagnosable illnesses, father's age has also been associated with neuroanatomical measures relevant to schizophrenia research. Paternal age above 30 is inversely correlated with grey matter volume in offspring, particular when measured in terms of cortical surface area (Shaw et al., 2012). Father's age is also associated with lower scores on a variety of neurocognitive tests, e.g., tests of intelligence and academic achievement (Saha et al., 2009).

Moderators of father's age and offspring outcomes. The strength of the relationship observed between father's age and offspring outcomes can depend upon which moderator variables are measured and how they are controlled. Different causal explanations are likely to underlie the effects of different moderators: the research described in this section will be used to illustrate potential moderators and their relevance to this dissertation. Some moderators are likely to be explicable using epidemiological

facts about an illness. For example, the adjusted odds ratio of autism in female offspring of older fathers is roughly three times greater than the adjusted odds ratio for male offspring of older fathers (Reichenberg et al., 2006). This difference in the odds ratio between male and female offspring may reflect the low base rate of autism in female offspring as opposed to a greater effect of father's age upon female offspring measured in absolute terms. In other words, a 1% absolute increase in the rate of autism disorders among both male and female offspring of older fathers would necessarily result in a relatively larger proportional increase among female offspring, among whom the disorder is more rare, thus inflating the adjusted odds ratio.

In contrast, other moderators may reflect genuine differences in the biological effects of father's age in various subpopulations. For example, maternal-fetal blood incompatibility may increase schizophrenia risk in male, but not female, offspring (Insel, Brown, Bresnahan, Schaefer, & Susser, 2005). If maternal-fetal blood incompatibility was associated with father's age, then the greater impact of father's age on male offspring would reflect genuine biological differences between male and female offspring.

Summary of father's age and offspring outcomes. Looking across studies of father's age and negative offspring outcomes reveals several common trends. First, many father's age-associated disorders are known to involve abnormal fetal development (e.g., cleft palate abnormalities and autism spectrum disorders). Secondly, many of these disorders are thought to potentially result from a variety of etiological pathways (e.g., maternal infections vs. genetic abnormalities). These trends suggest that careful consideration of the developmental processes known to go awry in father's age-

associated illnesses may provide clues as to how having an older father might predispose an individual towards developing schizophrenia-related neuropathology.

Causal Underpinnings of the Relationship between Father's Age and Offspring Outcomes

Confound hypotheses. Before considering potential causal explanations for the correlation between father's age and schizophrenia, it's important to address potential confounded variables which could account for this effect. Researchers who argue that the relationship between father's age and schizophrenia is a spurious one suggest that the observed correlation is the result of "operational confounding," i.e., that measuring father's age inadvertently measures something else, and that it is this unobserved variable which accounts for the relationship between father's age and schizophrenia. Some authors have argued that age-related epigenetic and obstetric factors are examples of such confounded variables which explain the link between father's age and schizophrenia (McGrath et al., 2014). Other authors have argued that age-related genetic and obstetric factors represent the biological underpinnings of the father's age effect in the same way that brain neuropathology mediates the effects of familial genetic risk on schizophrenia symptoms.

To avoid semantic or conceptual confusion, this dissertation will employ a straightforward working definition of confounding as it relates to father's age and schizophrenia. For the purposes of this dissertation, the father's age-schizophrenia relationship is spurious (i.e., better explained by confounded variables) to the extent that evidence suggests that the same fathers choosing to have children at a younger age would <u>not</u> lower their offspring's risk of developing schizophrenia. Reciprocally, the father's

age-schizophrenia relationship is not spurious to the extent that research suggests that fathers choosing to have children at a younger age would decrease the total number of cases of schizophrenia in their offspring, whatever the intervening causal mechanisms are that mediate that effect.

As an illustrative example of this distinction, if it were the case that the correlation between a father's age and their offspring's risk for schizophrenia was fully explained by correlations between (a) paternal age and maternal age, and (b) maternal age and offspring schizophrenia, then a successful public health campaign that lowers the average father's age without any change in the average mother's age would have no effect upon the rate of schizophrenia in offspring. In contrast, if the offspring of older fathers are more likely to have obstetric complications due to a de novo mutations more common in the sperm of older fathers, then a successful public health campaign to lower the average father's age *would* decrease the number of cases of schizophrenia, even if the average mother's age did not change in the slightest.

The first, and possibly most obvious, potential confound between father's age and offspring outcomes is mother's age. Mother's age and father's age are strongly correlated (McGrath et al., 2014). Given the well-known correlations between mother's age and offspring outcomes, the possibility of mother's age explaining the father's age-schizophrenia link must be addressed. In the case of father's age and schizophrenia link, however, this possibility seems unlikely. The relationship between father's age and offspring schizophrenia has generally increased in strength in studies that have controlled for mother's age (Brown et al., 2002; Malaspina et al., 2001; Zammit et al., 2003). This continued or even strengthened statistical relationship between father's age and offspring

outcomes after controlling for mother's age has also been found in studies of other mental illnesses, including autism spectrum disorders and bipolar disorder (Sanders et al., 2012). While an in depth discussion of biological mechanisms will be postponed until a later section, it is worth noting here that in studies that have compared father's age and mother's age in predicting biological markers associated with schizophrenia risk (e.g., de novo mutations in the offspring), the relationship between mother's age and those biological markers has tended to disappear once father's age has been controlled for (Kong et al., 2012), further supporting the significance of father's age over mother's age.

The statistical relationship between father's age and schizophrenia risk is significant even though father's age correlates with several demographic variables known to decrease offspring risk for schizophrenia. These include parents' years of education and higher socio-economic status (Werner, Malaspina, & Rabinowitz, 2007). Unsurprisingly, studies which control for these protective factors often show an increased relationship between father's age and schizophrenia (B. Miller et al., 2011). In addition to being correlated with protective factors, however, father's age is also correlated with several purported risk factors for schizophrenia. One of the most cited potentially confounding factor is paternal social deficits. Relatives of schizophrenia probands (including their fathers) are more likely to display a variety of social and cognitive deficits, and these deficits are associated with delays in engaging in normative social and romantic relationships (Jaffe, Eaton, Straub, Marenco, & Weinberger, 2014). Thus, a man with schizophrenia risk genes may have difficulty with (or reduced interest in) securing a romantic partner, may marry at an older age, and may father his children at an older age. In this scenario, the relationship between the father's age and his offspring's risk for

schizophrenia would be explained by the behavioral manifestations of his schizophrenia diathesis (i.e., risk genes) and have nothing to do with his age, per se. While this possibility may contribute somewhat to the effects of advanced paternal age, studies suggest that it is not the primary contributor to the effect, as it remains significant even when father's level of social functioning is controlled for (B. Miller et al., 2011). In addition, studies which have compared siblings within families have found that children born later in the father's life span have a greater risk of developing psychotic illness, a finding which would be difficult to explain using the "schizophrenia risk genes lead to delayed fatherhood" hypothesis (D'Onofrio et al., 2014). Some autism researchers have also suggested that impaired paternal social functioning may explain the link between father's age and offspring risk for autism spectrum disorders (Piven, 2001). Just as in the schizophrenia literature, however, studies which have controlled for fathers' subclinical autistic traits have still found a relationship between father's age and offspring autism risk (Hultman et al., 2011); in fact, the relationship between father's age and offspring autism is strongest among autism probands without familial cases of autism (O'Roak et al., 2012).

In summary then, while the possibility that the relationship between father's age and offspring risk for schizophrenia is the result of a confounded variable is a legitimate concern that should continue to be tested, the majority of studies that have examined this possibility have found that the relationship between father's age and schizophrenia remains significant or even strengthens after controlling for potentially confounding factors. As such, this review will now turn to examining hypotheses that propose causal links between father's age and offspring risk.

Social transmission hypotheses. A subset of hypotheses which seek to explain the relationship between father's age and offspring risk for schizophrenia posit that older fathers engage in some sort of behavior which increases the risk for schizophrenia in their offspring. This possibility is not to be dismissed offhand, as there are several potent and well-established risk factors for psychotic illness which are potentially associated with parental behavior, e.g., childhood sexual abuse (Holtzman et al., 2013). Generally speaking, however, research has not supported the social transmission hypothesis of father's age and risk for schizophrenia. Firstly, as described earlier, father's age has remained a significant predictor in studies which have controlled for demographic variables (e.g., poverty). Secondly, when measured directly, established social risk factors for schizophrenia (e.g., unwanted pregnancy) have generally been found to be uncorrelated with father's age (Herman et al., 2006). Thirdly, father's age has also been associated with a host of congenital disorders where the father's behavior is less likely to increase risk for the illness (e.g., achondroplasia). Finally, adoption studies have shown no link between risk for schizophrenia and the adoptive father's age, while biological father's age remains associated with the offspring's risk for schizophrenia (Ek, Wicks, Magnusson, & Dalman, 2012). All of these findings suggest that even if certain schizophrenia predisposing social behaviors are associated with father's age, these cannot explain the entire relationship between father's age and schizophrenia risk. As such, this review now turns to potential biological explanations of this link.

Biological transmission hypotheses. Biological transmission hypotheses for father's age propose that (a) father's age is causally associated with schizophrenia risk and (b) that this relationship is partly explained by biological differences between the

sperm of younger and older fathers which has downstream effects upon the biology of their offspring which, in turn, predisposes those offspring to schizophrenia. As is so often the case in schizophrenia research, however, it is quite probable that more than one mechanism could be acting cumulatively to increase the risk of schizophrenia in the offspring of older fathers.

De novo mutations. De novo mutations in germ line sperm cells are common and can be found among many apparently healthy individuals (Kong et al., 2012). The following paragraphs will briefly review the biology of gamete production and how it differs in males and females. Understanding these differences will assist in the evaluation of biological transmission hypotheses.

In women, there are 22 mitotic cell divisions in utero (Crow, 2000). These divisions produce all of the ova that a women will go on to use during reproduction across her lifespan. In contrast, progenitor sperm cells undergo 30 divisions in utero, then resume mitotic cell division with the onset of puberty, dividing once every 16 days for the rest of the man's life (Goriely, McGrath, Hultman, Wilkie, & Malaspina, 2013). To put this in perspective, at age twenty, a man's germ line sperm cells will have undergone approximately 150 cell divisions, and by age fifty they will have undergone 840 cell divisions, once again in contrast to the 22 cell divisions of a woman's egg cells. This difference between the production of male and female gametes suggests that there may be differential effects of father's age and mother's age on offspring health outcomes.

The process by which males produce mature sperm cells is known as spermatogenesis. The process begins with a type A(d) spermatogonium, a diploid (i.e., containing 2 sets of 23 chromosomes) sperm cell which serves as the progenitor for all

other male sperm cells. The type A(d) spermatogonium undergoes mitosis (i.e., cell division resulting in two diploid cells) to produce another type A(d) spermatogonium and a type A(p) spermatogonium. The resulting type A(d) spermatogonium remains to ensure a constant supply of type A(d) spermatogonium cells for future sperm production. The resulting type A(p) spermatogonium cell undergoes mitosis to produce two diploid type B spermatogonia. These type B spermatogonia undergo mitosis to produce two diploid primary spermatocytes. These primary spermatocytes undergo meiosis, producing two haploid secondary spermatocytes, each containing only one set of 23 chromosomes instead of the usual two. The secondary spermatocytes undergo meiosis a final time to produce two haploid spermatids each. These spermatids then undergo a process of maturation known as spermiogenesis which transforms the spermatids into spermatozoa. These spermatozoa are the motile versions of sperm cells which will attempt to fertilize the ovum during sexual reproduction. The important message to take away from all of this is that there are repeated cell divisions throughout the generation of spermatozoa, and that each of these replications introduces the opportunity for copying errors and mutations. In addition to this, the type A(d) spermatogonia which began this process are themselves the product of previous cell divisions occurring every 16 days over the lifetime of a sexually mature male. Any errors which accumulate in these type A(d) spermatogonia over a man's lifetime will be passed down into his spermatozoa during spermatogenesis.

As just mentioned, the mitotic cell division responsible for creating new type A(d) spermatogonia (the germline cells that provide the basis for future sperm production) occasionally results in a variety of small mutations in the resulting cells, known as de

novo mutations. These errors can be small enough so as not to compromise the viability of the offspring produced from the resulting sperm cells, and yet may have significant effects on future offspring development. Examples of these mutations include point mutations (where a single nucleotide "letter" is replaced with another), small insertions or deletions of genetic material (where short stretches of nucleotides are removed or inserted into the DNA sequence), and copy number variations (where stretches of the DNA sequence are repeated; Goriely & Wilkie, 2012). There is also some evidence that mutated spermatogonia may divide at a higher rate than other spermatogonia and thus eventually come to predominate in the pool of germline cells that gives rise to spermatozoa (Goriely et al., 2013).

The number of de novo mutations in men's spermatozoa has been shown to increase linearly with age. Kong et al. (2012) sequenced the full genomes of 78 mother/father/offspring trios. They found that each additional year of father's age resulted in an average of 2 additional de novo mutations in their offspring, whereas mother's age was not correlated with number of de novo mutations once father's age was controlled for. This suggests that the vast majority of inherited de novo mutations can be found on chromosomes inherited from the father. It is worth noting that the non-significance of mother's age once father's age is controlled for is the same pattern found in most studies of schizophrenia and autism risk discussed earlier. Schizophrenia probands in Kong et al.'s study were also found to have de novo mutations in genes previously associated with psychotic illness, mutations which were not present in their unaffected siblings. Other studies of schizophrenia and autism probands have provided corresponding evidence of

higher rates of genetic mutation and structural variation (Sebat et al., 2007; Stefansson et al., 2008).

The de novo mutation hypothesis has several important virtues in explaining the link between father's age and schizophrenia risk. Firstly, it accounts for the asymmetry between mother's age and father's age, since de novo mutations accumulate within the father's sex cells but not in the mother's sex cells. Secondly, the random mutations associated with father's age should not be specific to "schizophrenia risk genes," which is consistent with the evidence that father's age is a general risk factor for a variety of illnesses. Thirdly, it is consistent with the finding that the majority of individuals with schizophrenia do not have a first or second degree relative with a psychotic illness (Sipos et al., 2004). Fourthly, evidence from studies of autism spectrum disorders provide similar findings supporting de novo mutations as a causal factor in explaining increased risk among offspring of older fathers (Sanders et al., 2012), lending further plausibility to the father's age-schizophrenia link. Overall then, predictions made from the biological facts of de novo mutations line up with the pattern of results found in studies of individuals with schizophrenia.

Epigenetic factors. Epigenetic processes associated with father's age are an alternate or complimentary biological explanation for the link between father's age and schizophrenia (Perrin, Brown, & Malaspina, 2007). Epigenetics refers to the study of processes that either (a) yield heritable changes in gene activity that are not caused by changes in the DNA sequence, or (b) stable, long-term alterations in the likelihood that a gene will be transcribed into a protein within a cell (Jirtle & Skinner, 2007). Genomic imprinting, a form of epigenetic regulation, is present in a markedly high number of

genes regulating neurodevelopment (Tsankova, Renthal, Kumar, & Nestler, 2007), suggesting that abnormal functioning in epigenetic mechanisms could have significant effects on neurodevelopment. DNA methylation (a form of imprinting) reduces the chance that RNA will transcribe the protein a gene transcribes for. Gene methylation patterns are thought to change over the course of an individual's life within somatic cells (i.e., cells that make up the adult body) but were generally thought to be erased and reestablished in spermatogenesis and oogenesis (Casillas, Lopatina, Andrews, & Tollefsbol, 2003). However, evidence has steadily accumulated that methylation patterns acquired during adult life are at least partially passed down to subsequent generations (Dias & Ressler, 2014). Additionally, the processes of erasure and resetting of methylation patterns within germ cells may become less effective as an individual ages due to, for example, altered levels of enzymes regulating methylation (Lopatina et al., 2002). Similarly to de novo genetic mutations, abnormal DNA-methylation patterns in sperm cells has been associated increasing paternal age, perhaps reflecting the accumulation of methylation changes through exposure to toxins or stressors over the course of a man's lifetime. These methylation changes may result directly from environmental exposure in the case of some toxins (Yauk et al., 2008), or through the effects of cortisol and other hormones in the case of stressful events (Gunnar & Quevedo, 2007).

Individuals with abnormal epigenetic functioning are at increased risk for several disorders, including schizophrenia (Mill et al., 2008). Epigenetic abnormalities have even been associated with so called "single-gene" disorders, where the aberrant gene may disrupt normal epigenetic processes (Reichenberg, Mill, & MacCabe, 2009). Gene

methylation patterns have been found to differ to a greater extent among monozygotic twins discordant for schizophrenia than among concordant twins (Dempster et al., 2011), which suggests one way in which individuals with identical genes may develop or fail to develop schizophrenia.

In summary then, epigenetic hypotheses attempting to explain the causal link between father's age and schizophrenia risk suggest (a) a similar pattern of predictions to those suggested by the de novo mutation hypothesis (e.g., increased spontaneous cases with increasing father's age), (b) that some pathological de novo mutations may result in aberrant epigenetic functioning, which would, in turn, disrupt normal neural development, (c) that epigenetic abnormalities themselves may accumulate over a father's lifespan and be passed down in some form to his offspring (in a manner similar to that of de novo mutations), and (d) that aberrant epigenetic functioning may also result directly from offspring or paternal exposure to stressors or toxins. These predictions, made using what is known about the biology of epigenetics, suggest that it will be difficult to tease apart the relationships between father's age, de novo mutations, epigenetic abnormalities, and offspring risk for schizophrenia. As such, this dissertation's hypotheses will be based on what can be inferred from what is known about de novo mutations, while acknowledging that epigenetic factors may be also be contributing to these effects.

Pathways between father's age, biological transmission, and offspring neuropathology. While de novo mutations and epigenetic abnormalities are good candidates for the biological risk factor transmitted from older fathers to their offspring, another important causal link between father's age and offspring schizophrenia remains to be explained. Namely, how do these de novo mutations, present in the single-cell

zygote, lead to the adult neuropathology underlying the symptoms of schizophrenia? In other words, a full account of the link between father's age and offspring schizophrenia should explain how de novo mutations in the father's sperm cells alter neurodevelopment, and how this altered neurodevelopment leads to the neuropathology underlying schizophrenia.

Unfortunately, there is unlikely to be a single pattern of aberrant neurodevelopment which mediates father's age and offspring schizophrenia. Millions of individual biochemical processes contribute to the development of a healthy human body and nervous system, all of which are carefully coordinated and timed across gestation, childhood, and adolescence (Rapoport, Giedd, & Gogtay, 2012). It is likely that there are many individual and inter-related developmental processes whose aberrant functioning may give rise to neuropathology which predisposes an individual to developing schizophrenia later in life. On top of the multiplicity of potential pathological developmental pathways, it is likely that several distinct forms of neuropathology can result in symptoms which meet criteria for schizophrenia. In other words, there are not only many paths that lead to the neuropathology of schizophrenia, there are also likely to be many neuropathologies (destinations) that can result in schizophrenia, each with its own multitude of developmental paths that lead to it. This is not a new idea. Eugen Bleuler believed that schizophrenia was an umbrella term for multiple etiologically-distinct conditions more than 100 years ago, as reflected in the title of his landmark "Dementia Praecox or the Group of Schizophrenias" (1911). While this conclusion may frustrate hopes for a single etiological explanation common to all cases of schizophrenia, it appears unavoidable in the face of the repeated heterogeneity found among individuals

with schizophrenia. Given this state of affairs, this review will not attempt to catalogue all the ways in which genetic abnormalities present in an older father's offspring at conception might cause changes in neural development that result in schizophrenia once the offspring reaches adulthood. Instead, it will simply attempt to provide a sampling of possible casual chains which might explain this association. This will hopefully demonstrate that de novo mutations are a plausible explanation for the relationship between father's age and offspring schizophrenia, as well as suggest findings that can be expected among individuals at clinical high risk (CHR) for psychosis.

The first and most obvious developmental mediator between father's age and offspring schizophrenia would be mutations in genes which encode proteins that are important for the proper functioning of neural circuits known to be disrupted in schizophrenia. For example, if a gene encoding for the production of NMDA receptors was mutated in such a way as to disrupt their normal functioning in the brain, this might lead to schizophrenia-like symptoms similar to those seen in individuals whose NMDA receptors are disrupted by pharmacological or immunological means (Hughes et al., 2010; Javitt, 2007). Increased rates of de novo mutations in such genes have been identified in schizophrenia probands with older fathers, evincing this as one path between father's age and schizophrenia in offspring (Kong et al., 2012). A second developmental pathway would involve de novo mutations in genes responsible for encoding proteins that play an important role in healthy neural development, e.g., proteins which selectively trigger programmed cell death during synaptic pruning (Paolicelli et al., 2011). The disruption of such genes would distort normal neurodevelopmental processes (e.g., cause excessive synaptic pruning) which would leave an individual with a schizophrenia-

predisposed brain (Ehrlich et al., 2014). A third possible pathway would be the disruption of genes whose products are essential to the proper functioning of non-neural biological systems, e.g., immune functioning (Anderson & Maes, 2013). The disruption of these systems then would have downstream effects upon neural development and functioning, which would, in turn, predispose an individual to schizophrenia. To give a hypothetical example, a disruption in a gene whose products are important for the regulation of the hypothalamic-pituitary-adrenal (HPA) axis might lead to abnormally high levels of cortisol, which could potentially alter normal neural development and predispose an individual to psychosis (D. A. Ross & Cetas, 2012). A fourth and related pathway between father's age and offspring schizophrenia would be the disruption of normal fetal development not specifically related to the central nervous system. Two hypothetical examples are a de novo mutation which increases the risk of oxygen deprivation during delivery (Cannon, Jones, & Murray, 2002) and a mutation which increases the risk of rhesus incompatibility, leading the mother's immune system to attack the developing fetus (Patterson, 2009). Each of the processes described above could also be disrupted by means other than a de novo mutation in the father's sperm cells, e.g., abnormal neural development due to malnutrition during pregnancy. As such, there is no a priori reason to suspect that the developmental and neural abnormalities that link father's age and offspring schizophrenia would be unique to the offspring of older fathers.

Background Summary and Hypotheses

The preceding background summary provided an overview of the clinical high risk (CHR) syndrome, of the correlation between father's age and increased risk for

schizophrenia in their offspring, and of the potential biological underpinnings of that relationship. Based on this review, this dissertation proposes four hypotheses.

1. Father's age at conception will be positively correlated with attenuated positive symptoms in CHR individuals, while mother's age will not.
2. Father's age at conception will be positively correlated with negative symptoms in CHR individuals, while mother's age will not.
3. Father's age at conception will be associated with poorer social functioning in CHR individuals, while mother's age will not.
4. Father's age at conception will be higher among CHR individuals with no known family history of psychotic illness as compared with CHR individuals with a family history of psychotic illness

Method

Participants

The sample is comprised of 766 participants who met CHR criteria using the Structured Interview for Prodromal Symptoms (SIPS), all of whom participated in the North American Prodrome Longitudinal Study (NAPLS; Addington et al., 2012).

Symptom and Functioning Measures

Structured Interview for Prodromal Symptoms (SIPS). The SIPS (T. J. Miller et al., 2003) is structured clinical interview comprised of 29 items assessing four symptom dimensions: positive (unusual thought content, suspiciousness, grandiosity, perceptual abnormalities, and disorganized communication), negative (social anhedonia, avolition, expression of emotion, experience of emotions and the self, ideational richness, and occupational functioning), disorganized (odd behavior and appearance, bizarre

thinking, trouble with focus and attention, and personal hygiene), and general (sleep disturbance, dysphoric mood, motor abnormalities, and impaired tolerance to normal stress). Each symptom is rated on a seven point scale that reflects its severity, frequency, and duration. For the positive symptom items, scores of 0 - 2 reflect absence to sub-prodromal symptoms, scores of 3 - 5 signify prodromal level symptoms, and a score of 6 indicates that the positive symptom is present at a psychotic level. For items associated with the other symptom dimensions (negative, disorganized, and general) scores simply reflect symptom intensity as there is no such thing as "prodromal" or "psychotic" level negative, disorganized, and general symptoms (e.g., there is no such thing as psychotic motor abnormalities). Scores of 0 - 2 reflect that the symptom is absent, questionable, or mild, scores of 3 - 5 reflect that the symptom is moderate, moderately severe, or severe, and a score of 6 indicates that the symptom is very severe.

To identify those participants who met criteria for the CHR state, the criteria of prodromal syndromes (COPS). The qualifying CHR syndromes included attenuated positive symptom syndrome, genetic risk and deterioration syndrome, brief intermittent psychotic syndrome, and youth and schizotypy syndrome (T. J. Miller et al., 2003). Individuals met criteria for attenuated positive symptom syndrome if they had experienced an onset or worsening of attenuated positive symptoms within the last 12 months, with those symptoms occurring with a frequency of at least once per week. Individuals met criteria for the genetic risk and deterioration syndrome if they were at genetic risk for psychosis, defined as meeting criteria for schizotypal personality disorder (SPD) or having a first-degree relative diagnosed with a psychotic disorder, as well as a decline of at least 30% in global functioning within the last 12 months. Individuals met

criteria for brief intermittent psychotic syndrome if they had experienced the recent onset of brief, self-limiting positive symptoms of psychotic intensity that did not meet the threshold required for diagnosis of a psychotic disorder. Individuals met criteria for the youth and schizotypy syndrome if they were 19-years-old or younger and met criteria for schizotypal personality disorder.

Global Functioning: Role and Social (GF:R and GF:S). GF:R and GF:S are two measures designed to assess role and social functioning within a CHR sample (Cornblatt et al., 2007). They have the virtue of measuring role and social functioning independently, as opposed to the conflation of the two present in the commonly-used global assessment of functioning (GAF) scale (Hall, 1995). These scales have demonstrated reliability and validity in a CHR population.

Structured Clinical Interview for DSM-IV Disorders Axis I Disorders (SCID-I) and the Diagnostic Interview for Personality Disorders (DIPD). Modified versions of the SCID-I and DIPD were used determine whether participants met current or lifetime diagnostic criteria for axis I and axis II disorders. This served several important functions. It allowed for the exclusion of individuals who had previously met criteria for a psychotic illness, to diagnose schizotypal personality disorder (a criteria for several of the CHR syndromes), and to determine what diagnoses were commonly comorbid with the CHR syndrome.

Familial Measures

Demographics and parental ages. Parents' ages were ascertained during an interview for demographic information conducted with all participants. Mother's age and father's age at the time of the interview was recorded both by asking for the parent's

current age as well as parent's date of birth. To better ensure that parents' ages were accurate, each parent's age at participant's birth was calculated in two ways: 1) by subtracting the parent's reported current age from the participant's age at baseline, 2) by determining the number of years and months between participant's reported date of birth and their parent's reported date of birth. The parent's age was set as the mean of these two estimates. If a discrepancy was found of two years or greater between these two estimates for a parent's age, attempts were made to contact site coordinators to find out if the data had been mis-entered. When the two estimates of a parent's age could not be reconciled using this method, the participant's data were excluded from the analysis. If a participant did not know a parent's birthday but was able to give their parent's age (or, if deceased, the age they would have been at the time of the base line assessment), their data were still included. Educational attainment and socio-economic status variables were also collected for participants and their parents.

For this dissertation's analyses, only participants with fathers aged 20-years-old or older at the participant's birth were included. This decision was based upon the previously described meta-analysis of McGrath and colleges (2014) which showed that offspring of particularly young fathers were also at increased risk for negative outcome. While this cut off did mean excluding a small portion of the eligible participants (around 3%, detailed numbers below), studies have shown that the underlying mechanisms for young father's age risk are likely to differ from those at work among older fathers, which would require separate interpretation (e.g., younger father's age might reflect high sensation seeking behavior in the father) which differ from the from the proposed

mechanisms that are the focus of this dissertation (i.e., de novo mutations in older father's sperm).

Family Interview for Genetic Studies (FIGS). Family pedigree and mental health status were collected for first and second degree relatives of participants using the FIGS (Maxwell, 1992). The FIGS is a semi-structured interview, during which a list of first and second degree relatives is constructed and their ages at the time of the interview are recorded. For deceased relatives, age at death was recorded. A set of questions was then used by the interviewer to rate whether these relatives did not, possibly, probably, or definitely met criteria for major depressive disorder, bipolar disorder, schizophrenia, psychosis generally, or organic psychosis. Raters could also note that insufficient information was available to determine whether a relative suffered from one of these disorders. Participants were considered to have a relative with a disorder if at least one of their first or second degree relatives was rated as possibly, probably, or definitely having that disorder.

Procedures

The NAPLS study protocol was approved by institutional review boards at each of the eight sites participating in the study (Emory University, Harvard University, University of Calgary, University of California Los Angeles, University of California San Diego, University of North Carolina, Yale University, and Zucker Hillside Hospital). Participants provided informed consent or assent, with parental informed consent also required for minors who wished to participate. Participants received the SIPS, FIGS, and other clinical measures at an initial screening interview to assess for the presence of one of the CHR syndromes described above. CHR participants were excluded if they had ever

met criteria for an Axis I psychotic disorder, and control participants were excluded if they met criteria for an Axis I psychotic disorder, had a first-degree relative with a current or past psychotic disorder, or had any positive symptoms at the prodromal level or higher. General exclusion criteria included the presence of a neurological disorder which could account for prodromal symptoms (e.g., brain tumor) or a full scale IQ < 70. Upon study entry, participants completed the remainder of the clinical and neuropsychological measures associated with their baseline appointment, usually within a few weeks of their screening.

Analyses and Results

Hypothesis One Analysis Plan

It was hypothesized that increased paternal age at conception (hereafter referred to as "father's age") would predict increased positive symptom scores among CHR individuals, while maternal age at conception (hereafter referred to as "mother's age") would not. Participants were included in the analyses for hypothesis one if they satisfied the following criteria: (a) available baseline information on positive symptom scores, mother's age, father's age, parents' education, and participant's age and (b) father's age greater than or equal to 20 years old.

Positive symptom scores were calculated in the following manner. Firstly, the raw scores for the first two positive subscales of the SIPS, unusual thought content and suspiciousness, were slightly recoded: 2 points were subtracted from each subscale score, with negative values recoded as 0. This had the effect of making sub-prodromal symptom scores (i.e., those equal to 0, 1, or 2) equal to 0, while prodromal-level scores ranged from 1 to 3. Psychotic-level symptoms were rated as 4 and were present only in the small

minority of CHR individuals who met the "brief intermittent and self-limiting psychotic symptoms" CHR syndrome criteria. The recoded scores for the two subscales were summed, yielding a summed score which ranged between 0 and 8. This method of recoding and summing was used on the basis of a recent analysis of the NAPLS data set that showed this computed score to have excellent utility in predicting outcomes (e.g., development of full blown psychotic illness) among CHR individuals (Jefferies et al., 2014).

In order to identify relevant covariates of maternal and paternal ages, zero-order correlations were computed between participant's age, parents' ages, averaged parents' education level, and positive symptom scores. Variables that were significantly correlated with positive symptom scores or with father's age were used as covariates in subsequent analyses. Variables correlated with a father's age but not the dependent variable were included in order to test for potential suppression effects (e.g., while education may only be weakly correlated with positive symptoms, its inclusion in the model may allow for a better estimate of the effect of parents' ages). Another reason these covariates were included was to address the possibility raised by several investigators that the father's age effect is a spurious product of demographic correlations with father's age: including these variables would facilitate testing whether or not this was in fact the case.

Averaged parents' education was calculated as the average of the two parents' scores on a 1 - 9 point rating scale of highest level of formal education completed by the parent (e.g., some high school = 4, completed high school = 5, etc.). These education levels and their values are shown in Table 2. Averaged parents' education level was used instead of a separate variables for each parent's education because mother's and father's

education levels were strongly correlated (r = .46) and in order to reduce the number of variables included in subsequent regression analyses. Of note, follow-up analyses (described below) were conducted using separate variables for mother's and father's education.

Rationale for regression models used in hypothesis one analysis. Given that positive symptom scores were not normally distributed, but rather had a significant right skew and no negative values (see Figure 1), Poisson and negative binomial distributions were tested as models for the data. Poisson regression assumes that observations are distributed in a Poisson distribution as opposed to the familiar normal/Gaussian distribution. As summarized by Atkins and Gallop (2007) Poisson distributions differ from the normal distribution in that: (1) they are probability distributions for non-negative numbers, (2) the mean of the distribution strongly controls the shape of the distribution: a low mean will produce a strongly right-skewed distribution as observations pile up near zero, while a high mean will produce a roughly Gaussian distribution, and (3) while the mean and variance are estimated independently in a normal distribution, the variance is assumed to be equal to the mean in a Poisson distribution. Real-world data often does not match the third assumption of the Poisson distribution (e.g., two populations may have equal means but one may have greater variance): when variance exceeds that expected from the mean, the data is said to be overdispersed. An extension of Poisson regression, negative binomial regression, may be used to model such over-dispersed data and provide a separate estimate of the mean and variance statistics for a sample. To determine whether data is significantly overdispersed and thus better described by a negative binomial distribution, two tests can be employed. Firstly, one can

examine whether the dispersion parameter theta (Θ) estimated by the negative-binomial regression is significant, indicating that the data is significantly more over-dispersed then what would be expected by chance. Secondly, one can perform a Vuong test (described in detail below) to compare the information value of the two models and see if the addition of the dispersion parameter significantly improves the model's ability to model the data. Taken together, Poisson and negative binomial regression provide useful and well validated regression models for count data and other data (such as positive symptom scores) which are not well described by ordinary least squares regression.

In addition to calculating Poisson and negative binomial regression models, zero-inflated versions of these models were tested given (a) the excess of zero values for the positive symptom scores and (b) the possibility that the predictors would have a different relationship with positive symptoms among individuals with sub-prodromal symptoms of unusual thought content and suspiciousness (i.e., those with a positive symptom score of zero). Zero-inflated models are mixture models which attempt to provide one set of coefficient estimates for predicting whether a dependent variable observation will be zero or non-zero (typically using a logistic regression model) and a second set of coefficient estimates for predicting the observation's value (typically using Poisson or negative binomial regression; Atkins & Gallop, 2007).

To illustrate the utility of zero-inflated models, consider a hypothetical study of the flu. In this study, the dependent variable is the number of days an individual suffers from the flu during the month of December. The number of plane trips taken by an individual might predict whether or not the individual contracts the flu during December and, thus, whether the individual has a non-zero value for flu days (i.e., one or more days

sick with the flu). However, the number of plane trips might not be correlated with the number of days it takes for the individual to recover from the flu and, thus, might not be correlated with the number of flu days an individual experiences. Zero-inflated models can even successfully model data sets where an independent variable predicts that an individual will have a non-zero (positive) value on the dependent variable and where the same independent variable is negatively correlated with the dependent variable. Returning to our hypothetical flu study, it might be the case that individuals who fly more frequently tend to be younger and healthier individuals, while those who fly infrequently are more likely to be older or in poorer health. In this example, while increasing numbers of plane trips flown would make an individual more likely to catch the flu in the first place, frequent-flyers' flus would tend to be shorter in duration. In contrast, those who took few or no plane trips would be less likely to contract the flu, but infrequent-flyers who contract the flu would be more likely to spend many days recovering from their infection. Zero-inflated models allow for the detection of effects such as this which are difficult or impossible to detect and model using non-zero inflated forms of regression. Procedures for determining whether data is better described by zero-inflation models will be described in the following paragraph.

To summarize then, four regression models were computed for the data: Poisson, zero-inflated Poisson, negative binomial, and zero-inflated negative binomial. To determine which of these models best fit the data, the method employed by Yurgil et al. (2014) to predict PTSD outcomes was adopted. Their analytic strategy is also described in greater detail in Atkins and Gallop's "Rethinking how family researchers model infrequent outcomes: A tutorial on count regression and zero-inflated models" (2007).

Briefly, the process was as follows. First, the four regression models predicting positive symptom scores using mother's age, father's age, participant's age, and parents averaged education were computed. Then, Vuong closeness tests (Vuong, 1989) were performed to compare all of the models in a pairwise fashion. Vuong closeness tests provide a probabilistic statement about whether one of two models is significantly more likely to better reflect the true underlying structure of data. The virtue of the Vuong test is that it can be used to compare non-nested models (e.g., zero-inflation models and non-zero inflation models) using an information gain criterion. This information gain criterion appropriately penalizes more complex models for their greater likelihood of capturing random variance. Vuong tests produce a *p* value which, if significant, specifies that one of the two models is more likely to reflect the underlying structure of the data. Once the "best model" for the data was determined using Vuong tests in a pairwise comparison process, this best model was used to make estimates of regression coefficients and as the basis for all subsequent analyses.

One such subsequent analysis using the best model was a hierarchical analysis to determine whether including both parent's ages significantly improved the overall fit of the model. This hierarchical analysis also allowed for the observation of how adding the second parent's age affected the coefficients for the other parent's age (i.e., how adding mother's age to the model affected the coefficient for father's age and vice versa). This was accomplished via two block-wise hierarchical analyses in which all variables save one parent's age were added in the first block, while the second parent's age was added in the second block. To determine whether adding the second parent's age significantly increased the overall model's predictive power, chi-square tests were used to determine

whether the log-likelihood increase in predictive utility for the second block was statistically significant. This chi-square test is analogous to the *F* test of the multiple R^2 change statistic used in hierarchical analysis of ordinary least squares regression. Changes in the coefficients for mother's and father's age between block 1 and block 2 of the hierarchical analyses were examined to determine if the addition of the second parent's age attenuated the first parent's age coefficient or even changed its direction.

An ordinary least squares regression was also computed to compare with the best model. This provided more easily interpreted coefficients for the overall influence of the independent variables on total positive symptom scores.

Hypothesis One Results

The initial dataset consisted of 765 CHR participants. One-hundred-and-one participants were excluded because they were missing one or both parent's ages (*n* missing both parents' ages = 46, *n* missing only father's age = 42, *n* missing only mother's age = 8) or because they had invalid values for mother's and/or father's age, i.e., their estimates of a parent's age computed using the two methods were more than 2 years discrepant (*n* with invalid age for both parents = 9, *n* with only invalid father's age = 6, *n* with only invalid mother's age = 7). Note that these *n*'s do not sum up to 101 because some participants both lacked a parent's age and had a discrepant age for the other parent. This left 664 CHR participants. Of these remaining participants, 10 were excluded because they lacked information about their mother or father's education level, leaving 654 CHR participants. A final 19 participants were excluded as their father's age was less than twenty years old, leaving a final sample of 635 CHR participants. This filtering

process is illustrated in Figure 2. Demographics for this sample of 635 CHR participants are shown in Table 3

Zero-order correlations were computed for the potential covariates (i.e., participant's age and averaged parents' education) and are shown in Table 4. Both averaged parents' education and participant's age were correlated with both mother's age and father's age. Participant's age was also correlated with positive symptom scores. As such, both participant's age and parents' average education were included as covariates in the models tested. Again, parent's education was included despite its non-significant zero-order correlation with positive symptom scores because (a) it is often proposed as a protective factor in studies of psychotic illness, perhaps because it suggests higher levels of functioning in parents and higher childhood SES, (b) paradoxically, several studies have shown that parent's level of education is *positively* correlated with symptom severity, a difficult to explain but not infrequently replicated finding (Byrne, Agerbo, Eaton, & Mortensen, 2004), and (c) because parental education was strongly correlated with both parents' ages. Given these facts, parents' education was included to test the possibility of its contributing to the overall predictive power of the model and possibly serving to unmask suppression effects related to parents' ages (e.g., positively correlated with parents age and negatively correlated with symptom severity).

The four potential regression models were calculated: Poisson, zero-inflated Poisson, negative binomial, and zero-inflated negative binomial. Pair-wise comparison's with Vuong tests showed that the zero-inflated Poisson regression was significantly better at modeling the data than each of the 3 other models (*p* values from each of these Vuong tests are shown in Table 5). In addition to this statistical testing suggesting the superiority

of the zero-inflated Poisson regression, examination of the coefficients of the zero and count models showed differential effects of the predictors (to be described in detail below), further supporting the idea that the zero inflated model added to the explanatory power of the analysis. Thus, zero-inflated Poisson regression was determined to be the best regression model and used in subsequent analyses.

Zero inflated Poisson regression—zero model. The coefficients from the zero model are shown in Table 6. The zero model consisted of a logistic regression that predicted the likelihood that a participant's positive symptom score would be zero. Coefficients were exponentiated and interpreted as the increased or decreased odds ratio of a zero score. The exponentiated zero model intercept reflects a 0.0097% base probability of having a positive symptom score of zero for a hypothetical participant with mean values for participant's age, father's age, mother's age, and averaged parental education (i.e., that the participant had average values on all predictors). This value might seem to be an extremely low base probability for having a positive symptom score of zero and thus possibly a statistical artifact. However, consider that only 53 of 635 CHR individuals had a zero score (i.e., 8.3%) and only 13 of those participants' ages (2%) were above the mean age of the non-zero score participants. If this is considered in concert with the powerful effect for each year of participant's age and mother's age (described below) on the odds of having a zero score, the reader can derive a sense for how the zero model's intercept could reasonably reflect such a low base probability of having a zero score. The take away message is that it is highly unlikely that a participant with average scores on all predictor variables would have a positive symptom score of zero, suggesting that CHR individuals with positive symptom scores of zero were both uncommon and

differed significantly from those with non-zero scores in terms of their age and their mother's age.

Turning now to the exponentiated coefficients for the predictor variables, each year of mother's age decreased the odds that a participant would have a positive symptom score of zero by a factor of 0.632 ($p < 0.05$). Every year of participant's age decreased the odds that a participant would have positive symptom score of zero by a factor of 0.340 ($p < 0.05$). To summarize then, the zero model suggested that younger participants with younger mothers were significantly more likely to have positive symptom scores of zero. There were no significant effects for father's age and parents' averaged education in the zero model.

Zero inflated Poisson regression—count model. The results of the count model, which predicted the value of positive symptom scores using a Poisson regression, are shown in Table 6. Exponentiated coefficients of the count model represent multiplicative changes in predicted positive symptom score per one unit change in a given predictor (e.g., one year of father's age). The intercept of the count model reflected a predicted positive symptom score of 2.63 given average values for participant's age, father's age, mother's age, and parents' education. Every year of mother's age decreased predicted positive symptom scores by a factor of 0.987 (i.e., a 1.3% decrease in positive symptom score per year of mother's age). Each year of participant's age increased predicted positive symptom scores by a factor of 1.012 per year of participant's age (1.2% increase in positive symptom score per year of participant's age). To summarize then, older participants with younger mothers were more likely to have higher positive symptom

scores. There were no significant effects for father's age and parent's education in the count model.

Hierarchical analysis of zero-inflated Poisson regression. The results of the hierarchical analysis of the zero-inflated Poisson regression are shown in Tables 7 and 8. Chi-square tests demonstrated that adding mother's age to the model significantly increased the model's predictive power while adding father's age did not. It is worth noting, however, that the predictive gain from including father's age did approach significance (*p* value of log likelihood of increase in predictive utility = .082). Also of note, the coefficient for mother's age in the count model was only significant after the addition of father's age to the model, possibly suggesting a mild suppression effect (i.e., that the positive correlation between mother's and father's age partially attenuates the negative correlation between mother's age and positive symptom score in the count model). In addition, the coefficient for father's age in the count model changed from negative to positive when mother's age was added in step 2, providing further evidence for this suppression effect.

Ordinary least squares regression and follow up analyses using separate values for mother's and father's education. An ordinary least squares regression was performed and its results were compared with those of the zero-inflated Poisson regression to aid in the interpretation of the zero-inflated Poisson regression's coefficients (results shown in Table 9). The ordinary least squares regression showed results generally consistent with the findings of the zero-inflated Poisson regression: older participants with younger mothers and older fathers were likely to have the highest positive symptom

scores, with only the coefficient for participant's age reaching statistical significance in the ordinary least squares model.

Follow-up analyses were conducted in which all of the regressions were re-conducted using separate values for mother's and father's education (as opposed to a single averaged value). This was done to test for the possibility that each parent's education level might have a different effect upon the dependent variable (e.g., that mother's education might have a unique effect upon their offspring's positive symptoms as compared with father's education). Including separate values for each parent's education level in this manner did not change the pattern of results for any of the above-described analyses (results available upon request). Given that parents' education did not correlate with positive symptom scores, a follow-up analysis consisting of the zero-inflated Poisson regression without parent's education was conducted. This produced coefficients for parents' ages and participant's age nearly identical to those found when parent's education was included.

Hypothesis One Summary and Conclusions

The analyses conducted for hypothesis one yielded the following results. A zero-inflated Poisson regression was significantly better at describing the data than the other three models that were tested. This was supported both by statistical testing (i.e., Vuong tests) and by explanatory gains made through the use of the zero-inflated model. Statistically speaking, the planned Vuong tests showing that the zero-inflated Poisson model was significantly more likely to represent the underlying relationship between the predictor variables and positive symptom scores. From an explanatory or conceptual perspective, employing the zero-inflated model allowed for the drawing of several

conclusions which would not have apparent using ordinary least squares regression: (a) participant's and mother's age are particularly robust predictors of zero vs. non-zero scores and less robust predictors of positive symptom severity (b) older mother's age had somewhat inverse effects in the zero and count models: it predicted a non-zero score and yet was negatively correlated with symptom severity, (c) the zero inflated model also allowed for the conclusion that participant's age, unlike mother's age, does not make inverse predictions in the zero and non-zero model, i.e., older participants were less likely to have a zero score and were more likely to have higher symptom scores. Father's age and parents' education were not significantly correlated with positive symptom scores in both the zero and count models.

In follow-up hierarchical analyses, mother's age was shown to significantly increase the overall prediction power of the zero-inflated Poisson regression, while the gain from adding father's age was only marginally significant. An ordinary least squares regression produced results consistent with the zero-inflated Poisson regression. Analyses conducted using separate values for each parent's education level did not change the pattern of results.

Thus mixed support was found for hypothesis one: maternal age generally did not predict increased positive symptom scores (in fact, it generally predicted reduced symptom scores), however paternal age did not significantly predict increased positive symptom scores. Hierarchical analyses suggested that the correlation between mother's and father's age might partially suppress the protective effects of mother's age on positive symptom scores in the count model.

A potential critique of the current analysis bears addressing here. The use of zero-inflated models may have risked over-fitting the data given their additional dimensions. While this is a genuine concern that should be addressed, three characteristics of this analysis make this problem unlikely. First, the Vuong test penalizes more complex models by adding a penalty term proportional to the number of dimensions the model contains (Vuong, 1989), counterbalancing the possibility of over-fitting. Secondly, the coefficients for the zero and count model portions of the zero-inflated Poisson regression differed in their size and (in the case of mother's age) direction, suggesting that the zero-inflated Poisson regression is conveying additional information about the pattern of results. Thirdly, the Vuong tests did not, in fact, prefer the most complex model. The most complex model would have been the zero-inflated negative binomial regression, which would have included the additional dispersion parameter theta (Θ). Indeed, the Vuong test produced an extremely strong preference for the simpler zero-inflated Poisson regression over the zero-inflated negative binomial regression ($p < .001$), the strongest difference found among all of the pairwise model comparisons that were performed. Thus, it seems unlikely that the zero-inflated Poisson regression was preferred simply due to its greater complexity compared with the competing models.

Hypothesis Two Analysis Plan

It was hypothesized that increased paternal age at conception (again, hereafter referred to as father's age) would predict increased negative symptom scores among CHR individuals, while mother's age would not. Inclusion criteria were the same as those for hypothesis one, except that baseline negative symptom ratings were required to be present.

Negative symptom score was calculated as the sum of the ratings for the SIPS's negative symptom subscales 1 through 5. The subscales were 0 to 6 ratings of social anhedonia, avolition, expression of emotion, experience of emotions and self, and ideational richness (i.e., ability to understand figurative meanings and engage in abstract thinking) respectively. The sixth negative symptom subscale, occupational functioning, was not included in order to focus more upon negative symptoms as opposed to functioning. The negative symptom scores were simply summed as there was no theoretical or statistical rationale to recode them (as had been done for the positive symptom score used in hypothesis one). To elaborate, the 0 to 6 rating for negative symptoms did not reflect non-prodromal, prodromal, and psychotic level symptoms as they had with the positive symptom ratings. Instead the simply reflected a continuum of symptom severity, ranging from absent to mild to extreme. Also unlike the positive symptom ratings, no special predictive utility has been shown for negative symptom ratings of three or higher as was shown for positive symptoms (Jefferies et al., 2014).

Relevant covariates of maternal and paternal ages were identified using zero-order correlations. Namely, zero-order correlations were computed between participant's age, averaged parents' education level, and negative symptom scores. Variables that were significantly correlated with negative symptom scores were considered to be relevant covariates and included as such in the analyses.

The statistical analysis planned for hypothesis two differed significantly from that planned for hypothesis one. This difference was due to two findings made during preliminary examinations of the negative symptom data: (1) zero values were both rare and did not have any particular theoretical relevance and (2) negative symptom scores

were far more regularly distributed than the positive symptom scores. The consequences of each of these findings upon the analyses and the rationale for those consequences will now be considered briefly.

Rationale for the regression models used in hypothesis two analysis. Zero-inflated models for negative symptom scores were not tested for theoretical and statistical reasons. Firstly, as has already been alluded to, there were no apriori theoretical reasons for assuming that zero and non-zero negative symptom scores differed in kind and/or would have a unique relationship to mother's age or father's age. While positive symptoms scores of one or higher indicated that a participant had at least one positive symptom of prodromal intensity, a one on the negative symptom scale did not reflect symptoms of prodromal intensity. Secondly, an examination of the distribution of the frequency of negative symptom scores (see Figure 3) showed no excess of zero scores on the negative symptom scale (i.e., *n* negative symptom score of zero = 17, *n* negative symptom score of one = 18) and that zero scores do not compose a large portion of the sample (i.e., 17 out of 635 participants). As such, coefficients for predicting zero values, even if significant, would have little if any value in explaining the major pattern of findings in the data set.

Preliminary analysis of the negative symptom scores suggested that ordinary least squares (OLS) regression was likely to be a valid analytic method for the data. A visual examination of the data set showed it to be roughly normally distributed, though admittedly right skewed. A regression of mother's age and father's age on negative symptom scores was conducted: visual examination of the plot of standardized residuals against standardized predicted values showed no evidence for significant

heteroscedasticity or non-linear effects. A Kolmogorov-Smirnov (K-S) test of the normality of the residual values was not significant ($d = 0.047$, $p > .13$), confirming that the residuals were not significantly non-normally distributed, a particularly strong finding given that the K-S test becomes overly sensitive with a large number of observations. A Breusch-Pagan test of homoscedasticity (Breusch & Pagan, 1979), another statistical test for homoscedasticity, was also not significant ($\chi^2 = 2.9$, $p > .088$). Examination of QQ plots also showed roughly normally distributed residuals. Given the repeated findings of normality using tests of assumptions of OLS regression, OLS regression was used as the primary analytic technique for testing hypothesis 2. Follow-up analyses were also conducted using Poisson and negative binomial regression to see if the findings would be consistent with those of the OLS regression.

Based on all of the considerations described in the above paragraph, the analytic strategy employed for hypothesis two was as follows. Zero-order correlations were run to identify relevant covariates. OLS regression was used to predict negative symptom scores using mother's age, father's age, and any relevant covariates. If the model was significant, hierarchical analyses were planned in order to test the relevant contributions of mother's age and father's age to the regression model's predictive ability using the same strategy employed in hypothesis one (i.e., adding one parent's age in block 1 and the other parent's age in block 2). Follow-up analyses included (a) re-running the analyses with the covariates used in hypothesis one so that the results of hypothesis one and two might be more directly compared and (b) running a series of analyses to determine whether Poisson or negative binomial regression were a better fit for the data.

Hypothesis Two Results

As with hypothesis one, 635 CHR participants had valid values for both parents' ages. Twenty-four individuals did not have baseline negative symptom ratings (i.e., they had not completed the entire SIPS interview for whatever reason), leaving an *N* of 611. Zero-order correlations were computed for the potential covariates (i.e., participant's age and averaged parents' education) and are shown in Table 10. Neither was significantly correlated with negative symptoms. As such, only mother's age and father's age were included in the regression model. The OLS regression using mother's age and father's age to predict negative symptom scores was not significant overall, F (2, 608) = 0.75, p > .47, and neither were the individual coefficients for father's age (t = -0.189, p > .84) or mother's age (t = 0.995, p > .31). Results for this regression can be seen in Table 11. A follow up OLS regression using the covariates from hypothesis one (participant's age and parents' education) was also not significant overall, F (4, 606) = 0.554, p > .69, and none of the individual coefficients were significant (results shown in Table 12).

In another set of follow-up analyses, Poisson and negative binomial regression models were compared with one another. A chi-square test of overdispersion of the data comparing the log-likelihoods of the Poisson and negative binomial regression was highly significant, $\chi^2 = 488.819$, $p < 2.2^{-16}$, indicating that negative binomial regression was more appropriate than the Poisson regression. Negative binomial regression produced findings very similar to the OLS regression. The overall significance test of the negative binomial regression model (i.e., a likelihood ratio test comparing the negative binomial model with a null model) was not significant: $\chi^2 = 1.324$, p > .51. Neither

parent's age coefficients in the negative binomial model were significant (results shown in Table 13).

Hypothesis Two Summary and Conclusions

The analyses conducted for hypothesis two uniformly demonstrated that father's age and mother's age did not significantly predict negative symptom scores. An OLS regression was not significant overall, and neither were the individual coefficients for parents' ages. Follow-up analyses using covariates for participant's age and parents' education were also not significant. A follow-up negative binomial regression also proved non-significant.

Hypothesis Three Analysis Plan

It was hypothesized that older father's age would predict a decreased current social functioning score (hereinafter referred to as social functioning) among CHR individuals, while older mother's age would not. Inclusion criteria were the same as those for hypothesis one, except that baseline social functioning ratings were required to be present. The social functioning score was operationalized as the 1 - 10 rating for current social functioning on the Global Functioning: Social measure.

Participant's age and averaged parents' education level were tested as potential covariates using zero-order correlations. Variables that were significantly correlated with social functioning were considered to be relevant covariates and included as such in the analyses. OLS regression was selected as the analytic strategy for hypothesis three given the relatively normal distribution of social functioning scores within the CHR populations (see Figure 4).

In summary, the analytic strategy employed for hypothesis three was as follows. Zero-order correlations were run to identify relevant covariates. OLS regression was then used to predict social functioning scores using father's age, mother's age, and any relevant covariates. If the model was significant, hierarchical analyses were planned in order to test the contributions of mother's age and father's age to the regression model's overall predictive ability using the same strategy employed in hypothesis one (i.e., adding one parent's age in block 1 and the other parent's age in block 2). Planned follow-up analyses included re-running the analyses with the covariates used in hypothesis one so that the results of hypothesis one and three might be more directly compared.

Hypothesis Three Results

As with hypothesis one, 635 CHR participants had valid values for parents' ages. An additional 6 participants were excluded because they lacked social functioning scores, leaving a final sample *N* of 629. Zero-order correlations were computed for the potential covariates (i.e., participant's age and averaged parents' education) and are shown in Table 14. Neither was significantly correlated with social functioning. As such, only mother's age and father's age were included in the regression model.

Assumptions of the OLS regression were then checked. Examination of the standardized residuals and predicted values plot showed some evidence of heteroscedasticity. However, a histogram of the standardized residuals and a non-significant Breusch-Pagan test of heteroscedasticity ($\chi^2 = 0.008$, $P > .92$) both evinced that there was no significant violation of homoscedasticity. Thus the regression was carried out and its results are reported here.

The OLS regression using mother's age and father's age to predict social functioning scores was not significant overall, F (2, 626) = 0.987, $p > .373$, and neither were the individual coefficients for father's age (t = -1.19, $p > .234$) or mother's age (t = .314, $p > .754$). Results for this regression can be seen in Table 15. A follow up OLS regression using the covariates from hypothesis one (participant's age and parents' education) was also not significant overall, F (4, 624) = .679, $p > .607$, and none of the individual coefficients were significant (results shown in Table 16).

Hypothesis Three Summary and Conclusions

The analyses conducted for hypothesis three uniformly demonstrated that father's age and mother's age did not significantly predict social functioning scores. An OLS regression was not significant overall, nor were the individual coefficients for each parent's age. Follow-up analyses using covariates for participant's age and parents' education were also not significant.

Hypothesis Four Analysis Plan

It was hypothesized that CHR individuals with a family history of psychosis (FHP+) would have significantly younger father's age than individuals without a family history of psychosis (FH-). The rationale for this hypothesis is that among individuals without a family history of psychosis, de novo mutations represent one etiological pathway whereby an individual might acquire the genetic and subsequent neurobiological risk substrate for psychosis without the need for a family agglomeration of psychosis risk alleles. As noted during the background literature review, a strengthened relationship between father's age and offspring outcomes has been noted among autism probands with

no family history of autism spectrum disorders (O'Roak et al., 2012), further prompting this hypothesis.

Participants were included in the analysis for hypothesis four if they met the inclusion criteria for hypothesis one and had no missing data on psychotic illness for a first degree relative. Instead of averaging both parent's education together, each parent's education was left as a separate variable to examine independent effects of each parent's education and because there was less concern that multicollinearity would negatively impact the analysis given the use of ANCOVA.

Family history of psychosis (FHP+) was operationally defined as having an interviewer rate a first or second degree relative as "possibly," "probably," or "definitely," having a psychotic illness, organic psychosis (i.e., psychosis with a likely somatic etiology such as brain injury), or schizophrenia on the Family Interview for Genetic Studies (FIGS).

Generally, individuals with no reported family history of psychotic illness were included in the no family history of psychosis (FHP-) group: however, individuals with a family history of mania but no family history of psychosis were not included in the FHP- group and thus excluded from the analysis. Two major rationales underlay this decision to exclude those with only a family history of mania: (a) ambiguity regarding the symptom status of family members identified as only having mania and (b) theoretical reasons based on the existing literature suggesting that, even when issues of diagnostic ambiguity are set aside, individuals with only a family history of mania would make an inappropriate control group. In relation to the issue of ambiguity, the instructions for the FIGS specify that if mania is identified in a family member, follow up questions should

be used to determine if that family member had psychotic symptoms during the manic episode and, if so, the family member should be coded as being positive for both mania and for psychotic illness. However, this fine grained distinction is difficult to make in practice, particularly when the informant is the participant and information on the relative in question can be limited to second hand information from other relatives. As such, there was concern that family members with a history of mania might have experienced psychotic symptoms that were unknown to the participant, especially given that approximately 58% of individuals who experience mania also experience psychotic symptoms (Dunayevich & Keck, 2000). Additionally, there was a concern that interviewers who discovered that a family member had a history of bipolar disorder might have simply rated the family member as having mania and moved on rather than separately evaluating for the presence of psychotic symptoms, which was understandable given that the need to make this secondary determination was not made particularly obvious in the instructions for the FIGS interview. Turning now to theoretical reasons for excluding individuals with a family history of mania but no family history of psychosis, the significant etiological overlap demonstrated between bipolar disorder and schizophrenia suggests that a family history of mania would have similar correlates as a family history of schizophrenia or other psychotic illness. Specifically, older father's age itself is an established as a risk factor for bipolar disorder (Dalman, 2009).

To test whether a group difference in father's age existed between FHP+ and FHP- individuals, analysis of covariance (ANCOVA) was employed. ANCOVA allows for the comparison of group means while partialling out the variance due to measured covariates. An assumption of the ANCOVA model, however, is that the independent

variable, i.e., family history of psychosis, is independent of the proposed covariates, e.g., parents' education (Field, 2009, p. 398). As such, prior to conducting the ANCOVA, *t*-tests were conducted comparing the means of the FHP+ and FHP- groups on mother's age, participant's age, and parent's education. If there were significant differences on these covariates between the FHP+ and FHP- groups, they were not included as covariates.

Hypothesis Four Results

As in hypothesis one, 635 CHR participants were found to have valid data for their parents' ages and education. Of these, 88 were missing information regarding psychotic illness in at least one first degree relative and were excluded, leaving 547 CHR participants. Of these 547 CHR participants, 164 had a family history of mania, of whom 63 did not also have a family history of psychosis and thus were excluded from the analysis. This left a final sample *N* of 484 CHR participants. Of these 484, 210 participants were in the family history of psychotic illness (FHP+) group and 274 participants were in the no family history of psychotic illness (FHP-) group.

Preliminary *t*-tests showed group differences on mother's age and participant's age between the FHP+ and FHP- groups. As such, these variables could not be used as covariates in the ANCOVA. This left father's education and mother's education to be included as covariates. Results of the t-tests and group means on covariates are shown in Table 17.

An ANCOVA was then performed with father's age as the dependent variable and FHP group membership as the independent variable. Mother's education and father's education served as covariates. Tests for the assumptions of the ANCOVA were

examined. Levene's test for equality of error variance was significant, F (1, 482) = 4.162, $p < .05$, suggesting unequal error variance between the two groups and thus that the assumption of homogeneity of variance might be violated. However, as noted by Field (2009, p. 150), Levene's test tends to be overly sensitive, particularly with large sample sizes, where small group differences in variance can none the less be found statistically significant. In such cases, Levene's test can indicate that there is a statistically significant difference in error variance, but this difference in error variance may not have an adverse effect on the validity of the ANCOVA. Thus, a secondary check suggested by Field was performed to determine if this violation was of practical significance to the analysis, namely Hartley's Homogeneity of Variance Test (Pearson & Hartley, 1954), also known as the variance ratio test. This test compares the ratio of the error variance between groups with a table of critical ratio values which depend upon the sample size and number of groups being compared. If the observed ratio is greater than this critical value, it is likely that the assumption of homogeneity has been violated to the point where it might affect the validity of the ANCOVA. The ratio of error variance between the two groups was calculated to be 1.35, well below the critical ratio (F_{max}) of 1.67, suggesting that the ANCOVA would be robust to the differing variance between the groups. In addition, tests of the homogeneity of slopes and multicollinearity were conducted and no violations of these assumptions were found.

The results of the ANCOVA can be found in Table 18. While the overall model was significant, F (3, 483) = 8.66, $p < .001$, the coefficient for family history of psychosis was not, $t = 1.38$, $p > .169$. A follow-up t-test comparing father's age between the two

FHP groups without any covariates was also not significant, t (1, 482) = 2.00, p > .158, results shown in Table 19.

Hypothesis Four Summary and Conclusions

The results of the ANCOVA conducted to test hypothesis four demonstrated that father's age did not significantly differ between individuals with and without a family history of psychosis (FHP). This was the case whether or not the covariates of mother's and father's education were included in the analysis.

Further supplemental analyses

Based on the results of analyses for hypotheses one through four, several follow-up analyses were conducted in order to further interpret these findings. These included a cross tabs analysis of family history of psychosis by zero vs. non-zero positive symptom score, a test for interaction effects between family history of psychosis and parents' ages in predicting outcome measures, and a comparison of participants with zero and non-zero positive symptom values on negative symptoms.

The rationale for examining the interaction of family history of psychosis and positive symptom score is as follows. First, the risk for psychosis conferred by the mutations that accumulate with advanced paternal age is assumed to entail a genetic mechanism that is at least partially separable from that involved in heritable risk alleles for psychosis. Consequently, for individuals with a family history of psychosis, de novo mutations, and thus paternal age, may be less predictive of CHR syndrome severity. Second, it is possible that individuals with a positive symptom score of zero are more likely to have a family history of psychosis due to the enrollment criteria for the study. Participants who met the "genetic high risk and functional decline syndrome" criteria

could be enrolled in the study with a positive symptom score of zero even if they did not exhibit any CHR-level perceptual abnormalities, grandiosity, or disorganized communication, so long as they had a family member with a possible history of psychotic illness and the participant had experienced a recent 30% decline in their Global Assessment of Functioning (GAF) score. This "positive symptom free" group of individuals represented a significant minority of individuals with a family history of psychosis and a positive symptom score of zero (12 out of 33, 36%). In contrast, all 19 individuals with a positive symptom score of zero and no family history of psychosis had CHR-level perceptual abnormalities, grandiosity, and/or disorganized communication, as they would not have been admitted to the study if they did not have such symptoms. Thus those with a family history of psychosis differed from those without a family history of psychosis in that it was possible for them to participate in the study even if they did not have any CHR-level attenuated positive symptoms. These criteria for study participation are relevant to the analysis of parent age effects for the following reason: if study admission criteria affected the relationship between the variables of family history of psychosis and positive symptoms (i.e., made participants more likely to have a positive symptom score of 0 if they had a family history of psychosis than if they did not), this may be influenced the relationship between parent's age and positive symptoms, especially as regards to parent's age predicting which individuals will have a positive symptom score of zero (i.e., the zero inflation model test in hypothesis one). This is particularly plausible given that family history of psychosis was previously shown to be correlated with mother's age. This possibility was investigated with supplemental analyses.

A cross tabs analysis was conducted in order to test the possibility that individuals with a positive symptom score of zero were more likely to have a family history of psychosis than would be expected by chance. Participants were classified as having either a zero or non-zero positive symptom score, and to either have or not have a family history of psychosis. The results of this analysis, including expected vs. observed cell counts, are shown in table 20. A Pearson Chi-Square test was highly significant, χ^2 (1, N = 549) = 10.773, $p < .001$. As predicted, a higher than expected number of individuals with a positive symptom score of zero had a family history of psychosis (26 observed, 16 expected). This finding supported the speculation in the preceding paragraph that individuals with positive symptom score of zero were more likely to have a family history of psychosis because they were more likely to meet criteria for entrance into the study.

Given the relationship between having a positive symptom score of zero and having a family history of psychosis, the interaction of family history of psychosis and parents' ages in predicting positive symptom scores was examined. Specifically, interaction terms combining parents' ages and family history of psychosis were examined to see if they would increase the predictive utility of parents' ages in predicting positive symptom scores, especially in predicting which individuals would have zero values on positive symptoms scores (i.e., the zero inflation portion of the ZIP). ZIPs were conducted with and without the addition of these interaction terms and their results are shown in table 21. The results of the first ZIP without interaction terms were in line with the ZIP conducted for hypothesis one: increased mother's age was associated with reduced likelihood of a positive symptom score of zero, but was associated with a lower

positive symptom score in the count model. However, in contrast to the analysis for hypothesis one, father's age was now a significant predictor in the zero-inflation model and approached significance in the count model: increased father's age was associated with increased likelihood of a positive symptom score of zero, and nearly significantly associated with having a higher positive symptom score in the count model, i.e., the reverse of the pattern for mother's age. Notably, this pattern of findings for father's age was simply a more significant version of the trend level findings for father's age found in the analysis for hypothesis one (i.e., the coefficients for father's age in this supplemental analysis and hypothesis one were in the same direction, however, they were statistically significant in this supplemental analysis). The increased statistical significance of father's age in this supplemental analysis may have been due to the fact that this supplemental analysis used the subset of participants who did not lack information on first degree relatives (since this was required to make the family history of psychosis determination). This subset may have had more accurate data regarding parents' ages or a greater proportion of particularly old fathers and this might explain the increased significance of father's age.

Now let us turn to the second step of the supplemental analysis, i.e., the ZIP model which includes interaction terms for family history of psychosis by each parent's age. The interaction terms were not significant within the count model, however they were highly significant within the zero inflation model, showing that the interaction between parent's ages and family history of psychosis was significant in predicting whether a participant would have positive symptom score of zero or not. This pattern (i.e., family history of psychosis being more relevant to the zero inflation model but not

the count model) is what was expected based upon the study participation-criteria rationale described above. A log-likelihood test of the increase in predictive utility of the model once the interaction terms were included indicated that the inclusion of the interaction terms significantly increased the model's ability to predict positive symptom scores (*p* value of log likelihood of increase in predictive utility = .014). Turning now to interpreting the interaction coefficients themselves, the interaction coefficients indicated that the effect of each parent's age was reversed if an individual had a family history of psychosis. For those without a family history of psychosis, individuals with older father and younger mothers were more likely to have a positive symptom scores of zero (the same pattern of findings as found in the model without interaction terms). In contrast, for individuals with a family history of psychosis, those with a younger father and an older mother were more likely to have a positive symptom score of zero, the opposite pattern of that found for the simple effects of mother's and father's age. It should be noted that both the parents' age coefficients and the interaction term coefficients were significant (or nearly significant in the case of the father's age coefficient) in the zero-inflation model, suggesting both a simple effect of parents' ages and an interaction effect between parents' ages and family history. Finally, an additional ZIP was conducted with the added intendent variable of family history of psychosis (results not shown here, available upon request). The pattern of finding remained the same when family history of psychosis was included, suggesting the significance of the interaction terms in the zero-inflation model is robust.

Given the usefulness of interaction terms combining parent's age with family history of psychosis in predicting positive symptoms, the ability of these interaction

terms to predict negative symptom and social functioning was also tested. The addition of interaction terms did not significantly improve the ability of parents' ages to predict negative symptoms (r^2 change .003, F = 0.970, p =.380), nor their ability to predict social functioning scores (r^2 change .004, F = 1.241, p =.290).

A supplemental analysis was conducted to determine whether participants with zero and non-zero positive symptom scores differed on their negative symptom scores. A t-test of this proved significant, (t = 3.171, df = 637, p = .002); mean negative symptom score of zero positive symptom group = 6.96 (SD 5.38), mean negative symptom score of non-zero positive symptoms group = 9.2 (SD 4.87).

Discussion

Several findings were made during the analyses conducted for this dissertation. First, it was found that increased mother's age at conception not only predicted the existence of prodromal-level positive symptoms in her CHR offspring, but also that these positive symptoms were of decreased intensity as compared with the offspring of younger mothers. Increased participant's age was positively correlated with both the presence and severity prodromal-level positive symptoms. Father's age, in contrast, was not significantly correlated with positive symptoms, although it's coefficient indicated a positive correlation with positive symptom intensity (once mother's age was controlled for) and a trend level finding suggested that it increased the regression model's overall ability to predict positive symptom scores. Secondly, it was found that neither father's age nor mother's age predicted negative symptom scores in CHR individuals. Thirdly, it was found that neither mother's age nor father's age predicted current social functioning.

Finally, it was shown that individuals with and without a family history of psychotic illness did not differ significantly in their father's age.

Findings Related to Participant's Age and Mother's Age

The first finding to be interpreted is the positive correlation between positive symptoms and participant's age. The finding is relatively straightforward to interpret as positive symptoms have generally been shown to increase in intensity over the age span included in this study, and more specifically that age is generally correlated with positive symptoms in populations at risk for and suffering from psychotic illness (Häfner, Maurer, Löffler, & Riecher-Rössler, 1993).

The next finding to interpret is that older mother's age was negatively correlated with positive symptom severity. Factors such as education (and closely associated SES) cannot easily explain this finding, as maternal education was essentially uncorrelated with positive symptom severity when also included in the model. So what is it that is protective about mother's age, at least in terms of positive symptom severity? One possible explanation is that woman who have children at a younger age are likely to be more impulsive (Moffitt, 2002), and that this impulsivity is correlated with excessive subcortical dopaminergic activity (Buckholtz et al., 2010). Younger mothers thus pass on their overactive dopaminergic system to their children, and it is this overactive dopaminergic system which predisposes their children to more severe positive symptoms. Another potential explanation for the increased level of positive symptoms in the offspring of younger mothers is that the offspring of younger mothers are at increased risk for obstetric complications (Fraser, Brockert, & Ward, 1995). These obstetric complications in turn have been shown to increase the risk for psychotic disorders in their

offspring, most likely through the disruption of normal neural development (Cannon et al., 2002).

Another finding related to mother's age is that older mothers were less likely to have offspring with zero values for positive symptoms. This is a difficult finding to interpret given the negative association between mother's age and positive symptom severity. One possible explanation is that older mothers were less likely to bring their children in for non-prodromal level symptoms, or that younger mothers were more likely to see their children's behavior as pathological when it was on the borderline of abnormality (recall that many of the participants with positive symptom scores of zero were adolescents, thus increasing the influence of parent's on participation in the study). Whatever the casual link between mother's age and positive symptom scores, it is clear that the results suggest that CHR individuals with a positive symptom score of zero may represent a somewhat unique and understudied subset of the CHR population with differential characteristics (e.g., significantly younger) and outcomes (less likely to convert to full blown psychotic illness).

Another pattern of finding to be interpreted is that mother's age and participant's age failed to predict negative symptom scores and current social functioning despite their significant prediction of positive symptom scores. One possibility that should be acknowledged is that the link between mother's age and positive symptoms is a Type I error and that there is no significant relationship between mother's age and any of the outcome variables. While this is a possibility that should be tested by attempting to replicate the findings, there are serval facts that suggest that this pattern of findings is not simply a Type I error. Positive symptom severity has been found to be mostly

independent of negative symptoms, cognitive dysfunction, and social/occupational functioning in individuals with psychotic illness (McGurk & Meltzer, 2000), making disparate findings across these domains in CHR individuals quite plausible. Building upon this initial assumption of plausibility, there are at least two families of explanations which can be advanced. One family of explanations posits that the causal link that unites maternal age and positive symptom (whatever it might be) does not exist between maternal age and negative symptoms / current social functioning. As an example of such a hypothesis, let us momentarily assume that the relationship between mother's age and positive symptoms is indeed due to excessive subcortical dopaminergic signaling in mothers who have children at a younger age, as was suggested earlier. In this hypothetical situation, abnormal subcortical dopaminergic activity would be less likely to have an influence upon negative symptoms (which are more closely tied with abnormalities in frontal regions) or social functioning (which are not closely tied with positive symptoms, and are more closely tied with cognitive functioning and negative symptoms). While this particular causal story is quite speculative given that the literature provides only moderate support for each of these causal links and no direct test of the hypothesis itself, the point of this example is not to propose a definitive interpretation of the discontinuity between positive symptoms and the other outcomes tested. Instead, this is just one possible explanation for how the findings related to positive symptoms could be genuinely significant while the correlations with negative symptoms and social functioning are not.

A second family of explanations may be raised to explain the pattern of findings related to mother's age. The second family of explanations proposes that, while the casual

link between mother's age and positive symptoms is also present between mother's age and negative symptoms / social functioning, there are unmeasured confounds of older mother's age which have little effect on positive symptoms but are relevant to the offspring's negative symptoms and social functioning. For example, older mothers may have lower levels of extraversion (Jokela, Alvergne, Pollet, & Lummaa, 2011) which, while uncorrelated with positive symptoms, may be correlated with non-clinical, personality-level manifestations of negative symptoms, i.e., reduced sociability and emotional expressiveness (S. R. Ross, Lutz, & Bailley, 2002). These sub-clinical negative symptom traits would be passed down to their offspring and thus affect their offspring's propensity towards isolation and lower emotional intensity in general (which would be reflected in the offspring's negative symptom score through measures of anhedonia, reduced emotional experience, and decreased emotional expressivity). Congruent with this idea, sub-clinical levels of negative symptoms receive a non-zero score on the negative symptom measure employed in this study (e.g., a person with mild levels of all 5 negative symptoms would have a score of 5). As such, an older mother that passes down normative personality traits for coolness, introversion, etc. may increase her offspring's negative symptom score and decrease their social functioning score, counterbalancing any protective influence of maternal age that are reflected on positive symptom scores.

So in summary then, the pattern of results found across hypotheses one, two, and three (i.e., a significant correlation between mother's age and positive symptoms and no significant correlation between mother's age and negative symptoms/social functioning) may be explained in one of two ways (a) the link between increased mother's age and positive symptoms is not relevant to negative symptoms and social functioning, and (b)

factors confounded with older mother's age, e.g., introversion, are relevant to their offspring's levels of negative symptoms and social functioning, but not to their offspring's level of positive symptoms. These possibilities are testable in future studies. For example, mother's personality traits (or sub-clinical negative symptoms / social functioning) could be measured and controlled for.

Findings Related to Father's Age

Let us now turn to potential explanations for the non-significance of father's age in predicting any of the dependent variables in hypotheses one, two, and three, beginning with reasons why father's age may in fact be linked with outcomes even though no significant findings were found. The analyses in this study assumed that father's age was linearly associated with outcomes given the established linear relationship between father's age and de novo mutations (Kong et al., 2012). However, it may be that the effects of de novo mutation, and thus of father's age on outcomes, may have a tipping point quality. A single de novo mutation, even in a critical genetic region related to brain development, may not sufficient to derail neurodevelopment to the extent necessary for the development of psychotic illness. The pattern of good functioning in complex systems until multiple component failures accumulate within the system, which is followed by a rapid decline in functioning, has been shown to accurately model many biological (and non-biological) systems and is mathematically described in a branch of systems analysis known as reliability theory (Gavrilov & Gavrilova, 2001). If such dynamics do in fact describe the effects of de novo mutations, then one might expect little correlation between father's age and positive symptoms below a certain father's age, but that the offspring of particularly older fathers, e.g., > 50-years-old, would have more

severe symptoms, because those offspring are likely to have multiple de novo mutations and thus have received the multiple hits necessary for serious derailment of neurodevelopment. Another possible scenario explaining a non-linear association would be if de novo mutations are not the key causal link between paternal age and offspring outcomes and, instead, it is the age-related breakdown of epigenetic mechanisms which causes the increased risk for negative offspring outcomes. As noted in the background literature review, it is known that the resetting of epigenetic imprinting is an important step in the generation of spermatozoa in men, and that this resetting process becomes more imperfect over time. It is likely that the decline in functioning of the epigenetic imprinting system is similar to that of other biological systems and follows the pattern described by reliability theory, i.e., good functioning and very gradual decline in the face of accumulating individual component failures until a critical point where functioning rapidly declines.

A final reason why one might not expect a linear effect of age despite the seemingly linear effect of age on de novo mutations has to do with the selfish sperm hypothesis briefly referenced during the literature review (Goriely & Wilkie, 2012). Some studies have shown that mutated germ line sperm cells may be begin to divide more rapidly than their unmutated counterparts. In a manner not unlike that found in oncogenesis, the mutated germ line sperm cells may eventually out reproduce the unmutated germ line sperm cells so that the majority of spermatozoa that are produced and eventually released during sexual reproduction derive from mutated germ line cells, while the healthy spermatozoa are essentially crowded out. The rapid rate of dividing of these mutated germ line sperm cells in turn increases the rate at which they develop

further de novo mutations with the simple assumption that each division has a small chance of an additional copy error being introduced. Just such a mechanism for increasing mutation rates in cancer cells is well documented (Lengauer, Kinzler, & Vogelstein, 1998).

Now let us turn to considering alternative explanations for the non-significance of father's age as a predictor, and specifically alternative explanations which do not posit the existence non-linear effects. Such alternative explanations must take into account both the non-significant findings of this dissertation and the well-established finding that father's age predicts increased risk of schizophrenia. One possibility is that, while father's age predicts increased risk for the development of schizophrenia, this increased risk is not reflected by increased positive symptom scores but instead through other clinical measures associated with risk for conversion (e.g., impaired scores on cognitive testing). Similar to the ideas suggested in relation to mother's age, older father's age may be confounded with personality traits for reduced impulsivity (Arslan, Penke, Johnson, Iacono, & McGue, 2014) and, correspondingly, genes for reduced levels of subcortical dopaminergic signaling. This would again allow for both the finding of nonsignificance (at least in relation to positive symptoms) while still accommodating findings of increased rates of schizophrenia. Alternatively, father's age may be correlated with offspring's risk of developing the CHR syndrome in the first place (thus increasing the total number of offspring who will go on to develop a psychotic illness) but not have any predictive utility in differentiating which CHR individuals will then go on to develop a psychotic illness. It should be noted that at least in the NAPLS sample, father's age did not differ significantly between the CHR and control groups: however, definitive testing

of this proposition would require a more representative sampling of the population as opposed to the self-selecting nature of control recruitment used in NAPLS.

Another interpretation of the non-significance of father's age relates to the type of sample used in this study as compared with the samples generally used in the extant literature. Individuals in the clinical high risk group were specifically selected on the basis of positive symptoms that were neither too attenuated (i.e., sub-prodromal level) nor too severe (i.e., psychotic). In contrast, previous studies of the father's age effect have generally been large, population-based cohort studies without such restrictions on symptom severity (McGrath et al., 2014). Given that germ line mutations would be likely to have non-specific effects across the entire range of psychopathology, these effects might be most readily detectable in unrestricted samples which included individuals across the full range of symptom severity and functioning.

The preceding interpretation of hypotheses one, two, and three may help to interpret the findings of hypothesis four. It may be the case that the effect of father's age on risk for psychosis is not linear but rather exhibits criticality. If so we should expect an excess of particularly old fathers in the family history of psychosis group but not necessarily a marked group mean difference. Another potential reason for the lack of a significant finding is that the operational definition of family history of psychosis employed in this dissertation may have been too broad to show a clear effect. A single first or second degree relative rated as "possibly" having a psychotic illness was sufficient to qualify as having family history of psychosis. Given that the group mean differences were in fact in the predicted direction, it may be worth retesting the hypothesis with an enriched sample, e.g., to meet criteria for family history of psychosis,

individuals must have a first degree relative diagnosed as probably or definitely having schizophrenia, while the criteria for no family history of psychosis remains the same. This would be akin to increasing the intensity of exposure to family history of psychosis and might yield a significant effect.

Limitations and Future Directions

Several limitations of the current work should be noted. A large number of participants were missing information on one or both of their parent's ages. Some of these participants were likely to have been raised by individuals other than their biological parents (e.g., were adopted). Such participants, if their biological parent's ages could have been determined, would have provided an opportunity to test the effects of biological parent's age independent of parenting behaviors. Another limitation was the use of a point measures of symptoms, which may have been more likely to be influenced by various confounds of parent's ages, as opposed to a more longitudinal measure (e.g., conversion to psychosis) that might be less influenced by such confounds. To give an example of this, if having a younger parent made a participant more likely to sign up for the study when they only had very mild symptoms (e.g., because younger parents were more likely to see such mild symptoms as pathological), this would be more likely to affect baseline values, but conversion rates might be less influenced by such self-selection effects. Thirdly, a more complex measure of genetic loading that took into account the frequency of cases of psychotic illness within the family pedigrees and weighed them with known consanguinity risk values, e.g., similar to the ones used successfully used to model other multi-factorial-polygenic-threshold disorders such as

heart disease (Vogel & Motulsky, 1986), may have been able to show a relationship between family history of psychosis and father's age.

Several lines of future research could build upon the results of this study. A natural next step would be to examine whether or not measuring mother and father's age improves our ability to predict whether a CHR individual will go on to develop a psychotic disorder above and beyond other established predictors. If older mother's age does indeed predict a lower risk of conversion beyond its influence on positive symptoms, it can be added to our existing algorithms at little cost of time or effort. This additional accuracy could help to further inform clinicians who wish to provide interventions with an appropriate mix of costs and benefits tailored to an individual's risk for the development of psychotic illness (e.g., avoiding prescribing antipsychotic medication when the risk of conversion is low).

Future studies may also wish to further examine the subset of CHR individuals with a positive symptom score of zero. This dissertation, along with the work of Jefferies et al. (2014), suggest that CHR individuals with a positive symptom score of zero differ in important ways from other CHR individuals (e.g., are significantly less likely to convert within two years, are younger, the differing effect of mother's age, etc.). As such, it is worth studying whether these individuals are, broadly speaking, false positives, at an earlier point in the risk period, or have an otherwise distinct trajectory. Potential future studies could examine zero score participant's cognitive testing profile and/or follow them for ten years or longer (the time horizon at which basic symptom measures have been able to predict conversion to psychosis; Klosterkötter, Hellmich, Steinmeyer, & Schultze-Lutter, 2001). Finally future studies may wish to drill down to discover what the

potential mechanism are that underlie the link between older maternal age and less intense positive symptoms in offspring, e.g., using physiological measures of subcortical dopaminergic signaling in offspring.

Conclusion

This dissertation set out to investigate potential clinical correlates of older father's age at conception in a clinical high risk population. Father's age was not a significant predictor of positive symptoms, negative symptoms, and social functioning. Family history of psychosis did not predict younger father's age. Increased mother's age at conception appeared to be associated not only with reduced intensity of positive symptoms but also, contrastingly, with increased odds for the presence of prodromal level positive symptoms in the first place.

While it may be hoped that further research will find ways to use mother's age and father's age to improve our predictive algorithms, it is unlikely that either will prove to be decisive protective or risk factors. Instead, if they do prove to have legitimate predictive utility, they likely be added to the long list of risk factors for psychotic illness, each of which has a minor effect upon an individual's risk. The majority of people exposed to a full suite of these risk factors (e.g., urbanicity, infection, childhood trauma, and malnutrition) will not go on to develop a psychotic disorder. It is clear then that the human brain is resistant to the development of psychotic illness even in the face of multiple insults. If necessary and sufficient causal factors do in fact exist, they are likely to lie at the level of neurobiology. As such, the challenge for researchers of psychotic illness will remain developing further insight into this group of disorders whose etiology remains characterized by probabilistic correlates, pleiotropy, and equifinality.

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Table 1

Key Psychosis Related Terms

Term	Definition
Psychosis	An abnormal mental state characterized by a loss of contact with reality. Symptoms of psychosis include delusions (fixed beliefs that are not amenable to change), paranoia (a subset of delusions centering around imagined threat from others), hallucinations (perception-like experiences that occur without an external stimulus), disorganized thinking (usually inferred from incoherent or illogical speech), and grossly disorganized/abnormal motor behavior (inability to engage in goal-directed behavior, catatonia). Psychosis can result from a variety of medical conditions, including autoimmune disorders (e.g., anti-NMDA receptor encephalitis) and endocrine conditions (e.g., hyperadrenocorticism). A variety of substances and organic insults can induce psychosis, including recreational drugs (e.g., cocaine-induced paranoid beliefs), medications (e.g., corticosteroids), and toxins (e.g., carbon monoxide poisoning).
Psychotic Disorders	Mental disorders in which psychosis is a prominent, though often intermittent, feature. These include some schizophrenia spectrum disorders (schizophrenia, schizoaffective disorder) and mood disorders with psychotic features (e.g., major depressive disorder with psychotic features).
Schizophrenia Spectrum Disorders	A group of mental disorders which are characterized by psychosis, negative symptoms, cognitive deficits, social impairment, and functional impairment. Also included among schizophrenia spectrum disorders are mental disorders or syndromes in which psychosis is not present but whose symptoms resemble attenuated versions of those found in more severe schizophrenia spectrum disorders: these include schizotypal personality disorder and the clinical high risk state. Schizophrenia spectrum disorders are likely to share some etiological and neurological underpinnings.

Term	Definition
Clinical High Risk (CHR) State	A syndrome associated with a significantly increased risk of developing a psychotic disorder (usually schizophrenia) within a few years. Several sets of diagnostic criteria may qualify an individual as being in the CHR state and these are sometimes referred to as CHR syndromes. CHR individuals usually suffer from attenuated versions of the positive and negative symptoms of schizophrenia spectrum disorders (e.g., excessive suspiciousness). In addition, they often experience non-specific symptoms including depression, mild cognitive impairment, moderately impaired functioning, and difficulties in social relationships. Other terms used to refer to the CHR state have included the schizophrenia prodrome and the ultra high risk (UHR) state.
Psychosis-Prone Individuals	Individuals who, barring some kind of preventative intervention, will go on to develop a psychotic illness. This is, of course, an idealized concept, as many unpredictable factors determine whether or not an individual will go on to develop a psychotic illness. One key goal of CHR research is to develop an improved predictive algorithm for identifying psychosis-prone individuals.
Positive Symptoms	A collective term for the symptoms of psychosis, i.e., delusions, hallucinations, disorganized thought, and behavior.
Negative Symptoms	A group of symptoms characterized by reduced interest and expressivity common in schizophrenia spectrum disorders. These include reduced emotional expression, avolition (reduced self-initiated purposeful activity), and anhedonia (reduced ability to experience pleasure).

Table 2

Parent's Education Rating Values

Value	Highest Level of Formal Education
1	No schooling
2	Some primary school
3	Completed primary school
4	Some high school
5	Completed high school
6	Some college / technical school / undergraduate
7	Completed college / technical school / undergraduate
8	Some graduate / professional school
9	Completed graduate / professional school

Table 3

Characteristics of CHR Individuals by Positive Symptom Score

Characteristic	Positive Symptom Score = 0 (*n* = 53)	Positive Symptom Score > 0 (*n* = 582)
Age (*SD*)	16.94 (3.7)	18.59 (4.3)
Father's Age (*SD*)	32.83 (7.2)	32.00 (6.3)
Mother's Age (*SD*)	29.64 (6.9)	29.16 (5.8)
Father's Education (*SD*)	6.34 (1.8)	6.27 (1.7)
Mother's Education (*SD*)	6.51 (1.7)	6.41 (1.6)
n Female (% of group)	21 (39.6%)	246 (42.3%)
n Non-White (% of group)	17 (32.1%)	234 (40.2%)

Table 4

Hypothesis One Summary of Correlation Coefficients Between Independent Variables / Covariates and Positive Symptom Score

r	Father's Age	Mother's Age	Participant's Age	Parents' Avg. Education	Positive Symptoms
Father's Age	—	.70***	-.03	.23***	-.02
Mother's Age		—	-.14***	.30***	-.06
Participant's Age			—	.06	.13***
Parents' Avg. Education				—	-.01
P1P2 Scores					—

*** $p < .001$

Table 5

Hypothesis One Pair-wise Vuong Closeness Tests Comparing Regression Models

	Comparison Model (*p* Value of Comparison)			
Base Model	1	2	3	4
1. Zero-Inflated Poisson Regression (ZIPR)	—	< .001***	0.042*	0.042*
2. Zero-Inflated Negative Binomial Regression (ZINBR)		—	0.042*	0.042*
3. Poisson Regression (PR)			—	0.015*
4. Negative Binomial Regression (NBR)				—

Note. Models include Father's Age, Mother's Age, Participant's Age, and Average Parents' Education

* $p < .05$, *** $p < .001$

Table 6

Hypothesis One Zero Inflated Poisson Regression Predictors of Positive Symptom Score

Count Model					
Variable	Estimate	SE	*p* Value	Predicted Positive Symptom Score	Ratio Change in Predicted Score for 1 Unit Increase (95% Conf.)
Intercept	0.944	0.202	< .001***	2.63	
Father's Age	0.006	0.006	.238		1.007 (0.996 - 1.017)
Mother's Age	-0.013	0.006	.036*		0.987 (0.975- 0.999)
Participant's Age	0.012	0.005	.043*		1.012 (1.001 – 1.024)
Parents' Education	-0.002	0.009	.832		0.9981 (0.980 - 1.016)
Zero Model†					
Variable	Estimate	SE	*p* Value	Predicted odds of having a 0 score	Ratio Change in Odds of Having a 0 Score for 1 Unit Increase
(Intercept)	20.335	8.853	< .001***	.000097	
Father's Age	0.153	0.111	.169		1.165 (0.937 - 1.450)
Mother's Age	-0.459	0.197	.020*		0.632 (0.429 - 0.930)
Participant's Age	-1.097	0.427	.010*		.340 (0.144 – 0.772)
Parents' Education	-0.066	0.214	.758		0.936 (0.615 – 1.425)

†: Note. In the zero model, positive coefficients for a predictor indicate that higher scores on this predictor are associated with a greater likelihood of having a zero score. Negative coefficients for a predictor indicate that higher scores on this predictor are associated with a lower likelihood of having a zero score. For example, the negative coefficient for participant's age suggests that the higher a participant's age, the less likely they are to have a zero score.

* $p < .05$, *** $p < .001$

Table 7

Hypothesis One Results of Hierarchical Analysis, Zero-Inflated Poisson Regression, Significance of Adding Father's Age

Step 1- Mom's age only

Count Model				**Zero Model**			
Variable	Estimate	SE	*p*	Variable	Estimate	SE	*p*
(Intercept)	0.970	0.025	< .001***	(Intercept)	-8.571	2.245	< .001***
Mother's Age	-0.009	0.005	.060	Mother's Age	-0.355	0.137	.010*
Participant's Age	0.012	0.006	.037*	Participant's Age	-0.980	0.328	.003**
Parents' Education	-0.001	0.009	.896	Parents' Education	-0.060	0.236	.801

Step 2- Coefficients with Father's age included†

Count Model				**Zero Model**			
Variable	Estimate	SE	*p*	Variable	Estimate	SE	*p*
(Intercept)	0.944	0.202	< .001***	(Intercept)	20.335	8.853	< .001***
Mother's Age	-0.013	0.006	.036*	Mother's Age	-0.459	0.197	.020*
Participant's Age	0.012	0.005	.043*	Participant's Age	-1.097	0.427	.010*
Parents' Education	-0.002	0.009	.832	Parents' Education	-0.066	0.214	.758

Log Likelihood of Increase in Predictive Utility	*p*
	.082

†: Note. Coefficients for father's age in step 2 not shown in order to ease visual comparison of coefficients. See Table 6 for father's age coefficients.

* $p < .05$, ** $p < .01$, *** $p < .001$

Table 8

Hypothesis One Results of Hierarchical Analysis, Zero-Inflated Poisson Regression, Significance of Adding Mother's Age

Step 1-
Father's age only

Count Model				**Zero Model**			
Variable	Estimate	SE	*p*	Variable	Estimate	SE	*p*
(Intercept)	0.966	0.025	< .001***	(Intercept)	-7.178	2.206	< .001***
Father's Age	-0.003	0.004	.498	Father's Age	-0.281	0.188	.134
Participant's Age	0.015	0.006	.010*	Participant's Age	-0.674	0.289	.020*
Parents' Education	-0.002	0.009	.826	Parents' Education	0.251	0.288	.384

Step 2-
Coefficients with mother's age included†

Count Model				**Zero Model**			
Variable	Estimate	SE	*p*	Variable	Estimate	SE	*p*
(Intercept)	0.944	0.202	< .001***	(Intercept)	20.334	8.853	< .001***
Father's Age	0.006	0.006	.238	Father's Age	0.153	0.111	.169
Participant's Age	0.012	0.005	.043*	Participant's Age	-1.097	0.427	.010*
Parents' Education	-0.002	0.009	.832	Parents' Education	-0.066	0.214	.758

Log Likelihood of Increase in Predictive Utility	*P*
	< .001***

†: Note. Coefficients for mother's age in step 2 not shown in order to ease visual comparison of coefficients. See Table 6 for mother's age coefficients
* $p < .05$, *** $p < .001$

Table 9

Hypothesis One Ordinary Least Squares Regression Predicting Positive Symptom Scores

Variable	Estimate	Std Error	*t*-Value	*p* Value
Constant	2.600	0.061	42.70	< .001***
Father's Age	0.012	0.014	0.87	.39
Mother's Age	-0.022	0.015	-1.43	.15
Participant's Age	0.046	0.015	3.14	.002**
Parents' Avg Education	-0.003	0.023	-0.13	.90

Multiple $R^2 = 0.022$, $F\ (4, 630) = 3.513$, $p = .008$**

** $p < .01$, *** $p < .001$

Table 10

Hypothesis Two Zero Order Correlations Between Predictors / Potential Covariates and Negative Symptom Scores

Variable	*r with negative symptom scores* (*p value)*
Father's Age	.029 (.47)
Mother's Age	.049 (.23)
Participant's Age	.027 (.51)
Parents' Avg Education	.013 (.75)

Table 11

Hypothesis Two Ordinary Least Squares Regression Predicting Negative Symptom Score

	Estimate	Std. Error	*t* value	*p*
(Intercept)	9.04	0.20	44.98	< .001***
Mother's Age	0.05	0.05	1.00	.32
Father's Age	-0.01	0.04	-0.19	.85

R^2= 0.002, F (2, 608) = 0.7525, p = .47
*** p < .001

Table 12

Hypothesis Two Follow-up Ordinary Least Squares Regression Predicting Negative Symptom Score with Hypothesis One Covariates

	Estimate	Std. Error	*t* value	*p*
(Intercept)	9.04	0.20	44.98	< .001***
Mother's Age	0.06	0.05	1.00	.32
Father's Age	-0.01	0.04	-0.19	.85
Participants' Age	0.04	0.05	0.85	.40
Parents' Avg. Education	-0.01	0.07	-0.11	.92

Multiple R^2= 0.004, F (4, 606) = 0.5543, p = .70

*** $p < .001$

Table 13

Hypothesis Two Follow-up Negative Binomial Regression Predicting Negative Symptom Score

	Estimate	Std. Error	*z* value	*p*
(Intercept)	2.202	0.024	92.125	< .001***
Mother's Age	0.005	0.006	0.942	.346
Father's Age	-0.001	0.005	-0.178	.859
Theta	4.196	0.378		

Comparison with null model $\chi^2 = 1.324$, $p = .516$
*** $p < .001$

Table 14

Hypothesis Three Zero Order Correlations Between Predictors / Potential Covariates and Social Functioning Scores

Variable	*r* with social functioning scores	*p*
Mother's Age	-.030	.228
Father's Age	-.055	.085
Participant's Age	-.026	.256
Avg. Parent's Education	.008	.421

Table 15

Hypothesis Three Ordinary Least Squares Regression Predicting Social Functioning Score

	Estimate	Std. Error	*t* value	*p*
(Intercept)	6.594	0.340	19.376	< .001***
Mother's Age	0.005	0.015	0.314	.754
Father's Age	-0.016	0.014	-1.190	.234

R^2= 0.003, F (2, 626) = 0.987, p = .373

*** $p < .001$

Table 16

Hypothesis Three Follow-up Ordinary Least Squares Regression Predicting Social Functioning Scores with Hypothesis One Covariates

	Estimate	Std. Error	*t* value	*p*
(Intercept)	6.708	0.482	13.915	< .001***
Mother's Age	0.001	0.015	0.086	.932
Father's Age	-0.016	0.014	-1.140	.255
Participants' Age	-0.011	0.015	-0.717	.474
Parents' Avg. Education	0.025	0.046	0.547	.584

Multiple R^2= 0.004, F (4, 624) = 0.679, p = .61

*** $p < .001$

Table 17

Hypothesis Four Family History of Psychosis Group Differences on Potential Covariates

Variable	*M FHP-* *M PHP+*	*Group Mean Difference*	*Std Error*	*t value*	*p*
Mother's Age	29.76 28.67	-1.09	0.525	2.083	.038*
Participant's Age	17.84 18.90	1.06	0.382	-2.787	.006**
Mother's Education	6.43 6.40	-0.03	0.147	0.233	.816
Father's Education	6.37 6.32	-0.05	0.159	0.304	. 761

* $p < .05$, ** $p < .01$

FHP- = No family history of psychosis, FHP+ = Family History of Psychosis

Note. Positive values indicate higher group means for positive family history of psychosis group.

Table 18

Hypothesis Four ANCOVA Comparing Father's Age between Family History of Psychosis Groups

Factor	***F***	***p***
Corrected Model	8.659	< .001***

Parameter	**Estimate**	**Std. Error**	***t***	***p***
Mother's Education	0.224	0.196	1.146	.252
Father's Education	0.667	0.182	3.664	< .001***
Family History of Psychosis	0.781	0.568	1.377	.169

Model R^2= .051.

*** $p < .001$

Table 19

Hypothesis Four T-Test Comparing Father's Age between Family History of Psychosis Groups

Groups	*M* FHP- *M* FHP+	*t*	*p*	eta^2
Family History of Psychosis	32.52 31.70	2.004	.158	.004

FHP- = No family history of Psychosis, FHP+ = Family History of Psychosis

Table 20

Supplemental Analysis- Cross Tab Analysis of Family History of Psychosis and Zero vs. Non-Zero Symptom Score

			No Family History of Psychosis	Family History of Psychosis	Total
Positive Symptom Score	= 0	Count	16	26	42
		Expected Count	25.9	16.1	42
	> 0	Count	323	184	507
		Expected Count	313.1	193.9	507

χ^2 (1, N = 549) = 10.773, p < .001

Table 21

Supplemental Analysis- Zero Inflated Poisson Regression for Positive Symptom Score with Family History x Parent's Age Interaction Terms

Step 1-
Without Interaction Terms

Count Model				**Zero Model**			
Variable	Estimate	SE	*p*	Variable	Estimate	SE	*p*
(Intercept)	0.833	0.208	.001***	(Intercept)	18.115	9.215	.049*
Mother's Age	-0.015	0.006	.025*	Mother's Age	-0.352	0.151	.019*
Father's Age	0.010	0.006	.079	Father's Age	0.240	0.117	.040*
Participant's Age	0.013	0.007	.057	Particpant's Age	-1.408	0.639	.028*

Step 2-
With Interaction Terms

Count Model				**Zero Model**			
Variable	Estimate	SE	*p*	Variable	Estimate	SE	*p*
(Intercept)	0.844	0.236	.000	(Intercept)	7.675	11.47	.504
Mother's Age	-0.016	0.008	.046*	Mother's Age	-0.389	0.160	.015*
Father's Age	0.013	0.008	.099	Father's Age	0.370	0.211	.080
Participant's Age	0.011	0.007	.112	Participant's Age	-0.914	0.408	.025*
Mother's Age x FHP	0.008	0.012	.503	Mother's Age x FHP	0.654	0.239	.006**
Father's Age x FHP	-0.007	0.011	.520	Father's Age x FHP	-0.525	0.187	.005**

Log Likelihood of Increase in Predictive Utility	*p*
	.014*

FHP- Family History of Psychosis
* $p < .05$, ** $p < .01$, *** $p < .001$

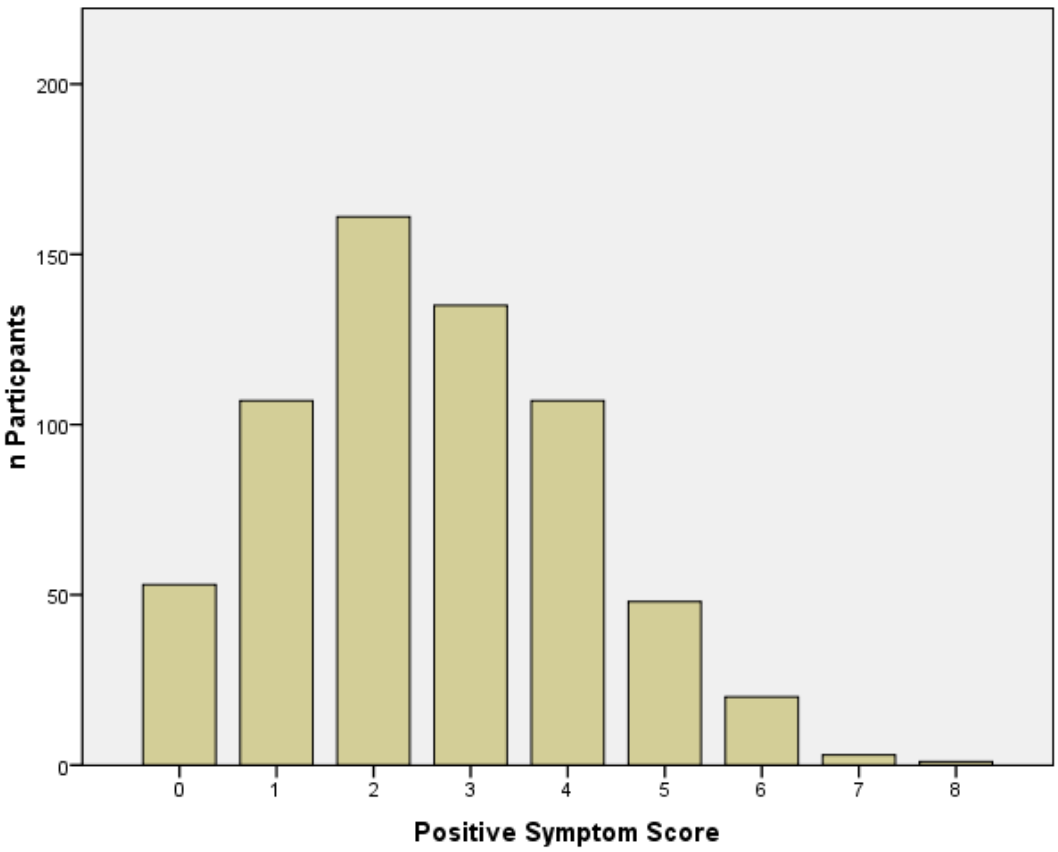

Figure 1. Bar plot of distribution of positive symptom scores among CHR participants**.** Positive symptom score is the recoded sum of the P1 and P2 subscales of the Structured Interview for Prodromal Symptoms as described in methods section.

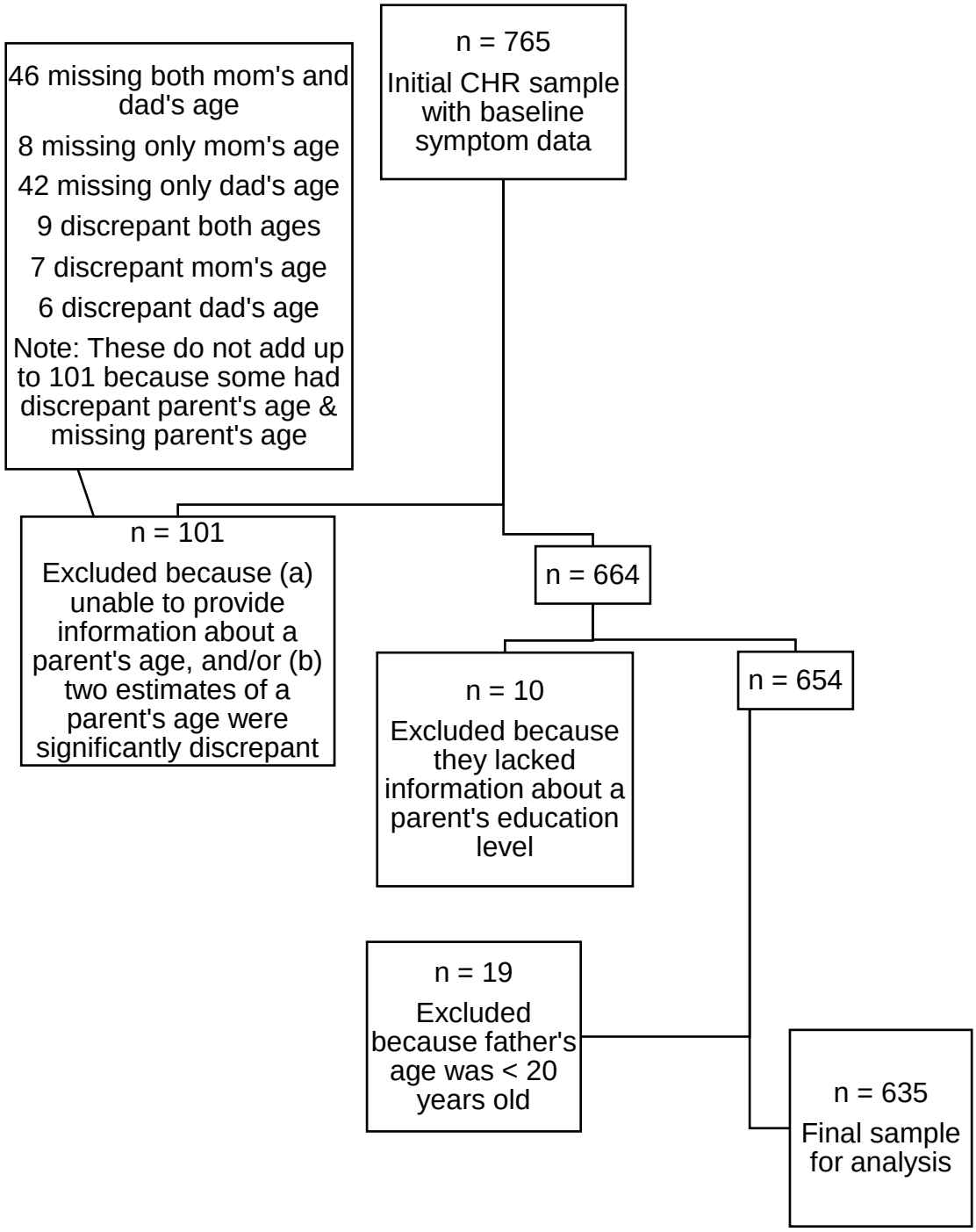

Figure 2. Subject exclusion flow chart.

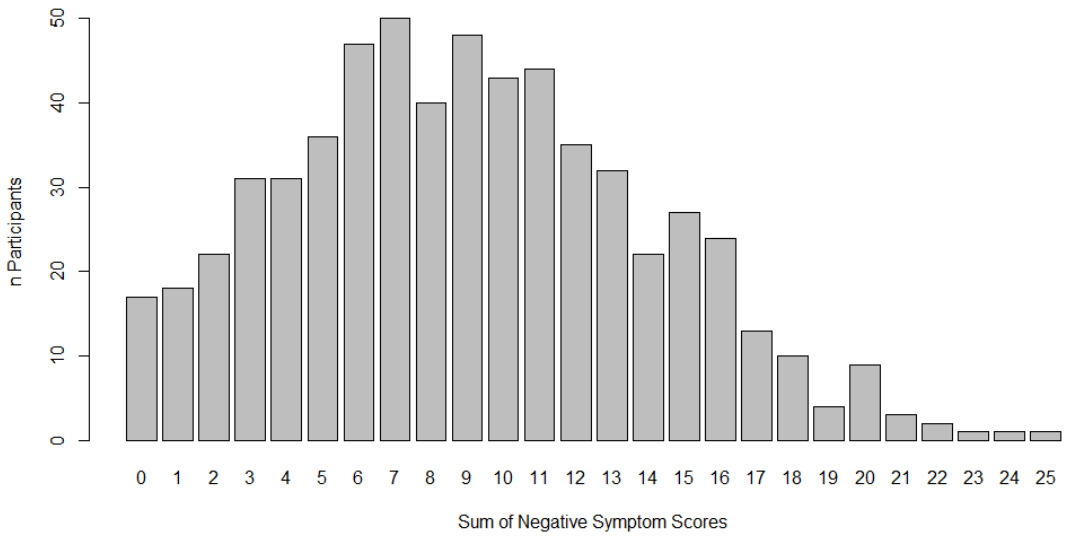

Figure 3. Bar plot of distribution of sum of negative symptom scores among CHR participants. Sum of negative symptom score was calculated as the raw sum of subscales N1 - N5 from the Structured Interview for Prodromal Symptoms.

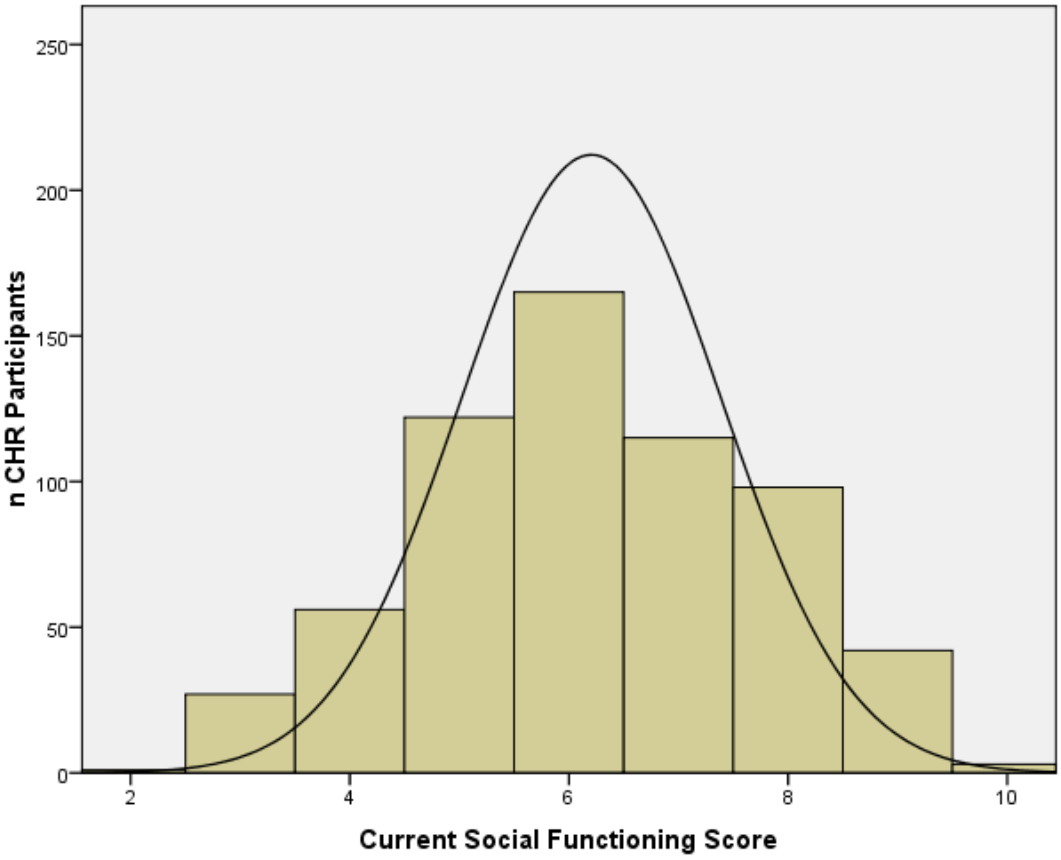

Figure 4. Histogram of current social functioning scores among CHR participants.