Advanced Paternal Age is a Risk Factor for Schizophrenia in a Cross-sectional Population Study of Iranians.

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Abstract

Since 1958 many, but not all studies have demonstrated that paternal age is a risk factor for schizophrenia. The small number of children in Western families makes risk comparisons between siblings born at different paternal ages difficult. In contrast, Eastern families have children both at early and later periods of life. Here, a cross sectional study was done in Iranians to investigate the paternal age effects and the frequency of schizophrenia in higher birth rank (i.e. older paternal age) vs. lower birth ranks.

Methods: Two hundred twenty schizophrenic patients and 220 control subjects matched for sex and age were employed in this study. Patients with neurological problems, substance abuse, mental retardation and mood disorder were excluded from both groups.

Results: The mean age of fathers at birth in cases (30 ± 6.26 years) was significantly more than the controls (26.45 ± 5.64 years; $p = 0.0001$). The age of 76 and 33 fathers at birth was over 32 in case and control groups, respectively. Individuals whose fathers' age at birth was more than 32 were at higher risk (2.77 times) for schizophrenia ($p<0.0001$, CI95%: 1.80-4.27). The maternal age at parturition was $26.1+-5.41$ vs. $25.07+-4.47$ ($p=0.02$) in cases vs. controls. Logistic regression analysis showed no maternal age effect in schizophrenia pathogenesis. Birth rank comparisons revealed; 35% vs. 24% of the cases vs. controls were in the third or upper birth rank ($p=0.01$).

Discussion: Further research is required to reveal whether DNA base and/or epigenetic changes in sperm account for the increased risk associated with elder fathers.

Key Words: Paternal age, Birth Rank, Schizophrenia, Epigenetic

Introduction

Schizophrenia is a chronic and disabling disorder with an ~1% incidence, and 120 million afflicted individuals worldwide (Saddock and Saddock, 2005). Hallucinations, delusions, and emotional, cognitive, and motor deficits are common symptoms that appear usually in late adolescence or early adulthood (Murray et al., 1996; Mc Leen, 2000). Many studies detected a genetic link to the disorder, although no single or small number of genes accounts for the majority of cases (Kato et al., 2005; Abdolmaleky et al., 2005). Systematic analyses of different pedigrees segregating schizophrenia suggested multiple genes with a complex inheritance pattern (Faraone et al., 2002; Ban, 2004). Child adoption studies supported a polygenic and multi-factorial etiology (Beckman and Franzek, 2000).

Environmental factors linked to schizophrenia include winter parturition (Torrey et al., 1997), maternal stress during pregnancy (Khashan et al., 2008, Malaspina et al., 2008a), second trimester and postnatal infection, labor and perinatal traumas, immigration, residency in urban areas etc. (Compton, 2004; McGrath, 2007), and paternal occupation and age (reviewed in Malespina, 2002). Paternal age has been linked to schizophrenia since 1958 (Johanson, 1958). Most studies including a recent meta-analysis have confirmed paternal age as a risk factor for disease (Hare and Moran, 1979, Brown et al., 2002, Dalman et al., 2002, Zammit et al., 2003, Byrne et al., 2003, Sipos et al,. 2004, El-Saadi et al., 2004, Tsuchiya et al., 2005, Wohl and Gorwood, 2007), however negative results also exist in the literature (Malama 1988). The link between paternal age and schizophrenia, argues that de novo changes to DNA can lead to schizophrenia, because the sole biological contribution of fathers to progeny is DNA. *De novo* mutations are linked to neuro-developmental disorders and cancers (Zhang et al., 1999; Hemminki et al., 1999; Wilkin et al., 1998) and may explain the high prevalence of schizophrenia despite the decreased fertility of schizophrenia patient (Malaspina et al., 2002).

Malaspina (2001) suggested that single base pair mutations, trinucleotide repeat expansion, or genetic imprinting changes might be responsible for the observed effect. However, DNA replication and epigenetic changes are closely linked at both the macromolecules level (e. g. DNA replication and global DNA methylation), and the metabolic level (e. g. synthesis of purines and dTTP and S-adenosyl methionine, the cofactor that donates a methyl group). Hence, it would not be surprising that paternal age influences both of these processes as well as others that overlap these processes.

In 2002, Malaspina et al. reported that the paternal age at birth of sporadic cases averaged 4.7 years older than familial cases of schizophrenia, and attributed ~27% of the sporadic cases to paternal age, and likely due to de novo mutations. However, Pulver et al. (2004) observed that paternal age is not different in (1) familial versus sporadic cases, and (2) first and second-degree paternal relatives of probands, and argued that these results did not support the linkage of paternal age to spontaneous forms of schizophrenia. It is quite clear that studies on large families and pre- and post- index generations are important to distinguish genetic from sporadic cases. However, it should be mentioned that, if advanced paternal age is a risk factor for schizophrenia, the frequency and coincidence of disease would be more in children of fathers with higher age that could be misinterpreted as genetic inheritance. This may obscure the detection of sporadic versus familial cases that could be overcome in part by the examination the effects of higher birth rank, accompanied with advanced paternal age, on schizophrenia pathogenesis in large families.

This study examined the relationship between paternal age and schizophrenia in an Iranian population, a distinct cultural group not previously studied. Typical Iranian families are large with a wide variation in paternal age not typically seen in western families. The study of large families provides us an opportunity to examine relationship between birth

order, paternal age and schizophrenia. If advanced paternal age is an important risk factor for schizophrenia, disease frequency should be greater in children with higher birth rank as well (i.e. higher paternal age). Hence, we examined the effects of higher birth rank and advanced paternal age on schizophrenia.

Method and Samples

This cross-sectional study was done in Tehran in 2005 and 2006 recruiting 220 patients (case group) with a diagnosis of schizophrenia from both psychiatric hospitals and private clinics. The control group was made up of 220 individuals from non-psychiatric hospital wards, matched for sex ($p=0.84$, chi-square test) and age ($p=0.37$, T-test) versus the case group. Individuals with any neurological problem, substance abuse, mental retardation or mood disorder were excluded from the case and control groups. Patients in an acute psychotic phase were excluded because of their inability to give consent. No organic problems were identified in any patients with schizophrenia by medical examinations. All cases and controls completed consent forms and data was collected on each subjected by trained medical students completing two instruments. The demographic questionnaire recorded sex, employment, education, paternal age, maternal age, paternal and maternal age at parturition, family history of psychotic disorders, number of children, birth rank within siblings, and age of disease onset. Both patient and his/her companions were asked about the presence of similar disorders in first and second-degree relatives. The second instrument was the SCID previously validated for diagnosis of schizophrenia on Iranian population.

Differences between study groups were examined using the chi-square test for categorical variables, and an independent sample t-test (or Mann-Whitney U-test) for continuous variables. Probability values of $P<0.05$ were considered statistically significant. Multiple logistic regression analysis was done with adjustment for independent

association of all factors with schizophrenia as a dependent variable. One model (included all independent variables at one stage) was developed to adjust for covariates. The analysis was done using SPSS software (version 11.5).

Results

The main goal of these experiments was to investigate the effect of parental age on the incidence of schizophrenia in an Iranian population using a cross sectional population approach (220 in each group) of patients seeking treatment. Patients were diagnosed using SCID validated for use with Iranian patients.

A greater number of cases (Table 1) had a birth rank of $\geq$ 3 versus controls (35% versus 24%, p = 0.01, OD = 1.7, CI = 1.12-2.5). Difference in the occurrence of psychiatric disorders in first-degree relatives was significant in case versus control groups (p = 0.04): 20.5% versus 13.2%, respectively (Table 2).

Paternal age at parturition of the proband was significantly different between the case and control groups. For instance, the mean paternal age at birth was 30$\pm$6.26 versus 26.45$\pm$5.64, in case versus control groups, respectively (P = 0.0001) (Table 3). A significantly higher number of fathers with an age of $\geq$32 were in the case group versus the control group (Tables 4 and 5). Analysis of the paternal age distribution (Figure 1) revealed that the fathers of the case group tended to have children at an earlier age, but that a greater number (76, 35%) of cases' fathers age were $\geq$32, than those in control group (No: 33, 15%). Individuals with fathers' ages at birth $\geq$32 were 2.77 times at higher risk for schizophrenia (p<0.0001, OD = 1.80-4.27, CI = 95%). The mean age of mothers at birth was marginally significantly (P = 0.02) between the case and control groups (26 versus 25.07, respectively). Logistic regression analysis (Table 6), adjusting for the effect of maternal age, birth rank and family history,

indicated that paternal age is an independent predisposing factor to schizophrenia; its odds ratio was 4.65 (2.36-9.19) with the confidence interval of 95%. This case control study providing data on a previously unexplored Iranian population support that the advanced paternal age and the birth rank of higher than 3 (i.e. higher age of father at birth) are linked to schizophrenia pathogenesis.

Discussion:

Our results linking advanced paternal age to schizophrenia are comparable to observations in other population. Further, logistic regression analysis suggested that higher birth rank along with advanced paternal ages was more of a risk factor for schizophrenia than maternal age.

Birth rank and short birth interval were found to be risk factors for schizophrenia (Bathaee et al., 1977; Gaughran et al., 2007; Smits et al., 2004). The affect of higher birth order/rank may reflect the costs of childbearing on a mother's long-term health as Christensen et al. (1998) analyzing twin data found support for the proverb "a tooth, a child". Higher birth rank affects may also be a function of advanced paternal and/or maternal age. Higher birth rank (and advanced paternal age) may account to the coincidence of disease in multiple siblings whereas such coincidences are taken as evidence for genetic inheritance. However, paternal age effects on schizophrenia pathogenesis may be mediated through both genetic and/or epigenetic mechanism (Perrin et al., 2007; Malaspina et al., 2008b).

About 50% of monozygotic twins (aka identical) afflicted by disease and begin life with identical genomes are discordant for schizophrenia, and when concordant do not have the same psychiatric phenotypes (Saddock and Saddock 2005). Additionally, all children of monozygotic twins concordant or discordant for schizophrenia have the same elevated

probability of becoming ill (Gottesman and Bertelsen, 1989; Kringlen and Cramer, 1989). These observations suggest that genetic susceptibility is transmitted to offspring, but environmental factors also contribute in disease occurrence and presentation. We detected elevated somatic mutation rates in twins discordant for schizophrenia around simple trinucleotide repeat sequences, e.g. $(CAG)_n$ (Nguyen et al., 2003). Note that this study was limited to $(CAG)_n$ repeat occurrences and we do not know whether the increased mutation rate extends to other sequences. However, studies by others have also detected an increase in neuronal aneuploidies in schizophrenic brains (Kunugi et al., 1999; Yurov el al., 2001). Several groups (see Bayer et al., 1999 for review) have suggested that the genetic-environmental linkage reflects the need for "two-hits" (genetic plus somatic changes) to DNA. Other studies showed that genetic and epigenetic changes occur with age (e.g.Tamura et al.; 2007, Abdolmaleky et al., 2008), and epigenetic differences exist between monozygotic twins (Petronis et al., 2003; Petronis, 2004).

Epigenetics refers to heritable but potentially reversible changes in DNA methylation, RNA (editing and interference), and protein (e.g. histone) modification and provide mechanisms for the environment to interact with the genome (for review see Abdolmaleky et al., 2004). The multiplicity of targets and modifications linked to epigenetic programming argues that perturbations to such pathways are likely to account for a large amount of phenotypic variations.

In sperm, dominant and co-dominant base change mutations will impact progeny, and subsequent generations in a simple Mendelian fashion. A recessive mutation will not be detected in progeny (except occasionally when a second mutated allele is present) unless the target gene is maternally imprinted and silenced. Epigenetic changes at imprinted genes could lead to inappropriate expression or lack of expression. The effects on subsequent generations

will be dependent on the parental origin of the change. Further, epigenetic DNA methylation is reset during passage through the germline and during embryonic and post natal development; hence, it is not clear what changes will be passed to subsequent generations (Reviewed by Abdolmaleky et al., 2004).

The dissection of paternal age affect in large families is advantageous given the plethora of potential DNA changes and the consequences in subsequent generations. The paternal age effect may be due to inadequate nutrition and/or exposure to environmental toxins during spermatogenesis. For instance, it is estimated that 30% of American diets are deficient in folate, particularly in women (Nath et al., 2006; Affenito et al., 2007), a nutrient required for the biosynthesis of TTP and DNA synthesis as well as purine nucleotides that are necessary for DNA and RNA synthesis and other cellular processes (e.g. epigenetic changes to RNA and proteins, energy transduction, and signally) and S-adenosyl methionine, the major methyl donor, and involved in epigenetic changes (i. e, methylation) to DNA, RNA and proteins. The success of genetic studies on rare human disease has prompted research on complex common disease to focus on genetic approaches while largely ignoring environmental factors that have potential to change not only DNA but also epigenetic changes to DNA, RNA and proteins. For instance, paternal age and occupations linked to schizophrenia in progeny may involve exposures that induce oxidative stress and impact folate metabolism (Waly et al, 2004). A comprehensive understanding of schizophrenia and other common complex diseases requires studies on the interaction of the environment with specific genotypes, some predisposing, to severe illness.

Substantial increases in life expectancy (Durrer1996, De Flora et al., 2005, Cutler et al., 2006) during the last century in developed countries may be attributed to better sanitation and nutrition, access to fresh water, the use of antibiotics and vaccinations against live micro-

elements, bacteria, parasites and viruses as well as elimination of some known toxins/microelements and lower fetal and childhood death rates. Now is the time to consider strategies against other non-living microelements: toxins, contaminant and nutritional misbalances threatening the current generation as (or more than) before via poorly known epigenetic mechanisms. Hopefully, these studies will provide reliable biological clues to design preventive remedies and new therapeutics in major mental diseases.

To test the relevance of de novo mutation versus epigenetic hypothesis, in addition to larger epidemiologic and genetic studies a whole genetic analysis of spermatozoids may be required to find the presence of de novo mutations in older age versus younger age, a job that is possible but very difficult and expensive with the current techniques, therefore less acceptable to be funded and accomplished. Although the same approach needs to be taken for the analyses of epigenetic alterations in spermatozoa in younger age versus the older age, elimination of the non-differentially methylated DNA using new modifications of methylated CpG island amplification techniques (e.g. Toyota et al., 1999; Toyota and Issa 2002) in the same individuals can make this analysis feasible and much more reasonable compared to genetic analyses. Such studies are required as several preventable known environmental and nutritional factors (e.g. folic acid deficiency) can induce epigenetic alterations that may impact the quality of the human life at large.

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Table 1. Analysis of Birth Rank (order).

Sample	N	Parity [N (%)]		
		1	2	>3
Case	220	48 (21.8)	95 (43.2)	77 (35.0)
Control	220	61 (27.7)	106 (48.2)	53 (24.1)
Total	440	109 (24.8)	201 (45.7)	130 (29.5)

Table 2. Frequency of Reported Familial Psychiatric Disorders

Sample	N	Family History [N (%)]	
		Yes	No
Case	220	45 (20.5)	175 (79.5)
Control	220	29 (13.2)	191 (86.8)
Total	440	74 (16.8)	366 (83.2)

Table 3. Mean and Range of Parental Age at Birth. Shown is mean $\pm$ standard deviation (SD), and minimum (min) and maximum (max) parental ages

	N	Paternal Ages			Maternal Ages		
		Mean $\pm$ SD	Min	Max	Mean $\pm$ SD	Min	Max
Case	220	30.54 $\pm$ 6.26	15	57	26.10 $\pm$ 5.41	14	46
Control	220	26.45 $\pm$ 5.64	18	47	25.07 $\pm$ 4.47	18	40
Total	440	28.50 $\pm$ 6.29	15	57	25.59 $\pm$ 4.98	14	46

Table 4. Samples Classes as a Function of Parental or Maternal Age at Birth

	Parental Age at Birth [Number (%)]				
	Paternal		Maternal		Total
	$\leq$ 32 yrs	$\geq$ 32 yrs	$\leq$ 32 yrs	$\geq$ 32 yrs	P=0.065
Case	144 (76)	76 (34.5)	195 (88.5)	25 (11.5)	220 (100)
Control	187 (85)	33 (15)	206 (93.5)	14 (6.5)	220 (100)

Table 5. Analysis of the effect of paternal age on schizophrenia[1]

	Beta	df	P value	OR	(with 95% CI)	
					Min	Max
Paternal age	1.54	1	0.0001	4.66	2.36	9.2
Maternal age	-0.07	1	0.065	0.93	0.88	1
Birth rank	0.05	1	0.77	1	0.73	1.5
Family history	0.48	1	0.07	1.62	0.95	2.7
constant	-0.38	1	0.48	0.68		

Abbreviations: Beta – beta error, df – degrees of freedom, P value – probability values, – [1]

Odd Ratio 95% CI (min) (max) – odds ratio with minimum and maximum values for the 95%
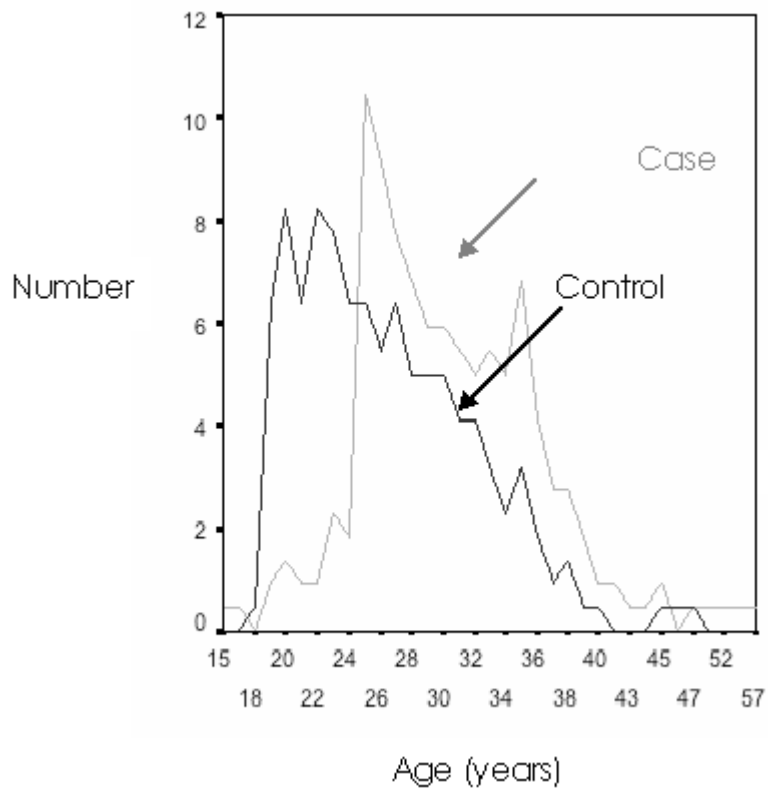
.confident interval

.**Figure 1.** Distribution of Paternal Age in case and control subjects