Telomere Length and Pulse Pressure in Newly Diagnosed, Antipsychotic-Naive Patients With Nonaffective Psychosis

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Introduction: Recent studies suggest that in addition to factors such as treatment side effects, suicide, and poor health habits, people with schizophrenia may have an increased risk of diabetes prior to antipsychotic treatment. Diabetes is associated with an increased pulse pressure (PP) and a shortened telomere. We tested the hypothesis that prior to antipsychotic treatment, schizophrenia and related disorders are associated with a shortened telomere, as well as an increased PP. Methods: Telomere content (which is highly correlated with telomere length) and PP were measured in newly diagnosed, antipsychotic-naive patients with schizophrenia and related disorders on first clinical contact and in matched control subjects. Both groups were also administered an oral glucose tolerance test. Results: Compared with control subjects, the patients with psychosis had decreased telomere content and an increased PP. As previously reported, they also had increased glucose concentrations at 2 hours. These differences could not be attributed to differences in age, ethnicity, smoking, gender, body mass index, neighborhood of residence, socioeconomic status, aerobic conditioning, or an increased cortisol concentration in the psychotic subjects. Discussion: These results suggest that prior to antipsychotic use, nonaffective psychosis is associated with reduced telomere content and increased PP, indices that have been linked to an increased risk of diabetes and hypertension.

Key words: schizophrenia/telomere/diabetes/psychosis/pulse pressure

Introduction

Schizophrenia is associated with an increased mortality rate, with cardiovascular disease accounting for many of the excess deaths. Many factors contribute to this problem, including increased rates of suicide, poor health habits, and high rates of smoking. Antipsychotic and antidepressant medications, which are widely used by people with schizophrenia, are also associated with weight gain, risk of diabetes, and possibly other cardiovascular risk factors.

However, some findings raise the possibility that physiological abnormalities associated with the disorder may also contribute to patients' increased mortality. The subtle but widespread physical anomalies found in schizophrenia2 lend plausibility to findings of abnormal glucose metabolism in antipsychotic-naive schizophrenia patients compared with control subjects in both recent4,5 and pre-antipsychotic era studies.6-12

An increased pulse pressure (PP), the difference between systolic and diastolic blood pressure (BP), is also a risk factor for cardiovascular mortality, as well as for dementia13 and all-causes mortality in the elderly.14 Although the physics of PP is complex, an important determinant of this measure is central artery elasticity15; with early atherosclerosis, there are commonly both a decreasing diastolic pressure and an increased systolic pressure. An increased PP is correlated with glucose intolerance.16 An increased PP is also associated with diabetes.16

Both an increased PP and glucose intolerance are associated with a shorter telomere length.17-19 The telomere is a noncoding structure consisting of TTAGGG tandem

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repeats at the end of mammalian chromosomes. A shortened telomere is also associated with poststroke mortality, dementia, cognitive decline, all-causes mortality in people older than 60 years, type 1 and type 2 diabetes, and coronary artery disease.

We hypothesized that compared with control subjects, newly diagnosed, antipsychotic-naive patients with non-affective psychosis would have a shortened telomere, as well as an increased PP. We examined patients with non-affective psychosis—ie, schizophrenia and related disorders—because these share clinical and genetic factors with schizophrenia, and most newly diagnosed patients with these disorders receive a diagnosis of schizophrenia within the first year after first clinical contact. We had previously reported that this patient population had an increased prevalence in diabetes or impaired glucose tolerance on the glucose tolerance test (GTT).

Methods

Subjects

Psychotic subjects were recruited at the time of their first contact with psychiatric services in a general academic hospital (Hospital Clinic of Barcelona). As part of the Spanish national health system, the hospital offers psychiatric services for everyone living in the surrounding catchment area (Esquerra Eixample) in the city of Barcelona. Esquerra Eixample is a relatively homogeneous middle class/upper middle class neighborhood in the center of the city. In Spain, there is also the option of seeking private care outside of the assigned catchment area. However, the Hospital Clinic serves as a regional referral center for psychosis, and in a survey of admissions to the emergency department of a large general hospital in an adjoining catchment area, there were no patients with psychosis from Esquerra Eixample.

These patients came from a larger study of this population. The subjects used in the present analysis were chosen from that ongoing study so that they would be matched for demographic variables, body mass index (BMI), and smoking. This matching was done blind to GTT and PP values. After this group matching was completed, their DNA was sent for telomere assays.

All subjects were interviewed using the Spanish translation of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) Axis I Disorders, clinician version. They were also administered the Dartmouth Assessment of Lifestyle Inventory, which quantifies substance abuse. Socioeconomic status (SES) was assessed with the Hollingshead-Redlich Scale.

Subjects in the psychosis group had a maximum cumulative (lifetime) antipsychotic exposure of 1 week and no antipsychotic use in the 30 days prior to the study. The psychosis subjects were allowed to receive antianxiety medication (lorazepam) the night before blood was drawn (N = 17), to a maximum of 3 mg, but not on the day of the blood sampling and GTT. Exclusion criteria for the control subjects included a history of a psychotic disorder or major depression or a current diagnosis of adjustment disorder.

Healthy control subjects were recruited using advertisements. In the larger study, control subjects were screened in an effort to match to the psychosis group BMI, age, gender, and smoking habit (average number of cigarettes per day). Additional inclusion and exclusion criteria for all subjects were (1) age from 18 to 64 years, (2) no history of diabetes or other serious medical or neurological condition associated with glucose intolerance or insulin resistance (eg, Cushing disease), (3) not taking a medication associated with insulin resistance (hydrochlorothiazide, furosemide, ethacrynic acid, metolazone, chlorothalidone, beta blockers, glucocorticoids, phenytoin, nicotinic acid, cyclosporine, pentamidine, or narcotics), (4) no history of cocaine use in the previous 30 days, (5) no lifetime diagnosis of schizophrenia or major depressive disorder, a current diagnosis of adjustment disorder, and (6) had not previously received an antipsychotic or antidepressant medication.

All subjects gave informed consent for participation in the study, which was conducted with the oversight of the institutional review boards of the Hospital Clinic of Barcelona, the University of Maryland Baltimore, and the Medical College of Georgia.

Metabolic Assessment

All subjects were given a 2-hour 75 g oral GTT, which began between 8 and 9 AM after an overnight fast. Fasting insulin, glycosylated hemoglobin (Hb1Ac), and cortisol blood levels were also recorded. Heart rate and BP (in mm Hg) were measured twice in the forearm, after 5 minutes of rest. Height and weight, while wearing underwear and without shoes, were recorded between the blood samplings.

Serum insulin level was measured in duplicate by monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunnes, Belgium). No cross-reaction with pro-insulin was detected. Hb1Ac was determined by chromatography (high-performance liquid chromatography, HA 8121; Menarini Diagnostici, Firenze, Italy; normal range 3.4%-5.5%). Cortisol was measured using a radioimmunoassay (Immuchem, Ivoz-Ramet, Belgium). BMI was calculated using the formula (weight [kg]/height [m²]). PP was calculated as systolic BP—diastolic BP. Homeostatic model assessments (HOMA) of steady-state beta-cell function (HOMA-%B) and insulin sensitivity (HOMA-%S) were calculated as percentages of a normal reference population of young nondiabetic subjects, using HOMA Calculator v2.2 (http://www.dtu.ox.ac.uk).
Telomere DNA Content

DNA telomere content (TC) was determined as previously described in blood leukocytes. Briefly, DNA was quantified with PicoGreen (Molecular BioProbes, Eugene, OR), a fluorometric dye, and known masses, 10–15 ng, were denatured at 56°C in 0.05 M NaOH/1.5 M NaCl, neutralized in 0.5 M Tris/1.5 M NaCl, applied, and UV cross-linked to Tropilon-Plus blotting membranes (Applied Biosystems, Foster City, CA). A telomere-specific oligonucleotide, end-labeled with fluorescein (5'-TTAGGG-3')4-FAM (IDT, Coralville, IA), was hybridized to the genomic DNA, and the membranes were washed to remove nonhybridizing oligonucleotides. Hybridized oligonucleotides were detected by using an alkaline phosphatase–conjugated antifluorescein antibody (Roche, Indianapolis, IN) that produces light when incubated with CDP-Star substrate (Applied Biosystems). Blots were exposed to Hyperfilm for 2 minutes (Amersham Pharmacia Biotech, Buckinghamshire, UK) and digitized by scanning. The intensity of the telomere hybridization signal was measured from the digitized images using Nucleotech Gel Expert Software 4.0 (Nucleotech, San Mateo, CA). TC for each sample is reported as a percentage of the median chemiluminescent signal from 6 replicate determinations of each patient DNA relative to the chemiluminescent signal in the same amount of a reference DNA standard (placental DNA) measured in parallel. The laboratory determining TC was blind to the subject’s clinical diagnosis. TC is directly proportional to telomere length measured by Southern blot (r = .904); however, TC can be measured with as little as 5 ng of genomic DNA and is insensitive to fragmentation of the DNA to less than 1 kilobase (kb) in length, and these measurements can be performed with DNA isolated from fresh, frozen, and paraffin-embedded tissues.

Statistical Analysis

Results are presented as mean ± SD. The 2 subject groups were compared using the nonpaired Student t test or the χ² test for comparisons of proportions. Significance was defined as P < .05 for all statistical tests, and these were performed using version 12.0 for Windows of the Statistical Package for Social Sciences computer program. The groups’ baseline glucose concentrations were nearly equivalent (see below), so for glucose tolerance the principal dependent variable was the 2-hour glucose concentration (2HG).

Results

There were 41 subjects with nonaffective psychosis (27 with schizophrenia, 9 schizoaffective disorder, 2 brief psychotic disorder, 2 delusional disorder, and 1 psychosis not otherwise specified) and 41 control subjects. The 2 groups were very similar with regard to demographics (except for SES), BMI, smoking, resting heart rate, and percentage from the catchment area (see table 1). In this relatively homogeneous sample, SES was not associated with PP, 2HG, or telomere length in univariate analysis (P > .15 for all 3 variables) and was not included in further analyses. All but 2 patients were completely naive for antipsychotic treatment; those 2 had minimal exposure, as described above. The 2 subjects who had received medication had received a maximum of 3 doses more than a year before the study was conducted.

The psychosis group had significantly decreased mean TC compared with control subjects (respective means [SD] 93.1% [12.1] vs 100.9% [15.2], each compared with the average of a reference population, which was defined as 100%; P = .011; figure 1). Telomere length is related to gender, but in our sample, there were no differences of TC between men and women; our 2 groups were also very similar with regard to gender composition. In the psychosis group, men and women had a similar TC (93.3% [13.9] vs 92.5% [7.2], respectively; P = .854), and no differences were found among the control group (100.0% [12.4] vs 102.9% [20.3]; P = .86). The psychosis group also had a significantly greater PP (in mm of Hg) of 47.9 (9.3) vs 41.8 (8.8); P = .003; figure

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Psychosis (N = 41)</th>
<th>Control (N = 41)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean (SD)</td>
<td>29.2 (9.2)</td>
<td>28.2 (6.6)</td>
<td>0.572</td>
</tr>
<tr>
<td>Male/female</td>
<td>28/13</td>
<td>28/13</td>
<td>1.000</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>87.8%</td>
<td>90.2%</td>
<td>1.000</td>
</tr>
<tr>
<td>Body mass index, mean (SD)</td>
<td>22.9 (4.1)</td>
<td>23.9 (3.1)</td>
<td>0.225</td>
</tr>
<tr>
<td>Number of cigarettes per day, mean (SD)</td>
<td>8.8 (9.6)</td>
<td>6.7 (8.6)</td>
<td>0.294</td>
</tr>
<tr>
<td>Residing in the hospital’s catchment area</td>
<td>29 (70.7%)</td>
<td>27 (65.9%)</td>
<td>0.406</td>
</tr>
<tr>
<td>Socioeconomic status (SD)</td>
<td>36.0 (15.6)</td>
<td>41.9 (13.6)</td>
<td>0.095</td>
</tr>
<tr>
<td>Resting heart rate, mean (SD)</td>
<td>77.1 (11.7)</td>
<td>73.1 (10.8)</td>
<td>0.112</td>
</tr>
<tr>
<td>Systolic blood pressure, mean (SD)</td>
<td>120.1 (11.7)</td>
<td>118.8 (11.9)</td>
<td>0.628</td>
</tr>
<tr>
<td>C-reactive protein, mean (SD)</td>
<td>0.202 (0.285)</td>
<td>0.212 (0.193)</td>
<td>0.856</td>
</tr>
</tbody>
</table>
2, as well as a significantly lower diastolic BP (72.2 [9.5] vs 77.0 [8.5]; \( P = .018 \)). There was no significant difference in systolic BP or resting heart rate (table 1). Within the psychosis group, 24 were treated with lorazepam while 17 were not. None of the BP scores (systolic, diastolic, and PP) were different between these 2 groups of psychotic subjects (\( P > .15 \) for all 3 comparisons).

As previously found, the psychosis and control groups did not differ on fasting measures of glucose tolerance. The respective values for fasting glucose concentration were 84.1 mg/dl (12.1) for the psychosis group and 83.3 mg/dl (8.2) for the comparison subjects (\( P = .72 \)). The values for fasting insulin were 11.4 (7.8) vs 9.5 (3.6; \( P = .175 \)), and the values for HbA1c were 4.4\% (0.38) vs 4.4\% (0.26; \( P = .759 \)). The homeostasis model measures of insulin resistance (HOMA-%S) and insulin release (HOMA-%B) were also not significantly different (\( P > .40 \) for both variables). In contrast, 2HG differed significantly (\( P = .001 \)) between the 2 groups: the psychotic group had a mean concentration of 108.3 (34.6) mg/dl, while the control subjects had a mean of 81.7 (21.0) mg/dl.

The psychosis group did not have a significantly higher plasma cortisol concentration than the control subjects (mean [SD]; 18.3 [5.2] vs 22.9 [7.3] mg/dl, respectively).

**Discussion**

In this study, newly diagnosed, antipsychotic-naive patients with schizophrenia and related disorders had shorter telomere length, a higher PP, and lower diastolic BP. As previously reported for this sample, they also had an increased 2HG. These differences could not be attributed to confounding by BMI, gender, age, ethnicity, psychotropic medications, smoking (which in our population was correlated with measures of abuse of other drugs, including alcohol; data not shown), aerobic conditioning as measured by resting heart rate, a higher cortisol concentration, area of residence, or drugs that affect glucose tolerance, most notably antipsychotics.

A limitation of our study was the relatively small sample size. Statistical power may have prevented finding group differences in the fasting measures, including HOMA-%B and HOMA-%S. On the other hand, given the relatively young age of our sample, it may be that glucose intolerance was in such an early stage of development that only a physiological challenge could reveal glucose-related differences in the 2 groups. Our failure to find a difference in fasting values, while finding a difference in a GTT, is consistent with previous literature. Another limitation of our results is that diet and some health habits were not formally assessed, and future studies of these abnormalities in schizophrenia would benefit from assessment of these health-related variables. However, most of the subjects were from the same middle class/upper middle class neighborhood in Barcelona. Spain is a country with a relatively homogeneous population with regard to socioeconomic factors, and medical care is available for free through government-supported clinics. The generalizability of these findings in an urban, Spanish, middle class/upper middle class population would, however, need to be tested in other populations.

The chief cause of excess premature mortality among patients with schizophrenia is cardiovascular disease. A shortened telomere, elevated PP, and abnormal glucose tolerance have all been associated with an increased risk of cardiovascular disease. Moreover, telomere length, which is highly correlated with telomere content, has been associated with both diabetes and cardiovascular disease. Two previous studies have recently
evaluated telomere length in patients with schizophrenia. Decreased telomere content has been found in patients with chronic schizophrenia\(^\text{30,31}\) and was associated with an increased severity of disease in such patients. However, confounding by smoking, the metabolic side effects associated with antipsychotic treatment, BMI, etc, could not be excluded in those studies. A shortened telomere is also associated with aging\(^\text{32}\) as well as post-stroke mortality, dementia, cognitive decline,\(^\text{39}\) all-causes mortality in people over the age of 60 years,\(^\text{21}\) type 1 diabetes\(^\text{13}\) and type 2 diabetes,\(^\text{23,34}\) and coronary artery disease.\(^\text{24}\)

Telomere length decreases during cycle of cell replication; this shortening is an important component of cell senescence. Oxidative stress, which accelerates aging, increases the amount of telomeric DNA lost during each replication cycle. There is some evidence that increased oxidation is associated with schizophrenia,\(^\text{35}\) which could explain the pathophysiology of shorter telomere length among psychotic patients because abnormal oxidation/reduction balance has also been associated with diabetes. Previous experiments\(^\text{36,37}\) have shown that the conversion from TC to telomere length is approximately that a score of 100% TC is correlated to 10 kb in telomere length. Therefore, each 1% difference in TC reflects approximately a 100-bp difference in telomere length.

An increased PP has previously been correlated with both telomere length\(^\text{19,22}\) and glucose dysregulation\(^\text{16}\) and has been proposed as a measure of biological as opposed to chronological age.\(^\text{14,36}\) We also found a significantly lower diastolic BP in the psychosis group but no significant difference in systolic pressure. A lower diastolic pressure is consistent with early vascular damage and is found in early stages of hypertension.\(^\text{37}\) Our results may reflect a loss of flexibility in the central, elastic arteries. However, it would be useful to apply other methods of determining arterial problems,\(^\text{15}\) such as pulse wave characteristics, the thickness of central arterial walls, and coronary calcification. Both an increased PP\(^\text{38}\) and a shortened telomere\(^\text{36}\) have been proposed as measures of biological as opposed to chronological age. Should these findings be replicated, they would provide evidence for the hypothesis we previously presented\(^\text{39}\) that schizophrenia is a syndrome of accelerated aging.

Neither do our data provide information on which of the abnormalities that we found occurs first nor do our results provide information on the mechanisms that are responsible for the comorbidity among these 3 abnormalities. One possible mechanism is that prenatal fetal programming produces all 3, perhaps because of shared risk genetic\(^\text{4,28}\) or environmental factors.\(^\text{40}\)

Nonaffective psychosis appears to be associated with reduced telomere content, higher 2HG levels, and increased PP, indices that have been linked to accelerated aging and a predisposition to diabetes and hypertension. Medication side effects, an increased suicide rate, poor health care, and poor health habits all contribute to the increased mortality of schizophrenia. However, the existence of these factors that impact on mortality does not exclude the possibility of a preexisting vulnerability to cardiovascular disease, as suggested by the abnormalities reported here.

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**References**


43. Conclusions: Telomere shortening and oxidative DNA damage are associated with chronic schizophrenia. *Schizophr Bull*. 2008;34:1024–1032.