New Evidence for the Involvement of Mitochondrial Inheritance in Schizophrenia: Results From a Cross-Sectional Study Evaluating the Risk of Illness in Relatives of Schizophrenia Patients

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ABSTRACT

Objective: One of the hypotheses about the genetic factors that contribute to schizophrenia involves mitochondrial DNA (mtDNA), an approximately 16,569-base pair molecule inherited only from the mother. If this hypothesis were true, one would expect a higher frequency of schizophrenia among matrilineal relatives who share mtDNA with a schizophrenia patient than among relatives who do not. This article reports the risk of presenting with schizophrenia, other psychiatric disorders, and conditions related to mitochondrial disorders in relatives who share mtDNA with a schizophrenia patient versus those who do not.

Method: We interviewed 100 schizophrenia patients (*DSM-IV* criteria) and 147 of their first-degree relatives from November 2007 to November 2009 to collect clinical data from patients and from both sides of each patient's pedigree. The study was conducted at of a psychiatric teaching hospital in Reus, Spain. Contingency tables were established, and odds ratios were calculated to estimate relative risk.

Results: Relatives who shared mtDNA with a schizophrenia patient had a higher risk of presenting with schizophrenia than those who did not share mtDNA (odds ratio [OR] = 3.05; 95% CI, 1.65–5.72; $P < .001$). Female but not male relatives who shared mtDNA with a schizophrenia patient also had a higher risk of unipolar depression (OR = 10.19; 95% CI, 4.07–32.80; $P < .001$), panic attack (OR = 15.52; 95% CI, 2.41–643.6; $P < .001$), and other anxiety disorders (OR = 4.14; 95% CI, 1.84–9.71; $P < .001$). Some conditions frequently associated with mitochondrial disorders were also more frequent among female relatives who shared mtDNA with a schizophrenia patient than among those who did not.

Conclusions: The results of this study support the hypothesis that mtDNA may be involved in schizophrenia. In females, mtDNA could also be involved in the development of other psychiatric and nonpsychiatric conditions. Further studies are needed to confirm the role of mtDNA in psychiatric disorders.

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Genetic factors have been proposed to be relevant in the development of schizophrenia, as first-degree relatives of schizophrenia patients are at higher risk for developing the illness (odds ratio [OR] = 9.8; 95% CI, 6.2–15.5), and the heritability of susceptibility for schizophrenia is about 81% (95% CI, 73%–90%).[1] A large number of genetic studies have been performed; however, the identification of specific variants or genetic mechanisms associated with schizophrenia has proven difficult. Most of the genetic studies thus far have focused on the nuclear genome and ignored the mitochondrial DNA (mtDNA; GenBank accession number NC_012920). Human mtDNA is a circular, double-stranded molecule of about 16,569 base pairs and encodes 37 genes: 2 ribosomal ribonucleic acids (RNAs), 22 specific transfer RNAs, and 13 polypeptides of the mitochondrial respiratory chain (MITOMAP, 2009). These 13 polypeptides represent a small fraction of the total number of respiratory chain subunits but are essential for its proper function and, therefore, for efficient generation of energy in the form of adenosine triphosphate.[2] Some characteristics of mtDNA are distinct from those of nuclear DNA: maternal inheritance, high substitution rate, polyploidy, and high level of polymorphism. These polymorphisms include some of those involved in the susceptibility to certain diseases and in the expression of some mtDNA mutations.[3] Specific mtDNA variants and mutations have been associated with, and implicated in, a number of human traits and multisystem disorders such as aging (Online Mendelian Inheritance in Man accession no. 502000), Alzheimer's disease (no. 502500), Parkinson's disease (no. 556500), and other classical mitochondrial disorders such as myoclonic epilepsy with ragged red fibers syndrome (MERRF, no. 545000), mitochondrial myopathy, encephalopathy, lactic acidosis, stroke (no. 540000), and Leber's hereditary optic neuropathy (no. 535000). Mutations in mtDNA can be spontaneous, maternally inherited, or the result of an inherited nuclear defect in the genes that maintain mtDNA, as several nuclear-encoded genes are involved in replicating and supplying deoxyribonucleotide triphosphates to maintain mtDNA copy number.

Several lines of evidence suggest that mtDNA could be involved in schizophrenia, recently reviewed by Verge et al[4]:

1. Some studies have indicated that rates of schizophrenia are higher among relatives of female patients compared to relatives of male patients.[5–7]
2. A wide range of studies has noted lower mating and offspring rates in schizophrenia patients than in the general population.[8–15] Recently, it has been hypothesized and demonstrated that the persistence of schizophrenia can be explained only if genetic susceptibility variants are located in the mtDNA and transmitted through female siblings.[16,17]
3. Alterations in mitochondrial morphometry, brain energy metabolism, and enzymatic activity of the respiratory chain

have been reported in schizophrenia, suggesting mitochondrial dysfunction as part of the pathophysiology of schizophrenia,[18] which could be related to mtDNA dysfunction.
4. Major psychiatric disorders, including schizophrenia, have been reported in adult patients with mitochondrial diseases.[4,19]

The aim of this study was to investigate whether matrilineal relatives who shared mtDNA with a schizophrenia patient had a higher frequency of schizophrenia, other psychiatric disorders, or clinical features associated with mitochondrial diseases than those relatives who did not. Confirmation of this hypothesis would support the idea that mtDNA variants and/or mutations could be involved in the development of schizophrenia.

Clinical Points

- Mitochondrial DNA is shared among matrilineal relatives, and the risk of developing schizophrenia is higher in matrilineal than non-matrilineal relatives of a schizophrenia patient.
- Female matrilineal relatives of a schizophrenia patient are at high risk of presenting with a depressive or anxiety disorder.
- Detection of high-risk individuals leads to the development of early intervention programs that have been shown to improve both prognosis and disease progression.

METHOD

Sample

This study was conducted from November 2007 to November 2009 at the inpatient and outpatient units of a psychiatric teaching hospital, Hospital Universitari Psiquiàtric Institut Pere Mata (Reus, Catalonia, Spain). A total of 100 white patients with a *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, diagnosis of schizophrenia and 147 of their first-degree relatives were interviewed. Patients diagnosed with a schizoaffective disorder, affective psychosis, or other presentations of psychosis were excluded from the study. Patients and their first-degree relatives were interviewed by the first author of the study, an experienced master's level psychiatrist. For 47 of the patients, both parents were interviewed; for 34 patients, only the mother was interviewed; for 7 patients, only the father was interviewed; and for the remaining 12 patients, a sibling was interviewed. Table 1 shows the sociodemographic and clinical characteristics of the patients.

Procedures

This research was approved by the Ethics Committee of the Hospital Universitari Sant Joan in Reus and also received the approval of the review board of the Institut Pere Mata. All patients were clinically stable and in a condition to understand the purpose of the study in accordance with the requirements of the Declaration of Helsinki. Informed consent was obtained from both the patients and their relatives.

Interviews and Questionnaires

Patients completed a semistructured interview to provide clinical, sociodemographic, and drug consumption data at the onset of the illness. Information regarding a family history of schizophrenia and/or other psychiatric disorders was obtained from direct interviews of patients and their first-degree relatives (in independent interviews), and this information was compared to identify any inconsistencies. First-degree relatives also completed a questionnaire focused on the conditions commonly reported among individuals with mtDNA mutations, which can be ascribed to the following categories: migraine headaches, peripheral neurovascular disorders, gastrointestinal dysmotility, neurologic disorders, cardiac abnormalities, skeletal muscle disorders, endocrine disorders, and constitutional disorders. This same questionnaire has been used in previous studies to identify maternal inheritance in disorders with presumed mitochondrial dysfunction.[20–22] At the beginning of the interview, a family pedigree was generated, and relatives were asked for a history of the clinical features associated with mitochondrial disorders for all first- and second-degree relatives of the schizophrenia patient. Workflow charts of data collection, guides, and data sheets used to interview the patients and relatives are available in the online supplementary material (available at PSYCHIATRIST.COM).

Data Analysis

Four groups were established in 2×2 contingency tables to compare the presence of schizophrenia and other psychiatric disorders in relatives depending on whether they shared mtDNA with a schizophrenia patient. (Figure 1 shows a genogram for relatives who shared mtDNA with a patient and for those who did not.) To avoid gender-related bias, male and female relatives were also analyzed separately. We calculated odds ratios (ORs) and exact 95% confidence intervals, and Fisher exact test was used if the number of counts or the expected value was less than 5.

The presence of other clinical conditions associated with inherited mtDNA disorders was compared between all of the relatives who shared mtDNA with a schizophrenia patient and all of the relatives who did not, between mothers (who shared mtDNA) and fathers (who did not), and between maternal grandmothers (who shared mtDNA) and paternal grandmothers (who did not). To avoid false positive results, conditions not related to mitochondrial disorders were included as control questions (eg, coronary artery disease, cancer, hypertension, and arthritis), and we compared the prevalence of these conditions between maternal grandfathers and paternal grandfathers.

The statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, Illinois) and EpiInfo 3.5.1 (Centers for Disease Control and Prevention, Atlanta, Georgia).

RESULTS

Matrilineal, Familial Non-Matrilineal, and Sporadic Schizophrenia

Of the 100 schizophrenia patients evaluated, 37 presented with familial schizophrenia (having at least 1 first- or 1 second-degree relative with schizophrenia), while 63 were sporadic cases (without a family history of schizophrenia). Among the familial schizophrenia patients, 17 presented with an apparent matrilineal pattern of inheritance (ie, antecedents of schizophrenia were present solely in relatives who shared mtDNA with the index case), while 20 presented without a matrilineal pattern of inheritance. Among these 20 patients, however, 12 had a relative who shared mtDNA and was affected by schizophrenia. Schizophrenia diagnosis was confirmed by medical records in 76.5% of the matrilineal relatives and in 80% of the non-matrilineal relatives. Clinical and sociodemographic variables were similar between matrilineal and other familial schizophrenia patients (Table 1).

Table 1. Sociodemographic and Clinical Characteristics of the Schizophrenia Patients Depending on the Pattern of Inheritance

	Familial		Sporadic,		
	Matrilineal,[a] n = 17	Other,[b] n = 20	n = 63	Test[c]	*P* Value
Male gender, n	10	13	42	0.149	.699
Age at assessment, mean ± SD, y	43.8 ± 13.1	42.6 ± 12.5	40.3 ± 11.6	0.277	.784
Age at disease onset, mean ± SD, y	22.3 ± 11.3	19.2 ± 6.5	19.2 ± 4.1	1.118	.298
Age at first admission, mean ± SD, y	27.3 ± 12.6	23.1 ± 6.4	23.0 ± 10.8	1.700	.200
Admissions to a psychiatric center, no.	5.9 ± 8.2	6.3 ± 6.6	5.2 ± 8.4	0.028	.868
Disease duration, mean ± SD, y	21.4 ± 12.9	23.4 ± 14.3	21.1 ± 12.2	0.195	.662
Suicide attempts, mean ± SD, no.	0.6 ± 1.1	0.8 ± 1.4	0.5 ± 0.8	0.235	.631
Schizophrenia subtype, n					
Paranoid	13	17	50		
Disorganized	2	2	3		
Undifferentiated	2	1	4	0.628	.731
Residual	0	0	5		
Catatonic	0	0	1		
Years of schooling, n					
1–8	15	11	31		
9–12	2	7	30	5.184	.075
> 12	0	2	2		
Substance use, abuse, or dependence, n					
Alcohol	9	12	32	0.187	.666
Tobacco	14	16	44	0.033	.855
Hallucinogens	1	2	12	0.209	.647
Amphetamines	0	0	9	NA	NA
Cannabis	8	6	25	1.137	.286
Cocaine	4	2	12	1.238	.266
Opiates	2	1	2	0.564	.452

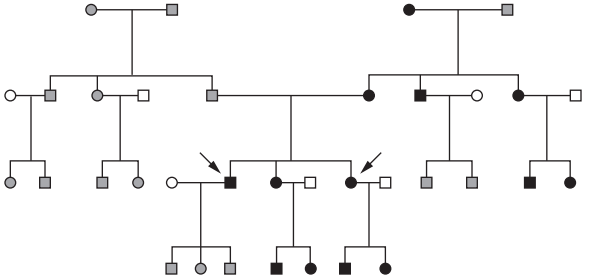
[a]Patients presenting a matrilineal pattern of inheritance.
[b]Patients presenting at least 1 non-matrilineal relative affected by schizophrenia in the family pedigree (however, in 12 of the 20 pedigrees there were also matrilineal relatives affected by schizophrenia).
[c]*t* Test or χ^2 test comparing variables between matrilineal and other familial schizophrenia patients.
Abbreviation: NA = not applicable.

Risk in Relatives Who Shared mtDNA With a Schizophrenia Patient

We interviewed 147 relatives of schizophrenia patients and retrieved information regarding first- and second-degree relatives on both sides of the pedigree. Among all of the relatives, 1,091 (656 females and 435 males) shared mtDNA with a schizophrenia patient and 1,300 (551 females and 749 males) did not (male:female ratio, 1:1.02; shared: not shared ratio, 1:1.19).

Risk of schizophrenia. Table 2 shows the kinship of relatives who presented with schizophrenia. Forty relatives (28 first-degree and 12 second-degree) who shared mtDNA with a schizophrenia patient also presented with schizophrenia themselves, whereas only 16 relatives (1 first-degree and 15 second-degree) who did not share mtDNA with a patient presented with schizophrenia. The number of relatives who presented with schizophrenia and the number who either shared or did not share mtDNA with a schizophrenia patient are presented in Table 3. Relatives who shared mtDNA with a patient were at a higher risk of presenting with schizophrenia than relatives who did not share mtDNA (OR = 3.05; 95% CI, 1.65–5.72; $P < .001$). Notably, this risk was slightly higher in male relatives (OR = 3.56; 95% CI, 1.57–8.24; $P < .001$) than in female relatives (OR = 2.86; 95% CI, 1.10–8.75; $P = .02$).

Figure 1. Genogram of First- and Second-Degree Relatives of a Male and Female Schizophrenia Patient[a]



[a]Arrows identify schizophrenia patients. Circles represent women; squares represent men. The black symbols indicate the biological relatives in the pedigree who shared mtDNA with the patients; the gray symbols indicate the biological relatives who did not share mtDNA with the patient.
Abbreviation: mtDNA = mitochondrial DNA.

Risk of other psychiatric disorders. Table 3 also shows the number of relatives presenting with other psychiatric disorders based on whether they shared mtDNA with a schizophrenia patient. Female relatives who shared mtDNA with a patient had a higher risk of unipolar depression (OR = 10.19; 95% CI, 4.07–32.80; $P < .001$), panic attack (OR = 15.52; 95% CI, 2.41–643.6; $P < .001$), and other anxiety disorders (OR = 4.14; 95% CI, 1.84–9.71; $P < .001$) compared to female relatives who did not share mtDNA with a patient. On the other hand, male relatives who shared mtDNA with a patient did not have a statistically higher risk of any of the psychiatric disorders listed in Table 3.

Risk of conditions associated with mitochondrial disorders. Information on conditions commonly associated with mitochondrial disorders was gathered from all first- and second-degree relatives in the pedigree. We compared the presence or absence of these conditions between the group of relatives who shared mtDNA with a patient and the group of relatives who did not (Table 4). When we compared the frequency of these conditions between mothers and fathers, headache, constipation, kinetosis, fibromyalgia, dysautonomia, arthritis, and muscular weakness were more frequent in mothers than in fathers, while heart disease was more frequent in fathers than in mothers. Most of the conditions evaluated were significantly more frequent in female relatives who shared mtDNA with a patient than in those female relatives who did not. Among the group of women who shared mtDNA with a patient, dysautonomia, constipation, headache, visual alterations not correctable by glasses or contacts, and hypertension were the 5 most frequently reported conditions. These conditions, together with kinetosis, arthritis, muscular weakness, and hypercholesterolemia, also showed the most statistically significant differences ($P < .001$) when frequencies were compared between women who shared mtDNA with a patient and those who did not (Table 4).

Table 2. Kinship and Number of Relatives Presenting With Schizophrenia

Sharing mtDNA With the Patient		Not Sharing mtDNA With the Patient	
First-Degree Relatives	[n]*	First-Degree Relatives	[n]*
Mother	8 [100]	Father	1 [100]
Brother	11 [113]		
Sister	7 [106]		
Son[a1]	2 [8]	Son[b1]	0 [4]
Daughter[a1]	0 [5]	Daughter[b1]	0 [7]
Second-Degree Relatives		Second-Degree Relatives	
Maternal grandmother	2 [100]	Paternal grandmother	0 [100]
		Paternal grandfather	1 [100]
		Maternal grandfather	1 [100]
Maternal aunt	2 [165]	Paternal aunt	3 [146]
Maternal uncle	4 [154]	Paternal uncle	3 [134]
Maternal cousin[a2]	3 [208]	Cousin[b2]	7 [530]
Niece[a3]	1 [67]	Niece[b3]	0 [41]
Nephew[a3]	0 [65]	Nephew[b3]	0 [38]
Total	40 [1,091]	Total	16 [1,300]

[a1]Son/daughter of a female patient; [b1]son/daughter of a male patient.
[a2]Son/daughter of a maternal aunt; [b2]son/daughter of a maternal or paternal uncle, or of a paternal aunt.
[a3]Son/daughter of a patient's sister; [b3]son/daughter of a patient's brother.
*The total number of relatives is indicated in brackets.
Abbreviation: mtDNA = mitochondrial DNA.

In the case of men, it is notable that conditions showing statistically significant differences between those who shared mtDNA with a patient and those who did not were more frequently reported in the group of men who did not share mtDNA with a patient. These conditions included cancer, heart disease, stroke, diabetes, constipation, vision alterations, dementia, and hypertension.

Maternal and paternal grandmothers showed statistical differences in terms of constipation and kinetosis, both of which were more frequent among maternal grandmothers. Maternal grandfathers presented no differences when compared to paternal grandfathers.

DISCUSSION

This study was based on the knowledge that schizophrenia is a complex disorder with multiple genetic and environmental factors involved in its development. The recent genome-wide association studies and copy number variation (CNV) studies have provided important evidence suggesting a role of both single-nucleotide polymorphisms (SNPs) and CNVs in schizophrenia genesis.[23] The contribution of significant associated SNPs and CNVs to schizophrenia, however, remains unknown. We hypothesized that the mitochondrial genome, in addition to nuclear sequences, could contain genetic susceptibility factors for schizophrenia. We investigated this possibility by comparing the number of relatives who presented with schizophrenia based on whether they shared mtDNA with a schizophrenia index case. We observed more individuals with schizophrenia in the group of relatives who shared mtDNA with a patient than in the group of relatives who did not. The first primary result of this study was that male relatives who shared mtDNA with a schizophrenia patient were at higher risk of developing the illness than male relatives who did not share mtDNA (OR = 3.56; 95% CI, 1.57–8.24; $P < .001$). Female relatives who shared mtDNA with a patient also had an increased risk of developing schizophrenia (OR = 2.86; 95% CI, 1.10–8.75; $P = .02$) when compared with female relatives who did not share mtDNA, but this risk was slightly lower than that observed for male relatives. The numbers of female and male relatives in our sample were quite similar, representing 50.5% and 49.5% of the total relatives, respectively. Likewise, the numbers of relatives who shared and did not share mtDNA with an index case were quite similar, representing 45.6% and 54.4% of the total relatives, respectively. In fact, the

Table 3. Psychiatric Disorders in Relatives of Schizophrenia Patients

	Men (n = 1,184)			Women (n = 1,207)		
Disorder, n (%)	Sharing mtDNA With the Patient (n = 435)	Not Sharing mtDNA With the Patient (n = 749)	*P* Value	Sharing mtDNA With the Patient (n = 656)	Not Sharing mtDNA With the Patient (n = 551)	*P* Value
Schizophrenia	20 (4.60)	10 (1.34)	< .005	20 (3.05)	6 (1.09)	< .05
Bipolar disorder	2 (0.46)	0 (0)	NS	2 (0.30)	1 (0.18)	NS
Unipolar depression	4 (0.92)	10 (1.33)	NS	56 (8.54)	5 (0.91)	< .001
Panic attack	3 (0.69)	2 (0.27)	NS	18 (2.74)	1 (0.18)	< .005
Other anxiety disorders	5 (1.15)	11 (1.47)	NS	38 (5.79)	8 (1.45)	< .001
Mental retardation	4 (0.92)	1 (0.13)	NS	3 (0.46)	0 (0)	NS

Abbreviation: NS = not significant.

Table 4. Conditions Present in Relatives of the 100 Schizophrenia Patients

Condition	Mothers, n	Fathers, n	*P* Value	Maternal Grandmothers, n	Paternal Grandmothers, n	*P* Value	Maternal Grandfathers, n	Paternal Grandfathers, n	*P* Value	Women Sharing mtDNA, %	Women Not Sharing mtDNA, %	*P* Value	Men Sharing mtDNA, %	Men Not Sharing mtDNA, %	*P* Value
Headache	33	7	<.001	6	2	NS	1	0	NS	8.4	0.7	<.001	1.4	1.2	NS
Migraine	11	1	NS	1	1	NS	0	0	NS	2.7	0.4	.001	0.5	0.3	NS
Constipation	36	14	<.001	8	0	.006	1	4	NS	10.2	0.7	<.001	0.2	2.9	.002
Diarrhea	4	5	NS	0	2	NS	1	0	NS	1.2	0.4	NS	0.5	0.8	NS
Abdominal pain	4	1	NS	1	1	NS	0	0	NS	1.2	0.2	.03	0.5	0.1	NS
Nausea	2	1	NS	1	1	NS	0	0	NS	0.8	0.2	NS	0.0	0.1	NS
Kinetosis	14	4	.02	6	0	.03	1	0	NS	4.3	0.4	<.001	1.4	0.7	NS
Severe fatigue	7	1	NS	0	0	NS	0	0	NS	1.7	0.2	.002	0.2	0.1	NS
Fibromyalgia	5	0	.03	0	0	NS	0	0	NS	1.7	0.0	.002	0.0	0.0	NS
Dysautonomia	39	18	.002	9	3	NS	3	0	NS	12.5	0.4	<.001	2.1	2.9	NS
Arthritis	9	1	.02	3	0	NS	0	0	NS	2.1	0.0	<.001	0.7	0.3	NS
Muscular weakness	13	1	.002	1	0	NS	0	0	NS	2.4	0.0	<.001	0.2	0.1	NS
Deafness < 60, y	6	6	NS	3	0	NS	5	1	NS	1.7	0.0	.002	0.7	1.7	NS
Vision alterations	19	20	NS	18	9	NS	6	6	NS	8.8	3.3	<.001	1.8	4.9	.01
Heart disease	5	30	<.001	9	5	NS	7	10	NS	2.4	1.6	NS	2.3	7.1	<.001
Stroke	7	5	NS	9	8	NS	13	8	NS	3.2	1.6	NS	0.2	3.5	<.001
Hypertension	28	19	NS	10	7	NS	4	3	NS	7.6	1.8	<.001	1.6	3.9	.04
Hypercholesterolemia	18	15	NS	5	3	NS	3	0	NS	5.0	1.1	<.001	2.8	2.7	NS
Kidney disease	10	9	NS	4	1	NS	3	1	NS	3.0	0.4	.001	0.7	2.0	NS
Cancer	17	20	NS	12	13	NS	17	18	NS	6.8	4.9	NS	2.3	9.2	<.001
Hypoglycemia	4	1	NS	1	1	NS	1	0	NS	1.1	0.2	NS	0.0	0.3	NS
Hypothyroidism	8	2	NS	2	0	NS	0	0	NS	2.1	0.5	.04	0.2	0.3	NS
Diabetes	18	16	NS	9	10	NS	6	3	NS	6.4	2.7	.004	1.4	5.5	<.001
Alzheimer's disease	0	1	NS	3	0	NS	1	2	NS	0.5	0.2	NS	0.0	0.5	NS
Dementia	4	2	NS	9	3	NS	2	1	NS	2.4	0.5	.02	0.0	1.2	.03
Parkinson's disease	1	1	NS	2	0	NS	2	2	NS	0.6	0.2	NS	0.0	0.7	NS

Abbreviation: mtDNA = mitochondrial DNA, NS = not significant.

number of women who shared mtDNA with a schizophrenia patient represented 54.4% of the total number of women and, in the case of men, 36.7% of the total number of men. Therefore, the number of subjects in the comparison groups should not account for the statistical differences observed.

We also compared the number of relatives who presented with other psychiatric disorders based on whether they shared mtDNA with a schizophrenia index case. Male relatives who shared mtDNA with a schizophrenia patient were not at risk for other psychiatric disorders. This was not the case, however, for female relatives who shared mtDNA with a patient, as they also had an increased risk of unipolar depression (OR = 10.19; CI, 4.07–32.80; $P < .001$), panic attack (OR = 15.52; 95% CI, 2.41–643.6; $P < .001$), and other anxiety disorders (OR = 4.14; 95% CI, 1.84–9.71; $P < .001$). Epidemiologic studies identified that women are 2 to 3 times more likely to suffer from these disorders. We also found that depression and anxiety are more frequent in female than male relatives, with frequencies of 10.4% and 3.0%, respectively. Therefore, it is possible that the small number of male relatives presenting with depression or anxiety disorders in our study lacked sufficient power to identify statistical differences. Also, gender bias is well recognized but poorly understood in mitochondrial disorders. One example is Leber's hereditary optic neuropathy, in which male gene carriers are about 4 times more likely to develop blindness than female gene carriers. It has been proposed that nuclear genetic modifier loci produce subtle anatomic, hormonal, or physiological variations between males and females and interact with mutations or variants of mtDNA, thereby influencing the clinical expression of disease.[24] A large number of studies have indicated that mtDNA might be involved in depression and anxiety disorders. In previous studies, high incidences of depression and anxiety have been observed among the matrilineal relatives of patients presenting with a mitochondrial disorder allegedly inherited from the mother. It has been hypothesized that the same mtDNA sequence variants that predispose family members toward the development of mitochondrial disorders also predispose them to these psychiatric disorders.[20,21] Likewise, depressive mood disorders have been associated with the A8344G mutation in individual patients of a MERRF pedigree,[25] and major depression has been identified in pediatric patients diagnosed with mutations in *MTND1*, *MTTK*, and a common 4,977-base pair deletion

in the mtDNA. In this study, the authors hypothesized that abnormal central nervous system energy metabolism in these patients was the underlying cause of the mood disorder.[26] In recent years, the study of mitochondrial dysfunction in psychiatric disorders has emerged as several hallmarks of decreased energy metabolism, such as decreased pH and increased lactate levels, have been observed in patients with psychiatric disorders. Moreover, both structural and functional magnetic resonance imaging studies have identified mitochondrial abnormalities in the brains of patients presenting with psychiatric disorders.[27,28] In addition, studies of mitochondrial function in muscle correlate strongly with data related to somatic symptoms in patients with major depressive disorders, suggesting once more that an energy deficiency might predispose to both somatic and mood disorders.[29] Recently, a modest maternal bias in the development of depression has been found in the analysis of pedigrees of patients presenting with recurrent early-onset depression (OR = 2.0; 95% CI, 1.5–2.6; $P < .001$).[30]

In terms of the nonpsychiatric conditions present among relatives of schizophrenia patients, most of these conditions were more frequent among mothers than among fathers and more frequent among women who shared mtDNA with a schizophrenia patient than among women who did not. Other studies have also reported that women have a higher prevalence of chronic pain conditions and depression and higher pain severity than men, including fibromyalgia, arthritis/rheumatism, back problems, and migraine headache.[31] Comorbidity of chronic pain conditions and depression-anxiety disorders has been reported elsewhere.[32] Furthermore, several studies have hypothesized the possible involvement of mtDNA in the conditions listed in Table 4. For headache, the questionnaire used in the present study first asked about frequent headache episodes to determine if migraine headaches were present; among all types of headache, only some types of migraine have been associated with mitochondrial inheritance.[33] The results obtained, with higher significant results for headache than migraine, might be associated with the difficulty in gathering enough clinical features to classify the type of headache as migraine. Susceptibility to kinetosis (motion sickness) is also higher in women than in men, and there is an association between personal and familial history of motion sickness and migraine.[34,35] One study reported an association between the mtDNA variant 7472insC with early-onset sensorineural hearing loss. Remarkably, most of the patients studied were susceptible to motion sickness.[36] Constipation is a common gastrointestinal tract symptom in patients with mitochondrial disorders, and it has been hypothesized that mtDNA variants may be associated with gastric emptying.[37] Primary symptoms of dysautonomia include excessive fatigue, polydipsia, vertigo, and feelings of anxiety or panic, among others. These symptoms were more frequent in women who shared mtDNA with a schizophrenia patient than in those who did not. Therefore, it is tempting to speculate that a general mitochondrial dysfunction can be a common mechanism associated with several conditions.

Regarding schizophrenia, a general hypothesis should take into account mtDNA and nuclear genetic variants interacting with environmental factors such as cigarette smoking or alcohol consumption, which can lead to mutations in mtDNA that can then lead to mitochondrial dysfunction at the level of adenosine triphosphate production, apoptosis, calcium homeostasis, or the level of reactive oxygen species. The above scenario can have implications in myelination, synaptic function, or neurotransmission. In addition to investigating nuclear genetic factors, future studies should analyze the mtDNA sequence, the presence of mtDNA rearrangements, and the expression of mtDNA genes, particularly in schizophrenia patients with apparent maternal transmission of the disease.

Limitations

Based on their medical records, a diagnosis of schizophrenia was confirmed in about 80% of the schizophrenia relatives. However, other psychiatric diagnoses and conditions related to mitochondrial disorders were not confirmed by this method, and a possible recall bias might have been introduced in the study.

The higher frequency of individuals presenting with conditions such as heart disease, stroke, hypertension, cancer, and diabetes in the group of men who did not share mtDNA with a schizophrenia patient compared to those who did could be attributed to the advanced age of these men who did not share mtDNA with a patient, as both grandfathers were included in this group. This age factor is a limitation of the present study. However, the age difference between the groups in the present study would be expected to favor the null hypothesis and would not account for our findings, as the prevalence of the chronic conditions studied could be expected to increase, not decrease, with age.

The present study supports the hypothesis that mtDNA could be involved in schizophrenia, but we did not directly analyze this genetic material. There is a paucity of genetic studies analyzing mtDNA. The primary results of such efforts have indicated an overrepresentation of the HV haplogroup,[38] an association between early disease onset and the J-T haplogroup,[39] and significantly lower expression of 11 of the 13 mitochondria-encoded transcripts in schizophrenia patients compared to control subjects.[40]

CONCLUSIONS

In this study, we found that both female and male relatives who shared mtDNA with a schizophrenia patient had an increased risk of schizophrenia compared to relatives who did not. Female but not male relatives who shared mtDNA with a schizophrenia patient were at increased risk of unipolar depression, panic attack, and other anxiety disorders. Female but not male relatives who shared mtDNA with a schizophrenia patient had an increased incidence of conditions frequently associated with mitochondrial disorders. All of these results are in accordance with previous studies, suggesting that mitochondrial dysfunction could be involved

in schizophrenia and other related psychiatric disorders such as anxiety and depression. Further studies analyzing mtDNA variants, expression, and depletion are needed to elucidate the implications of mtDNA dysfunction in psychiatric disorders.

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Supplementary material: Workflow charts of data collection, guides, and data sheets used to interview the patients and relatives are available at PSYCHIATRIST.COM.

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See supplementary material for this article at PSYCHIATRIST.COM.

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Supplementary Material

Article Title: New Evidence for the Involvement of Mitochondrial Inheritance in Schizophrenia: Results From a Cross-Sectional Study Evaluating the Risk of Illness in Relatives of Schizophrenia Patients

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List of Supplementary Material for the article

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Workflow of data collection

1. Identification of a clinically stable patient

A. Direct interview of patient

2. Explanation of the purpose of the study. Obtaining of Informed Consent
3. Obtaining clinical, socio-demographic and drug consumption data
4. Obtaining information regarding schizophrenia or other psychotic disorders present in relatives
5. Obtaining information regarding other psychiatric disorders present in relatives

B. Direct interview of relative(s)

6. Explanation of the purpose of the study. Obtaining of Informed Consent
7. Obtaining clinical data about onset and evolution of illness of the patient
8. Build-up of the pedigree
9. Obtaining information regarding schizophrenia or other psychotic disorders present in the pedigree
10. Obtaining information regarding other psychiatric disorders present in the pedigree
11. Obtaining information regarding conditions related to mitochondrial disorders present in the pedigree

C. Contrasting data

12. Comparison of data retrieved from the patient and relatives
13. Confirming schizophrenia or psychotic disorders in relatives through medical records

Guide and data sheet for conducting direct interviews with patients

ANNEX 1: DATA SHEET FROM PATIENTS (regarding psychiatric disorders)

A.4.

The following guide should be used to obtain information regarding schizophrenia or other psychotic disorders that might be present in relatives.

Please tell me whether anyone on the list that I will mention has ever had schizophrenia or other psychotic disorders. Read slowly and in the given order for every possible relative.

A.5.

Now please tell me whether anyone on the list that I will repeat has ever had a serious mental illness, emotional problem or nervous breakdown. Read again slowly and in the given order for each relative.

The following questions should be used either as a general comment (if the patient did not identify any psychiatric problem/diagnoses in relatives) or to investigate the specific psychiatric diagnoses (if there is any evidence of psychopathology in a specific relative). Also, the interviewer should ask clarifying questions to identify the probable psychiatric diagnosis of the relative.

1. **Has [any of your relatives/ (he/she)] ever seen a psychiatrist, psychologist or other health professional for a psychological or emotional problem?**
2. **What kind of problem(s)?**
3. **Was he/she treated for them?**
4. **Has he/she received pharmacological or psychological treatment?**
5. **Was he/she hospitalized?**
6. **How many times?**
7. **Do you know whether he/she has a specific psychiatric diagnosis?**
8. **Did he/she ever feel sad, blue, depressed or tired or have less energy?**
9. **Did he/she ever exhibit an abnormally elevated or irritable mood, arousal, energy level or reduced need for sleep?**
10. **Did he/she ever have effects of anxiety such as heart palpitations, chest pain, muscle weakness and tension, nausea, shortness of breath, stomach ache or headache? Has he/she ever experienced a panic attack?**
11. **Did he/she ever have sensory perception (hearing, sight, smell, taste or touch) in the absence of external stimuli, extravagant or unreal beliefs or conduct disorganization?**

Guide and data sheet for conducting direct interviews with relatives

ANNEX 2: DATA SHEET FROM RELATIVES (regarding psychiatric disorders)

ANNEX 3: QUESTIONNAIRE AND DATA SHEET FROM RELATIVES (regarding conditions that are related to mitochondrial disorders)

B.8.

Construct a family tree including all possible relatives of the patient. Write the first name of each relative. Begin by asking about the kinship of the interviewed relative with the patient. Follow with the patient's father and mother, brother(s) and sister(s), son(s) and daughter(s), paternal grandfather and grandmother, maternal grandfather and grandmother, nephews and nieces, uncles and aunts and cousins.

B.9.

The following guide should be used to obtain information about the patient's family. The interviewer should be flexible in deciding what to ask and whether it is necessary to continue the interview with respect to a particular family member. The questions should be formulated for each relative to ensure that none is omitted. Obviously, use the appropriate name and gender. Start with first-degree relatives.

Please tell me whether any of the members of this pedigree has ever had schizophrenia or any other psychotic disorder.

Start asking about the patient's father and mother, then his/her brothers and sisters, sons and daughters, paternal grandfather and grandmother, maternal grandfather and grandmother, nephews and nieces, uncles and aunts and cousins.

B.10.

Now please tell me whether any of the pedigree members has ever had a serious mental illness, emotional problem or nervous breakdown?

The following questions should be used either for a general comment (if the relative does not identify any psychiatric problem/diagnoses in his/her relatives) or to investigate the specific psychiatric diagnoses (if there is evidence of psychopathology in a specific relative).

1. **Has [any of your relatives/ (he/she)] ever seen a psychiatrist, psychologist or other health professional for a psychological or emotional problem?**
2. **What kind of problem?**
3. **Were he/she treated for the problem?**
4. **Has he/she received pharmacological or psychological treatment?**
5. **Were he/(she hospitalized?**
6. **How many times?**
7. **Do you know whether he/she has a specific psychiatric diagnosis?**
8. **Did he/she ever feel sad, blue, depressed or tired or have less energy?**
9. **Did he/she ever exhibit an abnormally elevated or irritable mood, arousal, energy level or reduced need for sleep?**
10. **Did he/she ever have effects of anxiety such as heart palpitations, chest pain, muscle weakness and tension, nausea, shortness of breath, stomach ache or headaches? Has he/she ever experienced a panic attack?**
11. **Did he/she ever have sensory perception (hearing, sight, smell, taste or touch) in the absence of external stimuli, extravagant or unreal beliefs or conduct disorganization?**

B.11.

The following questionnaire should be used to obtain information regarding conditions related to mitochondrial disorders present in the pedigree. Start asking the interviewed relative about the presence of the conditions in themselves, the patient and other biological relatives (follow this order: the patient's father and mother, brothers and sisters, sons and daughters, paternal grandfather and grandmother, maternal grandfather and grandmother, nephews and nieces, uncles and aunts and cousins). Mark with a cross in the questionnaire table if the condition is present in the patient and/or the interviewed relative. If they are present in other relatives, record this information (the corresponding number of the condition) in the corresponding tables in ANNEX 3.

QUESTIONNAIRE

		Patient	Relative	
1	What is your relationship with the patient? _____	XXXXXX		
2	What is your current age? _____/_____years. What is your birth date?_____/_____	XXXXXX	XXXX	XXXX
	HEADACHES			
3	Have you suffered from ongoing headaches (including at least 5)? If no, skip to question #4.			
	If so, were the headaches usually associated with any of the following:	XXXXXX	XXXX	XXXX
3a	Lasting between 4 and 72 hours each?			
3b	Occurring only on one side of your head?			
3c	Pulsating or throbbing?			
3d	Severe enough to change your usual daily activity?			
3e	Aggravated by walking on stairs or other similar routines?			
3f	Nausea and/or vomiting during the headache?			
3g	Increased pain with bright lights during the headache?			
3h	Increased pain with loud noises during the headache?			
3i	In the hour just prior to the onset of the headaches, did you experience any of the following: visual changes including flashes of light, tingling, numbness, weakness or speech difficulty?			
4	Have you ever been diagnosed with "migraine" by a physician?			
	BOWEL FUNCTION			
5	Have you had ongoing problems with constipation for which you were treated with medications or diet?			
6	Have you had ongoing problems with diarrhea for which you were treated with medications or diet?			
7	In the last year, have you had abdominal pain or discomfort for at least 12 weeks (not necessarily in a row)? If no, skip to question #8.			
	If so, was it associated with any of the following:	XXXXXX	XXXX	XXXX
7a	Change in stool consistency (softer, harder)?			
7b	Change in stooling frequency (more or less often)?			
7c	Relieved with defecation (stooling)?			
8	Have you ever been diagnosed with "irritable bowel" by a physician?			
9	Has a small portion of food made you feel full right away?			
10	Have you ever been diagnosed with "delayed gastric emptying" by a physician?			
11	Have you suffered from 5 or more similar episodes of nausea and vomiting? If no, skip to #12.			
11a	During these episodes, were you lethargic or sleepy?			
11b	Did the nausea and vomiting essentially go away between episodes?			
12	Have you frequently suffered from motion sickness in cars, boats or planes (at least 25% of trips)?			
	SOFT TISSUES & FATIGUE			
13	At any time in your life, did you experience severe fatigue for 6 months or longer? If no, skip to #14.			
13a	Did the fatigue affect both physical and mental function?			
13b	Was the fatigue present at least half (50%) of the time?			
13c	Did you find it difficult to walk 5 blocks?			
13d	Was the fatigue severe enough to interfere with work or recreational activities?			
14	Have you ever been diagnosed with "chronic fatigue" or "Epstein-Barr" by a physician?			
15	Have you ever been diagnosed with "fibromyalgia" by a physician?			
16	Have you ever had any troublesome color change or swelling of any part of your body?			

17	Have your hands and/or feet frequently felt cold?			
18	Have you ever suffered from frequent and painful muscle cramps?			
18a	If you answered yes to #16, #17, or #18: At any given time, did it affect one side of the body more than the other?			
19	Have you ever been diagnosed with "Raynaud's" by a physician?			
20	Have you ever been diagnosed with "arthritis" by a physician?			
	NERVES & BRAIN			
21	Have you ever had fainting spells?			
22	Have you ever had droopy eyelids?			
23	Have you ever had a seizure?			
24	Have you had frequent unexplained high or low body temperatures as measured by a thermometer?			
25	Have you had muscle weakness or muscle fatigue on exertion?			
26	Were you ever diagnosed with mental retardation?			
27	Have you ever been diagnosed with "attention deficit disorder" with or without "hyperactivity" ("ADD" or "ADHD") by a professional such as a physician or psychologist?			
28	Have you ever been tested and found to have a learning disability, including dyslexia?			
29	Have you ever been diagnosed with "autism", "autistic features" or "Asperger syndrome" by a professional such as a physician or a psychologist?			
	MENTAL HEALTH			
30	Have you ever been diagnosed with "anxiety" or "anxiety disorder" by a professional such as a physician or a psychologist? If no, skip to #31.			
30a	Were you treated with medications for your anxiety?			
31	Have you ever been diagnosed with "schizophrenia" by a professional such as a physician or a psychologist?			
32	Have you ever had times when you had a lot of energy during which you did things that you regretted later (mania)?			
33	Have you ever been diagnosed with "bipolar disorder" or "manic-depression" by a professional such as a physician or a psychologist?			
34	Have you ever been diagnosed with "depression" by a professional such as a physician or a psychologist? If no, skip to #35.			
34a	Were you treated with medications for your depression?			
34b	Did your symptoms of depression last for more than 6 months?			
	While you were depressed, did you experience any of the following nearly every day:	XXXXXX	XXXX	XXXX
34c	Depressed mood (sadness)?			
34d	Less interest or pleasure in nearly all activities?			
34e	Substantial change in appetite and/or weight?			
34f	Substantially more or less sleep?			
34g	Faster or slower activity levels as observed by others?			
34h	Tiredness or loss of energy?			
34i	Felt worthless or inappropriately guilty?			
34j	Trouble thinking or concentrating?			
35	Have you ever been diagnosed with "panic attacks" by a professional such as a physician or a psychologist? If no, skip to #36.			
35a	Were you treated with medications for your panic attacks?			
	EARS & EYES			
36	Have you ever suffered from hearing loss? If no, skip to #37.			
36a	If so, how old were you when hearing loss started?_____	XXXXXX	XXXX	XXXX
36b	Was the hearing loss worse in one ear compared to the other?			
37	Have you ever heard frequent ringing in your ears (tinnitus)?			
38	Have you had substantial problems with vision that was not correctable by glasses or contacts?			

39	Are you bothered by bright lights more than other people (not only during headaches)?			
40	Have you ever had cross-eyedness (strabismus)?			
	HORMONES			
41	Do you generally have problems if you miss a meal? If no, skip to #42.			
41a	If so, what problems?_____	XXXXXX	XXXX	XXXX
42	Have you ever had hypoglycemia documented by a low blood sugar level?			
43	Have you ever been diagnosed with "hypothyroidism" by a physician?			
44	Have you ever been diagnosed with "diabetes" by a physician?			
45	Have you ever had growth hormone deficiency documented by laboratory testing?			
	HEART & BLOOD VESSELS			
46	Have you ever suffered a heart attack?			
47	Have you ever suffered a stroke?			
48	Have you frequently felt your heart racing, even when you were not anxious?			
49	Have you ever been diagnosed with "tachycardia" or an abnormally high heart rate by a physician?			
50	Have you ever been diagnosed with "cardiomyopathy" by a physician?			
51	Have you ever been treated with diet or medications for high blood pressure?			
52	Have you ever been told your cholesterol level was high?			
	OTHER			
53	Other than infections, have you ever had any kidney disease?			
54	On most nights, do you need to get out of bed in the middle of the night to urinate?			
55	Have you ever had cancer?			
56	Were you born with a birth defect that required therapy or surgery? If no, skip to #57.			
56a	If so, what birth defect?_____	XXXXXX	XXXX	XXXX
57	Have you ever been diagnosed with an immunodeficiency (decreased immune system) or have had severe or unusual infections?			
58	Have you ever been pregnant? If no, skip to #59			
58a	During any pregnancy, did you experience a substantial amount of nausea or vomiting? If no, skip to #59.			
58b	Was intravenous (iv) fluid needed because of nausea or vomiting?			
59	Have you ever had any significant medical problems not asked in this questionnaire? If no, skip to #60.			
59a	If so, what problems? _____	XXXXXX XXXXXX	XXXX XXXX	XXXX XXXX
60	Did any children in your family die suddenly from unknown causes? If no, skip to the end.			
60a	If so, at what age (months or years)?_____	XXXXXX	XXXX	XXXX
60b	How was this child related to you?_____	XXXXXX	XXXX	XXXX
60c	Was a diagnosis of sudden infant death syndrome (SIDS) or probable SIDS given?			

ANNEX 1:

Patient code: _____	**DATA SHEET FROM PATIENTS**		
Kinship	Schizophrenia (⊗ yes/ __ no)	Other psychotic disorder (⊗ yes/ __ no) Specify	Other psychiatric disorders (⊗ yes/ __ no) Specify
Father			
Mother			
Brother			
Sister			
Son[1]			
Daughter[1]			
Maternal grand-father			
Maternal grand-mother			
Paternal grand-father			
Paternal grand-mother			
Nephew (son of a brother)[1]			
Niece (daughter of a brother)[1]			
Nephew (son of a sister)[1]			
Niece (daughter of a sister)[1]			
Paternal uncle[1]			
Paternal aunt[1]			
Maternal uncle[1]			
Maternal aunt[1]			
Son of a maternal aunt[1]			
Daughter of a maternal aunt[1]			
Son of a maternal uncle[1]			
Daughter of a maternal uncle[1]			
Son of a paternal aunt[1]			
Daughter of a paternal aunt[1]			
Son of a paternal uncle[1]			
Daughter of a maternal uncle[1]			

[1] Indicate the number of individuals if more than one.

ANNEX 2:

Patient code: _____	**DATA SHEET FROM RELATIVES (regarding psychiatric disorders)**		
Kinship	Schizophrenia (⊗ yes/ __ no)	Other psychotic disorder (⊗ yes/ __ no) Specify	Other psychiatric disorders (⊗ yes/ __ no) Specify
Father			
Mother			
Brother			
Sister			
Son[1]			
Daughter[1]			
Maternal grand-father			
Maternal grand-mother			
Paternal grand-father			
Paternal grand-mother			
Nephew (son of a brother)[1]			
Niece (daughter of a brother)[1]			
Nephew (son of a sister)[1]			
Niece (daughter of a sister)[1]			
Paternal uncle[1]			
Paternal aunt[1]			
Maternal uncle[1]			
Maternal aunt[1]			
Son of a maternal aunt[1]			
Daughter of a maternal aunt[1]			
Son of a maternal uncle[1]			
Daughter of a maternal uncle[1]			
Son of a paternal aunt[1]			
Daughter of a paternal aunt[1]			
Son of a paternal uncle[1]			
Daughter of a maternal uncle[1]			

[1] Indicate the number of individuals if more than one.

ANNEX 3: Table 1

DATA SHEET FROM RELATIVES (regarding conditions related to mitochondrial disorders)								
Patient code: _____	**MATERNAL RELATIVES**							
Condition	Grand-father	Grand-mother	Uncle[1]	Aunt[1]	Son of a maternal aunt[1]	Daughter of a maternal aunt[1]	Son of a maternal uncle[1]	Daughter of a maternal uncle[1]

[1] Indicate the number of individuals if more than one.

ANNEX 3: Table 2

DATA SHEET FROM RELATIVES (regarding conditions related to mitochondrial disorders)								
Patient code: _____	**PATERNAL RELATIVES**							
Condition	Grand-father	Grand-mother	Uncle[1]	Aunt[1]	Son of a paternal aunt[1]	Daughter of a paternal aunt[1]	Son of a paternal uncle[1]	Daughter of a paternal uncle[1]

[1] Indicate the number of individuals if more than one.

ANNEX 3: Table 3

DATA SHEET FROM RELATIVES (regarding conditions related to mitochondrial disorders)										
Patient code: _____	**FIRST-DEGREE RELATIVES**						**OTHER**			
Condition	Mother	Father	Brother[1]	Sister[1]	Son[1]	Daughter[1]	Nephew (son of a brother)[1]	Niece (daughter of a brother)[1]	Nephew (son of a sister)[1]	Niece (daughter of a sister)[1]

[1] Indicate the number of individuals if more than one.