

ORIGINAL RESEARCH ARTICLE

Wolframin mutations and hospitalization for psychiatric illness

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Genetic predisposition plays an important role in most common psychiatric disorders. The identification of a specific gene associated with a psychiatric illness can lead to improved management of the gene-associated disorder. Mutations in the wolframin gene are associated with mental illness. Many patients with the Wolfram syndrome (WS), who are homozygous or compound heterozygous for wolframin mutations, have severe psychiatric symptoms. In WS families, close blood relatives, who have a high probability of carrying a single wolframin mutation, had a statistically significant excess, over spouse controls, of psychiatric hospitalizations, attempted and completed suicides, and self-reports of mental illness. Since heterozygous carriers of wolframin mutations are relatively frequent in the population according to the general Hardy–Weinberg principle, such mutations might be responsible for the illnesses of many psychiatric patients. The hypothesis that heterozygous carriers of a wolframin mutation are predisposed to psychiatric illness was tested in subjects from 25 WS families. In all, 11 relatives who had psychiatric hospitalizations could be genotyped through mutation analysis. Eight of these carried the wolframin mutation transmitted in their family, significantly (one-sided $P=0.0022$) more than the 3.0 expected if there were no association between psychiatric hospitalizations and mutations at this locus. All eight mutation-positive subjects had been hospitalized for a major depression. This confirmation of the association is not influenced by confounders, undetected stratification, or genetic heterogeneity. The relative risk of psychiatric hospitalization for depression was estimated to be 7.1 (95% CI 1.9–26.6) for carriers of a single wolframin mutation compared to noncarriers.

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Family, twin, and adoption studies have shown that genetic predisposition plays an important role in most common psychiatric disorders. Identifying any gene whose mutations predispose mutation carriers to a psychiatric disorder defines a precise metabolic pathway to target with preventive or therapeutic agents.

Mutations in the wolframin gene^{1,2} are associated with diverse behavioral and cognitive abnormalities.^{3–6} This gene, spanning about 33.4 kb of genomic DNA on chromosome 4p16.1, consists of eight exons, of which exons 2–7 and the major portion of exon 8 are coding. The gene's mRNA of 3.6 kb codes for a polypeptide of 890 amino acids, with a molecular mass of 100.29 kDa and nine to 11 possible transmembrane domains. The function of the wolframin protein product, expressed in all cell types, is unknown. In the brain, it is expressed predominantly in the

hippocampus CA1, amygdaloid areas, olfactory tubercles, and the superficial layer of the allocortex.⁷

Homozygotes or compound heterozygotes for wolframin mutations have the rare autosomal recessive Wolfram syndrome (WS),^{8,9} defined by the occurrence of *diabetes mellitus* and *progressive bilateral optic atrophy*. WS patients have diverse abnormalities of the central, autonomic, and peripheral nervous system,¹⁰ and a high frequency of severe, varied, psychiatric symptoms.³

It is important to assess the health effects of carrying a single wolframin mutation since carriers are much more frequent than homozygotes in the general population. The first evidence that WS heterozygous carriers are predisposed to psychiatric illness came from a study of 36 WS families in which the blood relatives had a significant excess of psychiatric hospitalizations documented from medical records, of suicides recorded on death certificates, and of self-reports of mental illness, compared to spouse controls.⁴ Each blood relative had a probability $\geq 25\%$ of carrying the WS mutation transmitted in his/her family, while each spouse control was considered to have the same probability as any

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member of the general population of carrying any WS mutation.

Later, the association between WS mutations and psychiatric hospitalizations was tested using the index-test method¹¹ and haplotypes to identify mutation carriers.⁵ We now report the definitive test of this association using mutation analysis to identify precisely carriers and noncarriers among WS blood relatives hospitalized for psychiatric illness. This analysis confirms that wolframin mutations are important in predisposing members of the general population to psychiatric illness.

Materials and methods

The index-test method¹¹ was used to confirm the association of wolframin mutations with hospitalization for psychiatric illness. The clinical data and DNA samples came from WS families; the WS patients are the 'index individuals' because they have two wolframin mutations, and thus *indicate* that such mutations are segregating in their family. Blood relatives in 25 WS families were sent questionnaires asking about psychiatric and cognitive difficulties, and about hospitalizations for mental illness.

The 'test cases' were the 11 subjects documented to have been hospitalized for mental illness through questionnaires, medical records, and telephone interviews. Three other potential test cases were excluded because their DNA or that of the WS proband and/or his/her parents, needed to determine which wolframin mutations are segregating in that family, was not available. The available clinical information demonstrated that all 11 test subjects had been hospitalized for depression.

DNA was extracted from cultured lymphocytes of the WS patients and their parents. Exons 2–8 were sequenced on an ABI 377 automated sequencer, using primers published previously.^{1,2} Each heterozygous variant was compared to the wild-type sequence (GenBank accession number Y18064) and its effect on the wolframin amino-acid sequence evaluated. Each mutation detected was compared to the comprehensive listing of known mutations in the WFS1 Gene Mutation and Polymorphism Database.¹² When the mutation on the maternal or paternal side of a family corresponding to that of the test subject was identified, the DNA segment containing that mutation was amplified for the test subject.

A test case was scored as positive if his/her DNA contained the same wolframin mutation as the proband's parent on the same side of the family. Each test case had a prior probability of carrying that mutation based on the genetic relationship between the test case and the index individual. The significance of the difference between the total number of observed positive test cases and the number of positive cases expected if there was no association, given by the sum of the prior probabilities for all cases, was tested using the exact binomial distribution.

Results

The wolframin mutations responsible for clinical WS in the index patients were found for all 11 maternal lines and for nine of the paternal lines (Table 1). In each family, the specific mutation that would have been transmitted to the test subject was identified. Figure 1 illustrates the relationships between test and

Table 1 Wolframin mutations in WS blood relatives hospitalized for psychiatric illness

Subject	Relation to proband	Probability of carrying the familial mutation	Maternal mutation	Paternal mutation	Subject's DNA
1 ^a	Cousin (maternal)	0.25	1944G>A, W648X	397G>A, A133T	Normal
2 ^a	Cousin (paternal)	0.25	1944G>A, W648X	397G>A, A133T	A133T ^b
3 ^c	Mother's half sib	0.25	1763G>A, W588X	2015T>C, L672P	W588X
4 ^c	Maternal aunt	0.5	1763G>A, W588X	2015T>C, L672P	W588X
5 ^c	Cousin (maternal)	0.25	1763G>A, W588X	2015T>C, L672P	W588X
6 ^d	Cousin (paternal)	0.25	2254G>T, E752X	2393, Ins ACG	Ins ACG ^e
7 ^d	Cousin (maternal)	0.25	2254G>T, E752X	1675G>A, A559T	Normal
8	Grandparent's sib (maternal)	0.25	817G>T, E273X	Not found	E273X ^b
9 ^f	Grandparent's sib (paternal)	0.25	1699, del CCTCTT	1230, del CTCT	del CTCT ^b
10 ^f	Grandparent's sib (paternal)	0.25	1699, del CCTCTT	1230, del CTCT	Normal
11	Cousin (maternal)	0.25	2254G>T, E752X	Not found	E752X ^b

^aThese subjects came from the maternal and paternal sides of one family.

^bListed in the WFS1 Gene Mutation and Polymorphism Database¹² as a mutation.

^cThese subjects came from the maternal side of one family.

^dThe probands' mother in the first family was the sister of the maternal grandmother of the proband in the second family.

^eFound in 1/99 'controls' from Coriell Institute (M Lesperance, personal communication).

^fThese subjects were siblings.

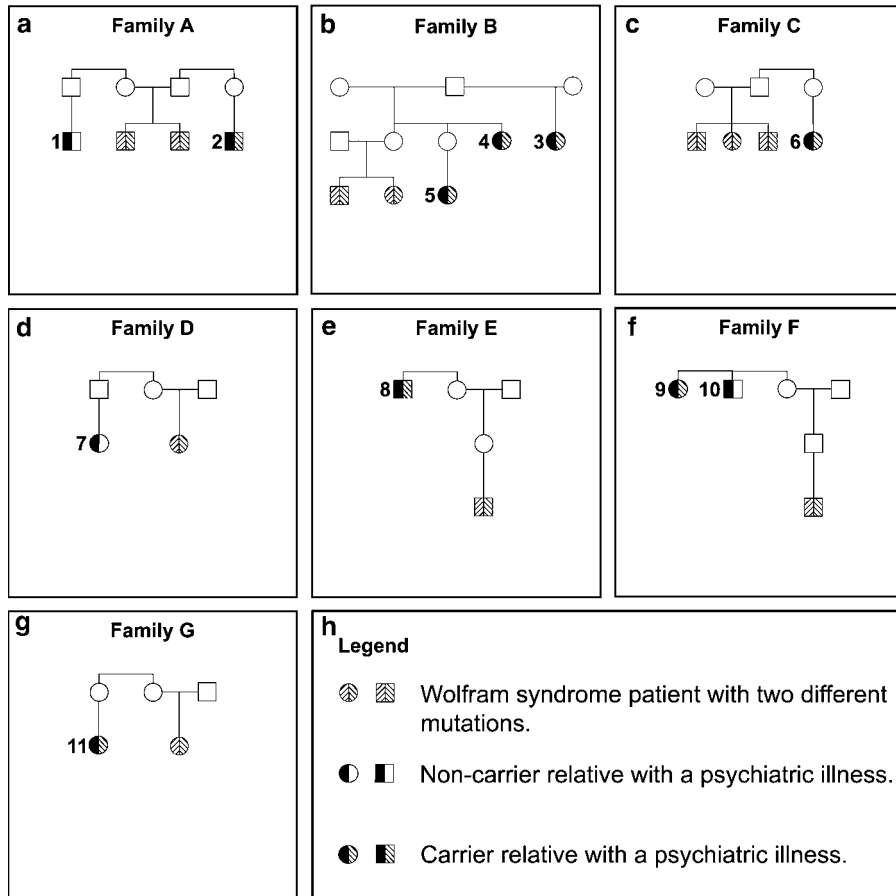


Figure 1 Relationship of test subjects hospitalized for psychiatric illness to the index individuals with the WS in the families whose mutation analysis is shown in Table 1. A black half-symbol designates each test subject, whose identifying number in the table is the Arabic number next to the subject's symbol. For each WS patient, the presence of two different WS mutations is shown by the crosshatching in different directions in each half of the patient's symbol. Relatives whose mutation status did not affect the index-test analysis are not shown.

index subjects in each nuclear family, and shows which test subjects carried the familial wolframin mutation.

Eight of the 11 test subjects had the wolframin mutation identified in his/her family. The expected number of carriers was 3.0 by summing over the 10 test cases with a prior probability of heterozygosity of 0.25 ($10 \times 0.25 = 2.5$) and the one with a prior probability of heterozygosity of 0.5 ($1 \times 0.5 = 0.5$) (column 3 in Table 1). The one-sided *P*-value was 0.0022 according to the exact binomial distribution and the odds ratio was 7.1 (95% CI 1.9–26.6), estimated as described in Swift *et al.*¹¹

Discussion

The highly significant excess of eight wolframin mutation carriers in 11 test subjects, found by direct sequencing of their DNA, over the 3.0 expected on the null hypothesis, demonstrates that these mutations are associated with hospitalization for psychiatric illness. No alternative explanation of this finding is possible, except for the 1/500 possibility ($P = 0.0022$) that it occurred by chance.

The test cases were selected from all families with identified wolframin mutations *only* because they had been hospitalized for a psychiatric illness. Parents in the WS families were identified as carriers only because they had a child with WS; they are random representatives of carriers of all wolframin mutations causing clinical WS in compound heterozygotes. With no disease association, wolframin mutations segregate according to Mendel's First Law: for example, 19 (63%) carriers were detected in 30 probands' siblings in WS families reported previously,^{1,5,13} not significantly different from the 67% expected.

The population frequency of wolframin mutations that predispose carriers to psychiatric illness is not yet known. Available population surveys^{14–18} suggest that the population frequency of truncating mutations is less than 1%, and that the population frequency of missense mutations is greater than 1%. If the population frequency of wolframin mutations that predispose carriers to psychiatric illness is about 1%, with an odds ratio of 7.1, wolframin mutation carriers would be estimated to be about 7% of patients hospitalized for depression. Previous estimates⁵ of

the relative risk and population frequency of depression associated with wolframin mutations based on haplotype analysis were less reliable; mutation analysis is more precise.

Several studies of mutation frequency in psychiatric patients appear to support the conclusion that wolframin mutations are numerically important in contributing to predisposition to psychiatric illness. Torres *et al*¹⁴ found 17 previously unreported wolframin missense mutations and seven synonymous base changes in exon 8 in 34 of 147 patients with schizophrenia or affective disorders. Crawford *et al*,¹⁵ using SSCP on liver biopsies, reported that nine of 204 suicides and two of 200 selected (no questionnaire evidence for depression) controls had specific missense mutations not found in 112 unselected controls. Three other missense mutations occurred at a relatively high frequency in all groups. Furlong *et al*¹⁶ detected the A559T wolframin mutation in 4/321 affective disorder patients, compared to none of 382 controls. They found no difference between groups in the frequency of the one common polymorphism that was the focus of their study. The A559T wolframin mutation was found in 2/147 patients in Torres *et al*,¹³ 1/204 in Crawford *et al*,¹⁴ 2/161 affective disorder patients in Serretti *et al*,¹⁷ and 0/184 Japanese bipolar patients.¹⁸ This mutation was present on the paternal side of one of the WS families in the present report. Sequeira *et al*¹⁹ found a significant excess of the homozygous H611R/H611R genotype in 111 suicide victims compared to 129 controls. The possibility that homozygotes for this mutation are predisposed to suicide is important because 20% of the general population have this genotype.

These cited studies that compared the wolframin mutation frequency in psychiatric patients to the frequency in a comparison group (if used) are typical population association studies. Results from studies with this design are not definitive because they are frequently not reproducible due to difficulties in matching, undetected stratification, and low statistical power.^{20–22} The studies that did not find evidence that wolframin mutations are associated with psychiatric illnesses screened, for only 1–4 polymorphic missense mutations^{17,18,23,24} had poor statistical power and limited mutation detection capability,²⁵ or sought 'linkage' by inspecting two families.²⁶

All published population surveys, however, raise an important question because their mutation screening detected only missense mutations in the diseased and comparison populations, suggesting that the frequency of truncating mutations is low in the general population. Truncating wolframin mutations are the most frequent mutation type in WS patients;^{1,2} this type was found in seven of the eight carrier test cases in the present study. It is possible that some missense wolframin mutations predispose to psychiatric illness, but do not lead to the clinically distinctive WS in homozygous or compound heterozygous individuals. In patients with the autosomal recessive syndrome, ataxia-telangiectasia (A-T) truncating

mutations are the most frequent type, as they are in WS. The predisposition of A-T heterozygotes to breast cancer was first shown in A-T families.²⁷ Later, it was shown that missense mutations predominate in breast cancer populations,²⁸ and that some missense mutations lead to mild, almost clinically undetectable, A-T in compound heterozygotes.^{29,30}

Thus, the crucial next step is determining which wolframin missense mutations predispose heterozygous carriers to psychiatric disorders. The population-based application of the index-test method¹¹ can do this with high statistical power and unassailable rigor. The present study applied the index-test method starting with index individuals who were WS patients, and finding the test cases among the blood relatives. The alternative population approach starts with the test cases, persons with a selected psychiatric disorder, and uses a single close blood relative as the index individual for each test case. The observed/expected comparison is carried out for the subset of index-test pairs in which a wolframin mutation is found in the DNA of the index individual. A specific missense mutation or group of mutations predispose to the selected psychiatric disorder if there is a significant excess of observed over expected mutation carriers among all pairs where the index individuals carry that mutation or group of mutations. The index-test method, unlike population association studies, is not affected by confounders, stratification, or genetic heterogeneity.

This specific confirmation that wolframin mutations predispose to hospitalizations for depression derives from the fact that the initial test group from WS families consisted of relatives hospitalized for psychiatric illness; it emerged later that each member of the group had been treated for major depressive disorder. In future studies, the index-test method could test whether mutations in this gene also predispose to the poor impulse control, problems with alcohol or illicit drugs, or abnormal thought processing symptoms self-reported at a high frequency by blood relatives in WS families (unpublished data).

Given the accumulating data supporting the hypothesis that wolframin mutations contribute significantly to the incidence of mental illness, it is important to replicate the findings reported here as soon as possible.

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