Molecular Psychiatry (2001) 6, 302–306

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

A missense mutation in a novel gene encoding a putative cation channel is associated with catatonic schizophrenia in a large pedigree

J Meyer[1], A Huberth[1], G Ortega[1], YV Syagailo[1], S Jatzke[1], R Mössner[1], TM Strom[2], I Ulzheimer-Teuber[1], G Stöber[1], A Schmitt[1] and KP Lesch[1]

[1]*Department of Psychiatry and Psychotherapy, University of Würzburg, D-97080 Würzburg, Germany;* [2]*GSF – National Research Center for Environment and Health, D-85764 Neuherberg, Germany*

Keywords: schizophrenia; genetics; 22q13.33; mutation; cation channel; heterogeneity

Schizophrenia is a common and etiologically heterogeneous disorder. Although inheritance of schizophrenic syndromes is complex with genetic and environmental factors contributing to the clinical phenotype, periodic catatonia, a familial subtype of catatonic schizophrenia, appears to be transmitted in an autosomal dominant manner. We report here that a Leu309Met mutation in WKL1, a positional candidate gene on chromosome 22q13.33 encoding a putative non-selective cation channel expressed exclusively in brain, co-segregates with periodic catatonia in an extended pedigree. Structural analyses revealed that this missense mutation results in conformational changes of the mutant protein. Our results not only underscore the importance of genetic mechanisms in the etiology of schizophrenic syndromes, but also provide a better understanding of the pathogenesis and incapacitating course of catatonic schizophrenia and related disorders. *Molecular Psychiatry* (2001) **6,** 302–306.

Periodic catatonia, a familial subtype of catatonic schizophrenia (MIM 605419) is a genetically heterogeneous disorder characterized by psychosis and psychomotor disturbances. Patients with periodic catatonia express a variable phenotype combining akinetic negativism, hyperkinesia with stereotypies and parakinetic movements as well as increased anxiety, impulsivity, and aggressiveness.[1] Acute psychotic episodes may be accompanied by hallucinations and delusions, while successive episodes lead to an increasingly severe catatonic residual state. Based on clinical evidence for genetic anticipation and on a cumulative morbidity risk of ~27% in first-degree relatives of patients, a major gene effect was predicted for some forms of periodic catatonia.[2,3]

Our group recently reported a genome-wide linkage scan of 12 German pedigrees with the periodic subtype of catatonic schizophrenia comprising 137 individuals including 57 affected.[4] In this sample, significant evidence for linkage was obtained on chromosome 15q15 by using non-parametric multipoint analysis methods (GENEHUNTER-PLUS LOD* score 3.57, $P = 0.000026$). For a second locus, mainly supported by a single large family (Figure 1), suggestive evidence for linkage was found for marker D22S1169 on chromosome 22q13.33 (LOD* score 1.85, $P = 0.0018$). Genotyping of additional chromosome 22-specific polymorphic markers narrowed the region of interest to the D22S1160-telomer interval comprising ~4 cM (J Meyer *et al*, unpublished data). KIAA0027, a partial cDNA deposited in GenBank[5] with a short, potentially unstable CTG stretch was among several positional candidates located within this region which were selected for further investigation. Genomic annotation of KIAA0027 (based on GenBank accession numbers D25217 for the cDNA and AL022327 for the genomic PAC clone RP3–355C18) revealed 12 exons spanning ~28 Mb.

In order to screen for gene variants leading to the schizophrenic phenotype we have amplified the 12 exons of KIAA0027 from genomic DNA of two affected individuals of the family depicted in Figure 1 and three unrelated controls by PCR. Analysis of PCR product size of exon 11 containing the CTG stretch did not show evidence for length variability of the nucleotide sequence. However, sequencing demonstrated heterozygosity for a 1121C→A transversion in both patients, resulting in a Leu309Met substitution (Figures 2 and 3). All available family members and a total of 327 unrelated controls (654 chromosomes) were genotyped for C1121A by using the *Sfa*NI restriction site created by the mutation. Seven affected individuals and obligate carriers were found to be heterozygous for the mutation, while six unaffected family members carry two wild-type alleles (Figure 1). The mutation was found to co-segregate with the syndrome in the pedigree as expected and in accordance with the haplotypes previously determined by the linkage scan, whereas C1121A was not detectable in the controls. The presence of the mutation in asymptomatic individuals was interpreted as examples of the previously reported incomplete penetrance, or atypical and relatively mild course of illness under, as yet, unspecified circumstances.[1] Screening of three additional core families, with confirmed sharing of the same chromosome 22-specific haplotypes in the affected family members for mutations in the entire KIAA0027 gene (coding and

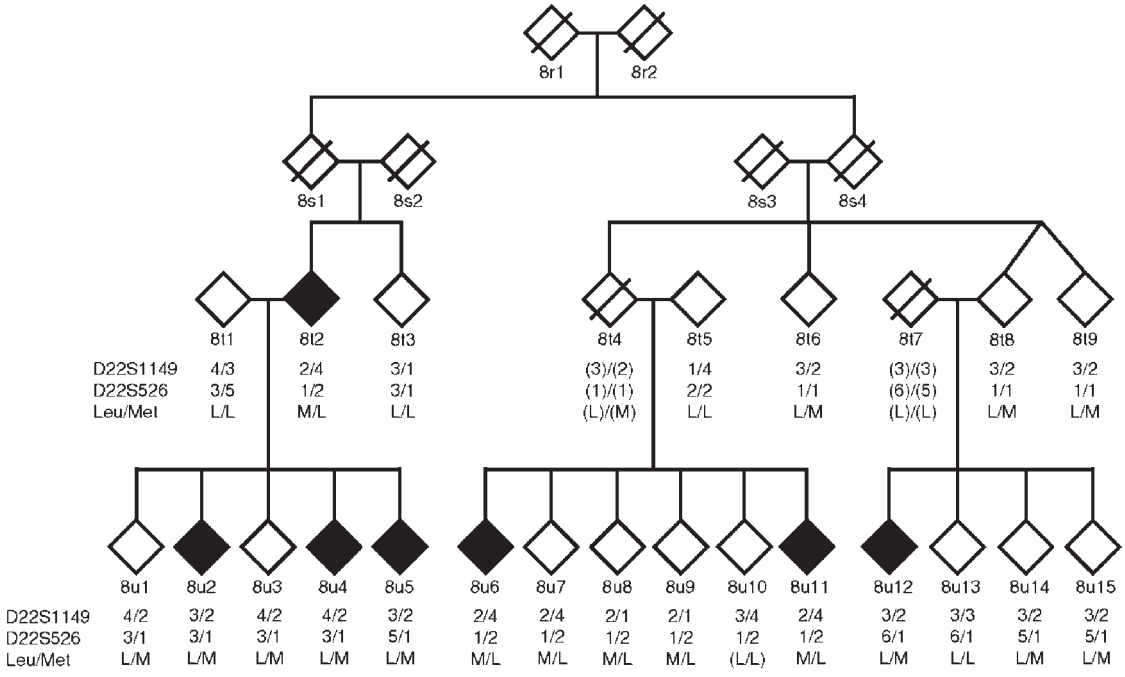

Figure 1 Segregation of the C1121A allele in a German pedigree with familial catatonic schizophrenia. Affected members are symbolised by black diamonds. The missense mutation encodes a methionine (M) instead of a leucine (L) amino-acid residue. Alleles of two adjacent polymorphic markers are provided (D22S1149 and D22S526). DNA of individual 8u10 was not available for mutation analysis and alleles were therefore deduced from data of the linkage scan. Assumed haplotypes of deceased persons are given in parenthesis.

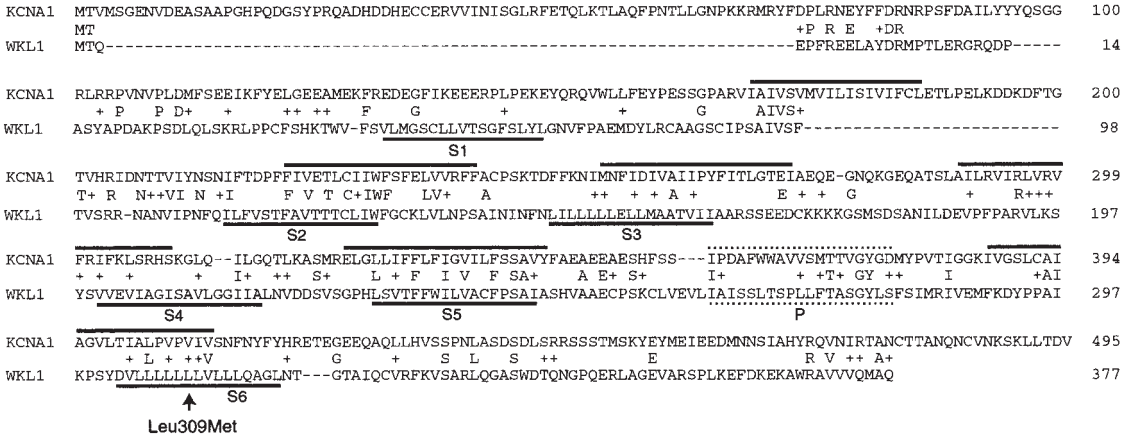

Figure 2 Predicted amino-acid sequence of WKL1 (GenBank accession number AF319633) and alignment with KCNA1 (L02750). Six potential transmembrane domains (S1–S6) and a putative pore region (P) are underlined. The location of the Leu309Met mutation in S6 is indicated.

non-coding regions, intron/exon boundaries), failed to detect C1121A or alternative mutations, thus further supporting the notion of remarkable genetic heterogeneity of periodic catatonia.

We have extended the 5′-UTR of KIAA0027 by 5′-RACE to an overall cDNA length of 3515 base pairs (GenBank accession number AF319633). In the expanded 5′-UTR a stop codon (TGA) was found in the previously reported open reading frame.[5] Therefore, the ATG codon at nucleotide position 197 should be considered as the correct translation initiation site, resulting in a protein of 377 amino-acid residues (Figure 4). This novel protein was provisionally designated WKL1.

Northern analysis of WKL1 mRNA revealed a single band with a size of ~3.6 kb, and transcripts were detected exclusively in human brain with highest signal intensities found in the amygdala, nucleus caudatus, thalamus, and hippocampus (Figure 4). The size of the WKL1 transcript is in agreement with the results obtained by the 5′-RACE technique, although the existence of splice variants in distinct brain regions or during neurodevelopment cannot be ruled out. More detailed assessment of WKL1 expression using both

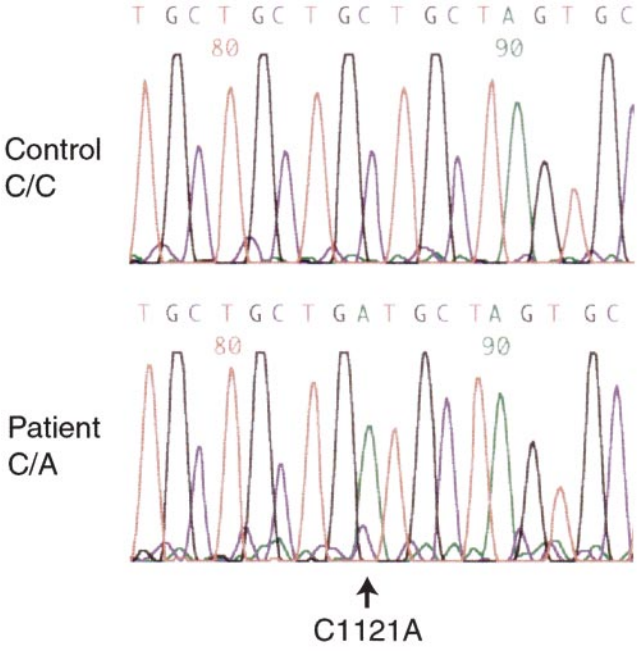

Figure 3 An electropherogram showing sequence of a portion of exon 11 revealed a C→A transversion at nucleotide position 1121 in patient 8u12.

Northern and *in situ* hybridization of human postmortem brain demonstrated transcripts also in entorhinal cortex, putamen, substantia nigra, and cerebellum (A Schmitt *et al*, manuscript in preparation). No detectable expression was found in peripheral tissues including heart and skeletal muscle (Figure 4).

Structure analysis of the revised WKL1 protein revealed six putative transmembrane domains (S1–S6) with a pore region (P) between S5 and S6 (Figure 2).[6] Although highest identity scores were obtained for the shaker-related human voltage-gated potassium channel KCNA1,[7,8] followed by other members of this extended gene family, characteristic features such as the voltage sensor in S4 and the selectivity filter in the P region are poorly conserved.[9,10] These characteristics suggest that the WKL1 protein may act as a non-selective neuronal cation channel, and thus may represent the first member of a novel subfamily distantly related to the shaker-related potassium channel superfamily.

Ion channels are critical for the initiation and termination of action potentials in neurons. For example, dysfunction of voltage-gated potassium channels results in impaired repolarization and increased excitability of neurons. Based on structure analyses, the Leu309Met mutation is located in the transmembrane domain S6, resulting in alterations of the secondary structure of WKL1 including decreased hydrophilicity and modified helix/sheet conformation (data not shown). The Leu309Met mutation is therefore likely to compromise channel function which, in turn, could lead to changes in neuronal excitability. Since mutations in potassium channel-encoding genes such as KCNA1 cause episodic (paroxysmal) ataxia,[11–13] and KCNQ2 benign familial neonatal epilepsy,[14] episodic psychomotor derangements such as hyperkinesia and parakinetic movements observed in the periodic subtype of catatonic schizophrenia support the notion that WKL1 encodes a cation channel, although this requires

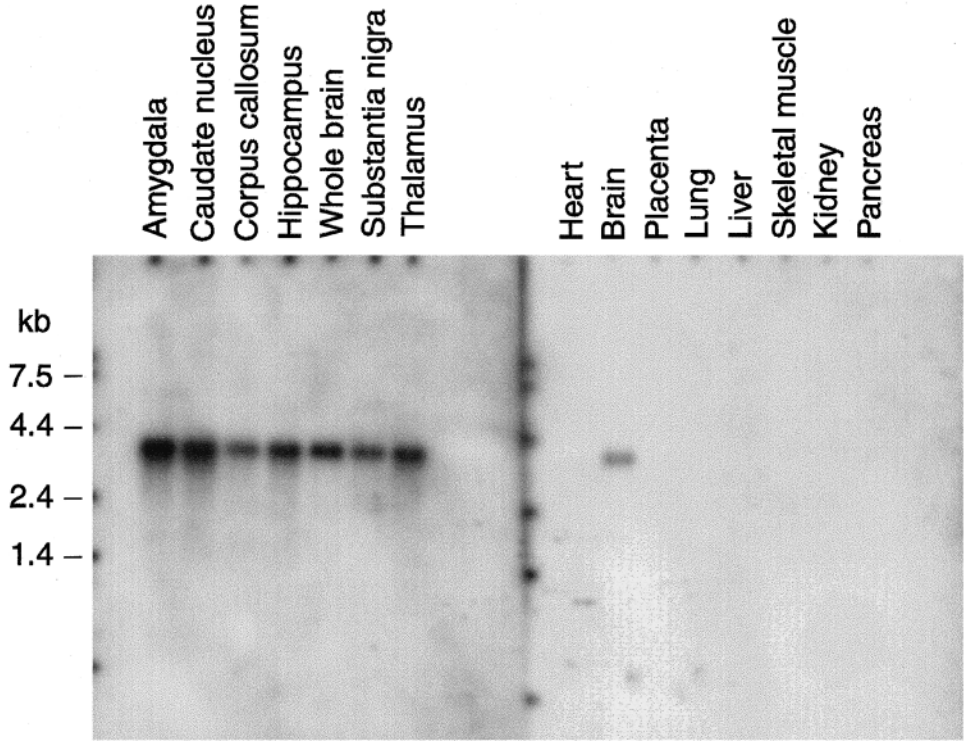

Figure 4 Northern analysis of WKL1 expression in various brain regions and peripheral tissues.

confirmation by elaborate electrophysiological analyses. Nevertheless, high expression of mutant WKL1 in the nigro-striatal motor and mesolimbic systems, leading to restricted channel function in these brain regions, would be in agreement with prominent psychopathologic features of periodic catatonia, such as psychomotor dysfunction, impaired cognitive functioning, and disinhibited or attenuated affective states.

The development of treatment resistance during successive episodes indicates a progressive component in the pathophysiology of schizophrenia.[15] Interestingly, significant changes in gating of shaker-type potassium channels by oxidation of methionine residues have been described.[16,17] Models for neurodegenerative processes in the long-term course of schizophrenia involve dopaminergic and glutamatergic projections between subcortical structures and the cortex, and psychotogenic effects of *N*-methyl-D-aspartate (NMDA) receptor antagonists.[15] Oxidation of the additional methionine residue in S6 of the mutant protein during psychotic episodes by NMDA receptor-mediated excitotoxicity and associated oxidative stress,[9,18,19] could lead to progressively impaired function of the WKL1 protein and further underscore the critical role of the Leu309Met mutation in the pathogenesis of catatonic schizophrenia. The incomplete penetrance, the occasionally atypical and relatively mild course of illness, and the considerable intrafamilial variability of the phenotype of periodic catatonia emphasize the relevance of secondary mechanisms, such as excitotoxicity-related oxidative stress, or indicate the segregation of modifier genes that interact with environmental factors, such as perinatal trauma and psychosocial stress. Evaluation of the role of WKL1 in other psychotic disorders with periodicity of episodes including bipolar and cycloid psychoses should be of high priority.

Taken together, our results provide evidence that haploinsufficiency of WKL1 causes a periodic subtype of catatonic schizophrenia. The Leu309Met mutation is the first in a pathogenetically relevant gene to be associated with a schizophrenic disorder that was discovered by linkage analysis followed by positional cloning. The identification of a schizophrenia-related gene will furnish a powerful tool to the understanding of the etiopathogenesis of catatonic schizophrenia and related disorders. Development of causal treatments of these devastating and cost-intensive disorders may now be a realistic prospect and an attainable goal.

Methods

Clinical

The multiplex family was ascertained as previously described.[4] The study was approved by the Ethics Committee of the University of Wuerzburg and all individuals participated after giving informed consent.

Mutation analysis

Exons of the WKL1 gene from patients and controls were amplified in a T-GRADIENT Thermocycler (Biometra, Göttingen, Germany). PCR was performed in a final volume of 25 μl containing 60 ng of genomic DNA, 10 pmol of each primer, 200 μM of each dNTP, 1.0 or 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris HCl (pH 8.3 at 25°C), 0.025 mg ml^{-1} BSA, 0.025% Tween 20, and 0.5 U of Taq DNA polymerase (Eurogentec, Seraing, Belgium). Resulting PCR products were sequenced using an ABI 310 automated sequencer (Applied Biosystems, Foster City, CA, USA). For C1121A confirmation and screening, exon 11 was amplified from DNA of 327 unrelated volunteers (654 chromosomes) and all family members except individual 8u10, whose DNA was no longer available for this study. Primers for exon 11 were 5′-TGGCTCGGTCACTTTTATTCC-3′ and 5′-CCCACAGGCTTCTCACCTC-3′, respectively. PCR products from the family were analysed by *Sfa*NI digestion followed by separating the resulting fragments on agarose gels stained with 2% ethidium bromide.

5′-Race

For 5′-RACE total RNA was extracted from adult human hippocampus using peqGOLD RNAPure solution (Peqlab, Erlangen, Germany). cDNA synthesis and 5′-RACE was carried out using the Smart RACE kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. Initial amplification was done with gene-specific primers RACE1 5′-CTGCTCTGC CGTTGGGAGCACTGG-3′ and RACE2 5′-GCCGTTGGG AGCACTGGTCTCTGG-3′, followed by half-nested PCR using gene-specific primer RACE3 5′-TGGTCTCT GGGTGAGGGACTTCCAG-3′. The completed WKL1 cDNA and deduced amino-acid sequence was analysed and compared with other relevant genes using the BLAST algorithm and the MacVector software package. Putative transmembrane domains were predicted using online protein analysis routines including SMART (http://smart.embl-heidelberg.de), EXPASY (http://expasy.proteome.org.au/tools/#align), and PSORT II (http://psort.nibb.ac.jp).

Northern blot

Human Northern blot membranes with equal amounts of poly A+ mRNA (Clontech) from various brain regions and peripheral tissues were hybridised with the ^{32}P-labeled insert of IMAGE clone 3222221 (Resource Center, Berlin) corresponding to ~700 bp of the 3′-end of the WKL1 gene using the protocol provided by the manufacturer. Hybridized membranes were exposed to photographic film (Kodak XR).

Acknowledgements

The authors would like to express their gratitude to the study participants and their family. They also thank P Riederer for sharing human brain samples, M Schartl and S Scherer for their help with the protein analysis, and C Müller-Reible for helpful discussions.

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Correspondence: KP Lesch, MD or J Meyer, Department of Psychiatry and Psychotherapy, University of Würzburg, Füchsleinstr 15, 97080 Würzburg, Germany. E-mail: kplesch@mail.uni-wuerzburg.de; jobst.meyer@mail.uni-wuerzburg.de
Received 19 December 2000; accepted 22 December 2000