European Journal of Human Genetics (1999) 7, 247–250

http://www.stockton-press.co.uk/ejhg

SHORT REPORT

No evidence for involvement of KCNN3 (*hSKCa3*) potassium channel gene in familial and isolated cases of schizophrenia

Frédérique Bonnet-Brilhault[1,2], Claudine Laurent[3], Dominique Campion[1,2], Florence Thibaut[2], Carole Lafargue[3], Françoise Charbonnier[1], Jean-François Deleuze[6], Jean-François Ménard[5], Maurice Jay[4], Michel Petit[2], Thierry Frebourg[1] and Jacques Mallet[3]

[1]*Génétique et Hématologie Moléculaires, IFRMP, Faculté de Médecine et de Pharmacie, Rouen*
[2]*Groupe de Recherche sur la Schizophrénie, Centre Hospitalier du Rouvray, Sotteville Lès Rouen*
[3]*Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs, Hôpital de la Pitié-Salpêtrière, Paris*
[4]*Centre Hospitalier spécialisé Saint Paul, la Réunion*
[5]*Unité de Biostatistiques, Centre Hospitalo-Universitaire de Rouen*
[6]*Département Génomique RP Rorer, Evry, France*

Several studies have reported in schizophrenia a decrease of age of onset in successive family generations, and this observation is consistent with anticipation. Anticipation is known to result from expansion of CAG repeats in several neurodegenerative disorders. Longer alleles of the KCNN3 gene, which contains a highly polymorphic CAG repeat, and encodes a neuronal small conductance calcium-activated potassium channel, have recently been shown to be over-represented in sporadic cases of schizophrenia. In this report, we tested the hypothesis of an association between longer alleles of CAG repeat in the KCNN3 gene and schizophrenia in 20 families with clinical evidence for anticipation and in 151 unrelated schizophrenic cases. No significant difference in the distributions of allele frequencies was observed between familial cases of schizophrenia and controls, and between unrelated cases and controls. Furthermore, no intergenerational CAG repeat instability was detected in the 20 families. Our results do not support the involvement of the KCNN3 (*hSKCa3*) gene in the etiology of schizophrenia.

Keywords: anticipation; schizophrenia; CAG repeat; KCNN3 gene; SKCa channels

Correspondence: Thierry Frebourg, Génétique et Hématologie Moléculaires, JE2006, Faculté de Médecine et de Pharmacie, 22 boulevard Gambetta, 76183 Rouen Cedex, France. Tel: 33 2 32 88 81 82; Fax: 33 2 32 88 80 80; E-mail: frebourg@chu-rouen.fr
Received 16 April 1998; revised 2 October 1998; accepted 12 October 1998

Introduction

Schizophrenia, which affects about 1% of the population,[1] is characterised by hallucinations, formal thought disorder and abnormal behaviour. There is convincing evidence that schizophrenia involves genetic factors, although the mode of inheritance of this disease remains unknown. Several studies have reported that, in multiplex families transmitting schizophrenia, the age of onset of the disease is earlier in succeeding generations.[2–5] This observation is consistent with genetic anticipation which suggests that inheritance of schizophrenia might be due, at least in part, to an expanded trinucleotide repeat mechanism.[6] Expansion of CAG trinucleotide repeats in coding regions has been identified in eight distinct neurological diseases, Huntington's disease, spinocerebellar ataxias, spinal bulbar muscular atrophy and dentatorubral pallidoluysian atrophy.[7] This type of mutation is remarkably unstable across the generations and in most of the cases the length of these sequences is correlated to the severity and age of onset of the disease.

The involvement of expanded CAG trinucleotide repeats in schizophrenia has received support from studies based on the Repeat Expansion Detection (RED) method, a multilocus assay which detects expansions larger than 40 repeats. These studies have found a statistically significant shift towards longer CAG/CTG repeats in schizophrenic subjects compared to control individuals, with an overlap in the repeat length between patients and controls.[8–10] Nevertheless, in a previous study, we did not replicate these results in a population of sporadic patients.[11] Furthermore, we did not detect any CAG expansion in 21 multiplex families with anticipation,[11] and this negative result was also reported by Bassett *et al*.[12] In order to identify candidate genes, investigators have screened for expansion in trinucleotide repeat-containing genes identified from human brain cDNA-libraries.[13–16] Recently, Chandy *et al*[17] have suggested that the KCNN3 (*hSKCa3*) gene, located on chromosome 1, might be a candidate gene for schizophrenia. The KCNN3 gene encodes a neuronal small conductance calcium-activated potassium channel, which is known to play a major role in determining the firing pattern of neurons via the generation of slow after-hyperpolarization and the regulation of intracellular calcium signals.[18] The 5' end of this gene contains two CAG repeats regions, and longer alleles of the second CAG repeat were reported to be over-represented in sporadic schizophrenic patients.[17] In the present report, we analysed the implication of the KCNN3 gene in 20 French families showing clinical evidence for anticipation and in a set of 151 unrelated schizophrenic cases.

Materials and Methods

Subjects

We analysed, in 20 multiplex families showing anticipation (7 from western France and 13 from the island of la Réunion),[4] 122 individuals, including 60 asymptomatic subjects and 62 schizophrenic patients, belonging either to the older (n = 23) or younger (n = 39) generation. Ascertainment and diagnostic assessment of these families have been discussed in detail in Thibaut *et al*.[4] One hundred and fifty-one unrelated schizophrenic patients (111 males and 40 females, aged 41 years ± 12.4 (mean ± SD), age of onset 22.6 years ± 5.7 (mean ± SD)), were also analysed. Diagnoses were made according to DSM-IIIR criteria[19] by two trained psychiatrists after clinical interviews following the French version of the Schedule for Affective and Schizophrenia Disorders Lifetime Version modified for the study of anxiety disorders (SADS-LA).[20] A control group was composed of 153 individuals, 116 males and 37 females, (age 38.8 years ± 12.3), free of neurological or psychiatric disease, and who had no family history of psychosis or neurodegenerative disorders.

All unrelated schizophrenic patients and controls were French Caucasians and originated in Normandy, in the west of France. Written informed consent was obtained from every individual.

DNA Isolation

Genomic DNA was prepared from blood by proteinase K digestion followed by ethanol precipitation using a standard procedure.[21]

PCR Amplification

The region of the KCNN3 gene (Genebank accession number G16005), containing the second CAG repeat, was PCR-amplified using 6FAM dye labelled sense (5' GACCCTTGCTGCAGCCTC 3'), and antisense (5' GGTCATTGAGATTGAGCTGG 3') primers. PCR were performed in a final volume of 25 µl containing 50 ng of DNA, 0.5 µM of each primer, 200 µM of dNTPs and 0.5 units of Taq polymerase (Appligene Oncor, Illkirch, France). The PCR consisted of 35 cycles of 20 s at 94°C, 20 s at 60°C, 40 s at 72°C, preceded by 3 min at 95°C and followed by 5 min at 72°C.

Measurement of Repeats Number

The PCR products (0.1 µl) and 0.5 µl of internal lane size standard (Genescan-2500 Rox, PE Applied Biosystems, Foster City, CA) were loaded on a 12 cm 6% denaturing polyacrylamide gel. The electrophoresis was performed at 1500 V for 5 h on an Applied Biosystems model 373A automated sequencer, and data were analysed using the Gene Scanner Model 672 Fluorescent Fragment Analyser (Applied Biosystems, Perkin-Elmer). To calculate the number of repeats from the size of the PCR fragments, PCR product derived from one homozygous patient was directly sequenced

using the PRISM AmpliTaq FS Ready Reaction Dye Terminator sequencing kit (Applied Biosystems, Perkin-Elmer).

Statistical Analysis

The non-parametric Mann-Whitney U test was used to compare distributions of CAG repeats number between the affected subjects belonging to the younger generation in multiplex families showing evidence of anticipation and the control group, and between the isolated cases and the control group. Since the hypotheses tested a unidirectional effect, one tailed *P*-values were used. A χ^2 test was also applied, using the CAG repeat size as a nominal variable, ie below and above 19 repeats (modal allele length). Power calculations were done using a χ^2 test of the dichotomised allele distributions.

Results

Among schizophrenic patients and controls, we identified 11 different alleles (13 to 24 CAG repeats corresponding to PCR fragments of 235 to 268 bp). As shown in Figure 1, no significant difference was found in the distributions of allele frequencies between younger generation schizophrenic patients of multiplex families and controls ($P = 0.32$), or between unrelated cases and controls ($P = 0.53$). Furthermore, no significant results were obtained when isolated or familial cases were compared with controls using 2×2 contingency tests ($X^2 = 0.033$, df = 1, NS and $X^2 = 0.247$, df = 1, NS, respectively). In families showing evidence of clinical anticipation, the pattern of transmission of the CAG repeats was consistent with Mendelian inheritance of parental alleles and no intergeneration repeat instability was observed.

Discussion

We analysed the involvement of the KCNN3 gene in unrelated schizophrenic cases and in patients belonging to multiplex families with clinical evidence of anticipation, respectively. Chandy *et al*[17] have recently reported that longer alleles of the second CAG repeat (range 20 to 28 repeats) present in the KCNN3 gene were overrepresented in unrelated cases of schizophrenia originating in France and North America. In this study we did not replicate these results. Considering that the odds ratio from the study by Chandy *et al* can be estimated approximately at 2, we calculated that a sample of our size had a power of 95% to detect such

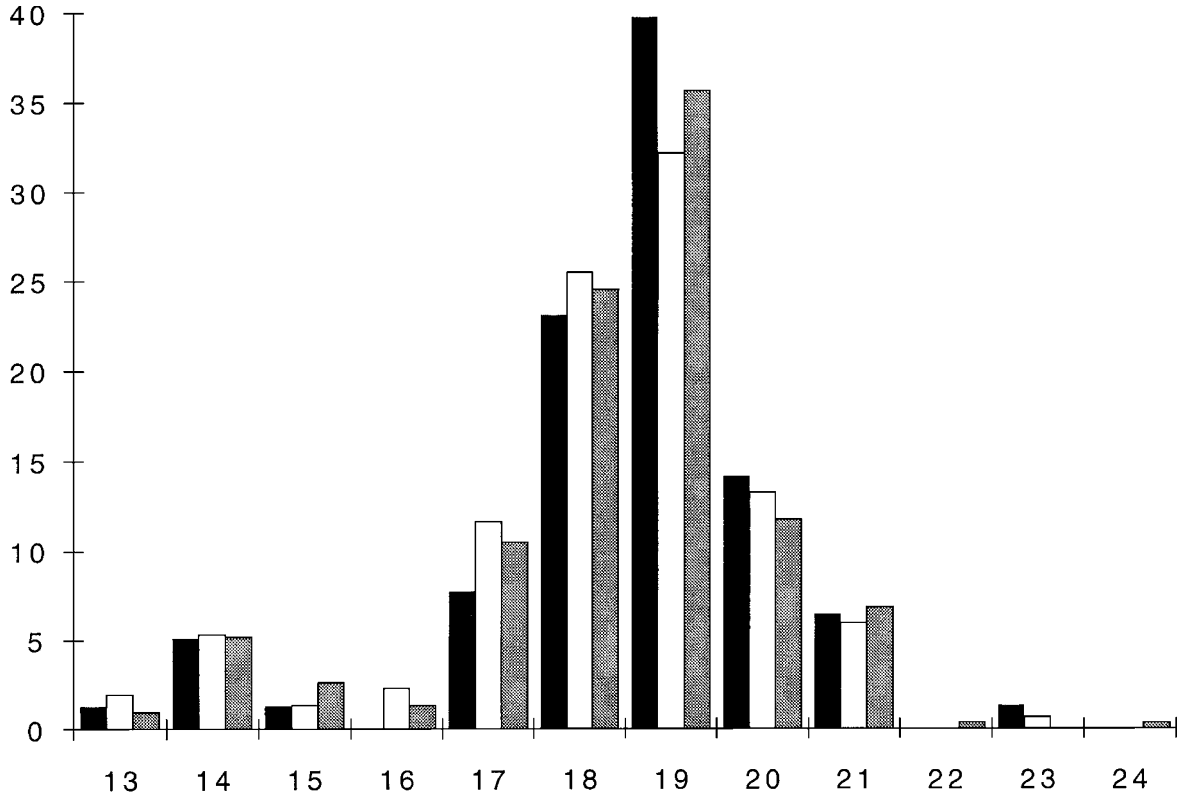

Figure 1 *Distributions of KCNN3 allelic frequencies in familial cases (black), sporadic cases (white) of schizophrenia and in controls (grey).*

an association. Recently Bowen *et al* studied 194 unrelated schizophrenic patients and 183 controls born in the UK or Eire. They interpreted their data as providing modest support (ie $\chi^2 = 2.820$, 1 df, $P = 0.047$, 1-tail. Mann-Whitney: $P = 0.11$, 1-tail) for the findings of Chandy *et al*. We observed that when the alleles were dichotomised into 19 and > 19, our Normandy controls did not differ from UK controls analysed in the Bowen *et al* study ($X^2 = 0.256$, 1 df, 2-tailed, NS), whereas both control groups significantly differ from the Alsatian control group used in the Chandy *et al* study (Normandy controls *vs* Alsatian controls: $X^2 = 6.135$, 1 df, 2-tailed, $P < 0.02$; UK controls *vs* Alsatian controls: $X^2 = 4.425$, 1 df, 2-tailed, $P < 0.05$). The significant shift towards longer alleles, reported by Chandy *et al* in schizophrenic subjects, was observed in Alsatian patients.[17] Although these results remained significant after pooling this sample with a smaller one from the USA, it should be stressed that statistical significance was only reached in the European group[17] whose controls differed from the other European controls.

To test specifically the involvement of the KCNN3 gene in genetic anticipation, we have also included in this study an appropriate population composed of 20 families of schizophrenic patients with clinical evidence of anticipation. We did not detect any intergenerational CAG repeats instability in these pedigrees. Altogether, our results do not support the involvement of the KCNN3 gene in the etiology of schizophrenia.

References

1 Kendler KS: Overview: a current perspective on twin studies of schizophrenia. *Am J Psychiatr* 1983; **140**: 1413–1425.
2 Ohara K, Xu HD, Mori N *et al*: Anticipation and imprinting in schizophrenia. *Biol Psychiatr* 1997; **9**: 760–766.
3 Bassett AS, Honer WG: Evidence for anticipation in schizophrenia. *Am J Hum Genet* 1994; **54**: 864–870.
4 Thibaut F, Martinez M, Petit M, Jay M, Campion D: Further evidence for anticipation in schizophrenia. *Psychiatr Res* 1995; **59**: 25–33.
5 Asherson P, Walsh C, Williams J *et al*: Imprinting and anticipation: are they relevant to genetic studies of schizophrenia? *Br J Psychiatry* 1994; **164**: 619–624.
6 O'Donovan MC, Owen MJ: Dynamic mutations and psychiatric genetics. *Psychol Med* 1996; **26**: 1–6.
7 Mitas M: Trinucleotide repeats associated with human disease. *Nucleic Acids Res* 1997; **25**: 2245–2253.
8 Morris AG, Gaitonde E, McKenna PJ, Mollon JD, Hunt DM: CAG repeat expansions and schizophrenia: association with disease in females and with early age at onset. *Hum Mol Genet* 1995; **4**: 1957–1961.
9 O'Donovan MC, Guy C, Craddock N *et al*: Expanded CAG repeats in schizophrenia and bipolar disorder. *Nat Genet* 1995; **10**: 380–381.
10 O'Donovan MC, Guy C, Craddock N *et al*: Confirmation of association between expanded CAG/CTG repeats and both schizophrenia and bipolar disorder. *Psychol Med* 1996; **26**: 1145–1153.
11 Laurent C, Zander C, Thibaut F *et al*: Anticipation in schizophrenia: no evidence of expanded CAG/CTG repeat sequences in French families and sporadic cases. *Am J Med Genet* 1998; **81**: 342–346.
12 Bassett AS, Husted J: Anticipation or ascertainment bias in schizophrenia? Penrose's familial mental illness sample. *Am J Hum Genet* 1997; **60**: 630–637.
13 Bowen T, Guy C, Speight G *et al*: Expansion of 50 CAG repeats excluded in schizophrenia by application of a highly efficient approach using repeat expansion detection and a PCR screening set. *Am J Hum Genet* 1996; **59**: 912–917.
14 Jain S, Leggo J, DeLisi LE *et al*: Analysis of thirteen trinucleotide repeat loci as candidate genes for schizophrenia and bipolar affective disorder. *Am J Med Genet* 1996; **67**: 139–146.
15 Gaitonde EJ, Sivagnanasundaram S, Morris AG, McKenna PJ, Mollon JD, Hunt DM: The number of triplet repeats in five brain-expressed loci with CAG repeats is not associated with schizophrenia. *Schizophr Res* 1997; **25**: 111–116.
16 Ohara K, Tani K, Tsukamoto T *et al*: Exclusion of five trinucleotide repeat (CAG and CCG) expansions in 17 families with schizophrenia. *Biol Psychiatr* 1997; **42**: 756–759.
17 Chandy KG, Fantino E, Wittekindt O *et al*: Isolation of a novel potassium channel gene hSKCa3 containing a polymorphic CAG repeat: a candidate for schizophrenia and bipolar disorder? *Mol Psychiatr* 1998; **3**: 32–37.
18 Nicoll RA: The coupling of neurotransmitter receptors to ion channels in the brain. *Science* 1988; **241**: 545–551.
19 American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edn, (DSMIIIR). 1987, Washington, DC. APA.
20 Fyer AJ, Endicott J, Manuzza S, Klein DF: Schedule for affective disorders and schizophrenia – lifetime version modified for the study of anxiety disorders. In: *Anxiety Disorders Clinic*. New York State Psychiatric Institute: New York, 1985.
21 Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press: New York, 1989.
22 Bowen T, Guy CA, Craddock N *et al*: Further support for an association between a polymorphic CAG repeat in the hKca3 gene and schizophrenia. *Mol Psychiatr* 1998; **3**: 266–269.