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Analysis of a polymorphism in the tuberous sclerosis (TSC2) gene does not predispose to schizophrenia

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Abstract The tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by the development of malformations in various organs including the brain. A polymorphism in the TSC2 gene has been found to be increased in gangliogliomas, a lesion which is associated with disturbed neuro-glial cell migration pattern. Since these pathomorphological changes are compatible with disturbed neuronal migration in schizophrenic brains, we investigated this polymorphism in 130 families with a schizophrenic index patient. A 222-bp fragment of genomic DNA containing the TSC2 variant was analyzed by SSCP. The analysis revealed that there is no association with schizophrenia.

Key words Schizophrenia · SSCP · TSC2

Introduction

Brains of tuberous sclerosis (TSC) patients show distinct cortical tubers, which stand out dramatically from the normal, orderly lamination of the cerebral cortex. The overall cortical topography within a tuber suggests abnormal neuronal migration (Short et al. 1995). Two genes that are disease associated have been identified: TSC1, which has recently been cloned, is located on chromosome 9q34 (van Slegtenhorst et al. 1997). TSC2 has been mapped to chromosome 16p13.3 (The European Chromosome 16 Tuberous Sclerosis Consortium 1993). The gene products of TSC2 have been analyzed (Wienecke et al. 1995) and found to be expressed in many neural structures (Kerfoort et al. 1996). The function of the protein tuberin is still unclear. Recently, a novel splice-site-associated polymorphism in the TSC2 gene was determined to be increased in gangliogliomas (Platten et al. 1997), a lesion which is composed of a mixture of abnormal nerve and glia cells. Interestingly, several studies have reported that gangliogliomas may be associated with schizophrenia (Bruton et al. 1990; Jellinger 1980). In addition, abnormal neuronal migration has been observed in several regions of schizophrenic brains (Akbarian et al. 1996; Honer et al. 1996; Jakob et al. 1986). These studies have stimulated us to investigate this TSC2 polymorphism using single-strand conformational polymorphism (SSCP) in families with schizophrenic index patients. This technique is widely used to detect polymorphisms in genomic DNA on the basis of differential mobility of single DNA strands after polyacryl gel electrophoresis. We performed SSCP analysis of TSC2 using DNA samples from 130 affected individuals and their parents.

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Materials and methods

Blood samples were obtained from 130 core families with one child suffering from schizophrenia. Patients were recruited from consecutive admissions to our inpatient clinics. All patients had been interviewed by experienced psychiatrists (M.A., M.R., W.M.) using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L; Endicott and Spitzer 1978). Lifetime "best estimate" diagnosis according to DSM-III-R criteria were based on multiple sources of information including personal structured interviews (SADS-L), medical records, and family history. Patients had a family history of schizophrenia obtained by direct interviews with relatives. The DNA was extracted from blood. A 222-bp fragment of genomic DNA containing the TSC2 variant was amplified using primers 5′-GGAGATGTAGATTCGGCGTC-3′ (located in intron 4) and 5′-CTGCGGAGCTGAACTTAGG-3′ (located in exon 5) for 30 cycles at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. PCR was performed in an automated thermocycler in a total volume of 10 ml containing 10 ng DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.4, 200 mM of each dNTP, 1 U Taq polymerase, 1.5 mM MgCl2, 0.1% gelatin and 20 pmol of each primer. PCR products were electrophoretically separated (20 W and 15 °C) on non-denaturating gels containing 12% polyacrylamide (29 acrylamide/1 bisacrylamide), 5% glycerol, and 1 × TBE buffer. The gels were visualized with silver staining (Budowle et al. 1991; von Deimling et al. 1993). Statistical analysis was performed using Student's two-tailed *t*-test.

Table 1 Allelic distribution of the TSC2 gene polymorphism. The percentage of transmitted alleles A1 is not significantly different from the percentage of non-transmitted parental A1 alleles (p = 0.85)

Allele	*N*	%
Controls (parental alleles non-transmitted to the index child)		
A1	33	12.69
A2	227	87.31
A1 + A2	260	100
Index child with schizophrenia (alleles transmitted from the parents)		
A1	32	12.30
A2	228	87.70
A1 + A2	260	100

Results

A 222-bp fragment of genomic DNA containing the TSC2 variant was amplified by PCR and analyzed by SSCP. We examined 130 families with one child suffering from schizophrenia. The parental DNA samples of these families served as healthy internal ethnic controls, in order to avoid uncertainties which may accompany population-based association studies. The frequency of the rare allelic polymorphism A2 vs the frequency of the frequent allele A1 was determined and used in statistical analysis. Haplotype relative risk (HRR) calculation in the 130 families, i.e., 260 alleles, showed that the allelic distribution in the control group (alleles not transmitted to the child) was for A2 = 33 and for A1 = 227 (12.69%). The allelic distribution in the disease group (alleles transmitted from the parents to the child) was A2 = 32 and A1 = 228 (12.30%). This calculation shows that the rare allele A2 in index patients has the same frequency as in non-transmitted control parental DNA, which reflects the overall allelic distribution in the entire population (p = 0.85; Table 1).

Discussion

We examined 130 core families with schizophrenia and the possible association with a polymorphism in the tuberous sclerosis gene 2 (TSC2). TSC2 is involved in the pathogenesis of the tuberous sclerosis complex, a disease which shows disordered neuronal migration in the central nervous system (Short et al. 1995). A splice-site-associated polymorphism in the TSC2 gene has been described to be increased in gangliogliomas (Platten et al. 1997). These rare brain tumors have also been observed in postmortem schizophrenic brains (Bruton et al. 1990; Jellinger 1980). The neuropathological changes in schizophrenic brains are still unclear; however, increasingly more reports in the literature have reported disturbed neuronal migration in different brain areas (Akbarian et al. 1996; Honer et al. 1996; Jakob and Beckman 1986). The rationale of the present report was to investigate the TSC2 polymorphism in a family-based study for association with schizophrenia. We did not find association of the TSC2 polymorphism with schizophrenia, because the distribution of the frequent A1 and the rare A2 allele showed the same distribution in the disease group (frequency = 12.30%) vs the parental control group (frequency = 12.69%). There was no significant difference between the two groups (p = 0.85).

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