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VARIATIONS IN GENES REGULATING NEURONAL MIGRATION PREDICT REDUCED PREFRONTAL COGNITION IN SCHIZOPHRENIA AND BIPOLAR SUBJECTS FROM MEDITERRANEAN SPAIN: A PRELIMINARY STUDY

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Abstract—Both neural development and prefrontal cortex function are known to be abnormal in schizophrenia and bipolar disorder. In order to test the hypothesis that these features may be related with genes that regulate neuronal migration, we analyzed two genomic regions: the lissencephaly critical region (chromosome 17p) encompassing the LIS1 gene and which is involved in human lissencephaly; and the genes related to the platelet-activating-factor, functionally related to LIS1, in 52 schizophrenic patients, 36 bipolar I patients and 65 normal control subjects. In addition, all patients and the 25 control subjects completed a neuropsychological battery. Thirteen (14.8%) patients showed genetic variations in either two markers related with lissencephaly or in the platelet-activating-factor receptor gene. These patients performed significantly worse in the Wisconsin Card Sorting Test–Perseverative Errors in comparison with patients with no lissencephaly critical region/platelet-activating-factor receptor variations. The presence of lissencephaly critical region/platelet-activating-factor receptor variations was parametrically related to perseverative errors, and this accounted for 17% of the variance (P=0.0001). Finally, logistic regression showed that poor Wisconsin Card Sorting Test–Perseverative Errors performance was the only predictor of belonging to the positive lissencephaly critical region/platelet-activating-factor receptor group. These preliminary findings suggest that the variations in genes involved in neuronal migration predict the severity of the prefrontal cognitive deficits in both disorders.

Key words: neuronal migration, prefrontal cognition, LIS1, platelet activating factor receptor, endophenotype, lissencephaly.

Over several decades, numerous family, twin and adoption studies have indicated that genetic factors play a major role in predisposing individuals for schizophrenia and bipolar disorder (Berrettini, 2002; Riley et al., 2003). Several of the susceptibility loci and genes associated with schizophrenia play a principal role in neuronal migration, connectivity and in the maintenance of the neural microcircuits (Harrison and Weinberger, 2005). Nowadays, schizophrenia could therefore be considered a genetic neurodevelopmental disorder (Rapoport et al., 2005). Although literature on neurodevelopmental alterations in bipolar disorders is less extensive, recent findings suggest anomalies in neuronal migration and connectivity (Harwood, 2003). In general, genes that regulate the migration of neurons could therefore be possible candidate-risk genes to be investigated in both disorders (Merikangas et al., 2002; Harrison and Weinberger, 2005).

Interestingly, the similarities between schizophrenia and bipolar disorder in genetic and developmental findings, neuropsychological abnormalities (Daban et al., 2006) and in other epidemiological features (Torrey, 1999), are part of a heuristic pattern of coincidences which implies that at least some cases of both disorders have common etiological antecedents, and supports a continuum (Crow, 1998) vs. a categorical (Kendler et al., 1998) view of psychotic illness.

Various studies on postmortem human brains provide evidence of a significant alteration of cell migration in the both prefrontal cortex and dentate gyrus from subjects with schizophrenia and bipolar disorder (Fatemi et al., 2000; Guidotti et al., 2000; Knable et al., 2001, 2004). Moreover, alterations in the neural migration in the cortex have been observed in lissencephaly, a severe brain malformation with tragic consequences (epilepsy, mental retardation and premature death; the association of lissencephaly with craniofacial malformations is known as Miller-Dieker syndrome) (Hong et al., 2000). In addition, the occurrence of schizophrenia-like symptoms and secondary-affective symptoms has been demonstrated in patients with epi-

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Abbreviations: ANCOVA, covariance analysis; CARS-M, Clinician-Administered Rating Scale for Mania Factor 1; HRSD, Hamilton Rating Scale for Depression; IQ, intelligence quotient; LCR, lissencephaly critical region; PANSS, Positive and Negative Syndrome ScalePCR, polymerase chain reaction; WAIS-R, Wechsler Adult Intelligence Scale–Revised; WCST, Wisconsin Card Sorting Test; WCST-PE, Wisconsin Card Sorting Test-Perseverative Errors.

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lepsy (Hyde and Lewis, 2003). Even more interestingly, the family history of epilepsy is a significant risk factor for schizophrenia (Qin et al., 2005); thus, the same genetic factor might induce schizophrenia as well as epilepsy. Furthermore, the current pharmacological treatment of many patients with schizophrenia or bipolar disorder includes the combinations of anticonvulsants and antipsychotics (Citrome et al., 2005). Therefore, a certain etiopathogenic overlap may exist among lissencephaly, schizophrenia and bipolar disorder.

Within the genomic region that is frequently deleted in lissencephaly (lissencephaly critical region, LCR in chromosome 17p13.3), the most well-known gene is *LIS1*, which interacts with dynein to mediate the coupling of the nucleus to the centrosome in neuronal migration. Furthermore, it binds both dynein and NUDEL (NDEL1) among many other interacting proteins (Shu et al., 2004). A most interesting point from the present study is that NUDEL1 has been shown to interact with the product of the *DISC1* gene (*Disrupted In Schizophrenia 1*; Morris et al., 2003; Ozeki et al., 2003). Interestingly, disruption of *DISC1* by a balanced chromosomal translocation (1;11)(q42;q14.3) was identified as a potential susceptibility factor for schizophrenia due to its co-segregation with this disease (St Clair et al., 1990; Millar et al., 2000; Blackwood et al., 2001). Moreover, other studies also described different *DISC1* variations related to schizoaffective and bipolar disorders (Hennah et al., 2003; Hodgkinson et al., 2004). The possibility of a direct role of *DISC1* in schizophrenia is further suggested by two recent studies. Callicott et al. (2005) showed that allelic variation within *DISC1* compromised hippocampal structure and function and, by virtue of this effect, would increase risk for schizophrenia. Finally, Sachs et al. (2005) detected a specific mutation in the *DISC1* in several members of a family. This mutation is predicted to cause a frame shift and truncated DISC1 protein.

There is considerable evidence that DISC1-NUDEL-LIS1 interaction plays an important role in neuronal migration, neurite architecture, and in intracellular transport (Ozeki et al., 2003; Brandon et al., 2004). Thus, functional alterations of this trimolecular complex could confer a variable degree of susceptibility for schizophrenia and other related disorders.

The *p53* gene (*TP53*) is also mapped into the LCR, and genetic variations of this gene have been related with a predisposition to develop schizophrenia and with its associated neurocognitive deficits (Chiu et al., 2001; Papiol et al., 2004; Ni et al., 2005).

Another set of interactions involved LIS1 and PAF (a phospholipid mediator, which acts by binding to a unique G-protein-coupled seven transmembrane receptor, PTAFR). LIS1 was identified as a subunit in the PAF–AH1B enzymatic complex. The PAF system (PAF, PAFAH and PTAFR) is functionally related to the *LIS1* gene within the molecular mechanisms involved in neuronal migration (Bix and Clark, 1998; Pahnke et al., 2004; Tokuoka et al., 2003; Yan et al., 2003).

On the other hand, converging lines of research suggest that an abnormal structure and function of the prefrontal cortex may be a cardinal feature of schizophrenia and bipolar disorder (Weinberger et al., 2001; Harrison, 2002). In this respect, cognitive and imaging endophenotype studies suggest that specific variations in genes could affect normal brain morphogenesis, prefrontal function, and subsequently, they could increase the risk of cognitive abnormalities, schizophrenia and bipolar disorder (Egan et al., 2001; Shifman et al., 2004; Harrison and Weinberger, 2005).

Consequently, we hypothesized (1) that patients with schizophrenia and bipolar disorder could show variations in some genes involved in neuronal migration, and (2) that these genetic variations could be functionally associated with a reduced prefrontal executive function. In order to test these hypotheses, we included the analyses of seven markers located in the critical region for lissencephaly (LCR), and those of the 13 markers of the PAF system-related genes (*PAFAH1B2*, *PAFH1B3* and *PTAFR*) in patients diagnosed with schizophrenia and bipolar type I disorder, compared with a group of normal control subjects. We also investigated eight cognitive domains.

EXPERIMENTAL PROCEDURES

Subjects

A written informed consent was obtained from all participants following an explanation of study procedures. The ethics committee of the University Clinic Hospital of Valencia (Spain) approved the research protocol. All subjects were aged between 18 and 60 years and were unrelated individuals of European ancestral birth, who were educated in Spain. Patients were recruited if they fulfilled the *Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition* (DSM-IV; American Psychiatric Association, 1994) criteria for either schizophrenia (n=52) or bipolar disorder type I (n=36), and if they had attended three different consecutive mental health units in the metropolitan area of Valencia over a 9-month period. Diagnoses were established with the Spanish version of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN-CATEGO; Vázquez-Barquero, 1993) after a minimum of one year's progress of the illness. The exclusion criteria used were: electro-convulsive therapy during the previous year, substance use disorders in the previous six months, and epilepsy, medical illness or a known organic cause that may account for either psychosis or cognitive abnormalities. All control subjects (n=65) were interviewed with the Spanish adaptation (Leal et al., 1988) of the Family History-Research Diagnostic Criteria (FH-RDC; Endicott et al., 1978) interview, and with the psychotic screening questionnaire of Bebbington and Nayani (1995) to confirm the absence of any family and personal history of psychiatric disorders, respectively.

Neuropsychological and clinical assessment

This assessment was performed according to Tabarés-Seisdedos et al. (2003) and Balanzá-Martínez et al. (2005). Briefly, all patients and the 25 control subjects completed a battery of 11 tests measuring eight cognitive domains with the following sequence: executive functions-abstraction (Wisconsin Card Sorting Test, WCST with four separate scores: Categories, Perseverative Errors, Non-Perseverative Errors and Total Errors), semantic and phonologic verbal fluency (Category Instant Generation Test, CIG; FAS Test from the Controlled Oral Word Association Test), working memory (the backward part of Digit Span Test from the Wechsler Adult Intelligence Scale–Revised or WAIS-R), verbal memory (VM) (the Babcock Story Recall Test), visual memory (the Rey-Osterrieth Complex Figure Test), visual-motor processing/attention (the Trail Making Test, parts A and B; the Stroop Color and

Table 1. PCR conditions

Tm	Markers
54 °C	ALFA1
58 °C	HLIS1, LIS1
60 °C	WI-7771, SHGC-74513, RH104513, GDB: 455460, D17S1566, D17S22, HLIS3, HLIS5, HLIS7, HLIS8
61 °C	D17S850E, RHI2602, WI-15104, D17S1267, RH18271, RH46116, A002039, G19939, RH70243, RH65106
62 °C	D17S379, RH71329, NIB1825, STS-AA011180, PTAFR, HLIS2, HLIS4, HLIS6, ALFA2

Thermal cycling amplification conditions for all markers consisted of an initial denaturing at 94 °C for 2 minutes, followed by 35 cycles at 94 °C for 45 s, annealing at a specific temperature (Tm) for each marker for 1 min, extension at 72 °C for 1 min; and finally an elongation step at 72 °C for 10 min.

Word Test with three separate tasks: Word reading trial, Color naming trial and Color-Word Interference trial; the Digit Symbol Substitution Test, DSST from the WAIS-R), sustained attention (from the Asarnow continuous performance test, the total number of hits minus the total number of false alarms was calculated) and motor speed (finger-tapping test). The test sequence was consistent for all subjects, and was administered on the same day the subjects were assessed by the clinical scales, and their blood was extracted for genetic analysis. The total time in which the neuropsychological battery was completed was approximately 90 min, divided by one or more pauses, as required. Patients were assessed while clinically stable. The Vocabulary Subtest from the WAIS-R was employed as an estimate of premorbid intelligence quotient (IQ). On the same day and for all patients, psychotic and other psychiatric symptoms were rated according to the Positive and Negative Syndrome Scale (PANSS; Peralta and Cuesta, 1994), depressive symptoms with the Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960), and to the manic symptoms with the Clinician-Administered Rating Scale for Mania factor 1 (CARS-M; Altman et al., 1994).

Molecular genetic analysis

All subjects were processed together.

PCR (polymerase chain reaction). In all cases, PCR was performed in a total volume of 15 μl containing 40–100 ng of DNA (genomic isolated from 200 μl of whole blood by a Qiagen kit protocol, QIAamp DNA Blood Mini Kit; Qiagen, Stanford, CA, USA), or cDNA obtained by reverse transcription from mRNA extracted from lymphocytes of 200 μl of whole blood using the TRIZOL protocol (TRIZOL reagent, Gibco BRL, U.S. patent no. 5.346.994), 12.5 pM of each primer, 125 μM of each dNTP and 0.5 U of *Taq* polymerase (Sigma Genomic *Taq* polymerase), in a buffer composed of 50 mM KCl, 2 mM$MgCl_2$, and 10 mM Tris. Reactions were repeated at least five times with each primer. The PCR conditions are found in Table 1.

Genomic regions. We studied a genomic region of 635 kilobases (kb) located in a critical region for lissencephaly (Cardoso et al., 2003; Fig. 1) in 17p13.3, including the *LIS1* gene (NT_010718.15). In addition, we studied the *PAFAH1B2* (NT_033899.7), *PAFAH1B3* (NT_011109.15) and *PTAFR* (NT_037485.3) genes. More specifically, we have analyzed four specific *LIS1* intragenic markers (Fig. 2A): D17S850E (UniSTS: 3456; Olivier et al., 2001); RH12602 (UniSTS: 41394; Agarwala et al., 2000); WI-15114 (UniSTS: 25888; Agarwala et al., 2000); and D17S1267 (UniSTS: 149804; Agarwala et al., 2000). In addition, we studied three *LIS1* related micro satellites, since they have been involved in the diagnosis of lissencephaly (Mutchinick et al., 1999; Pilz et al., 1998; Honda et al., 1998; Kohler et al., 1995; Batanian et al., 1990; Schwartz et al., 1990; vanTuinen et al., 1988). These markers were D17S1566, D17S22 and D17S379 (Fig. 2B; see Table 2 for details).

D17S1566 (UniSTS: 151857; Tsuchiya et al., 2000): is centromeric to *LIS1* and is located at the *T53* (*p53*) gene on chromosome 17p13.1 (GeneID: 7157; MIM: 191170), which plays an essential role in cell cycle regulation, specifically in the transition from G0 to G1. *p53* Is a tumor suppressor gene (Toh et al., 2005), which has been described as a risk-gene for schizophrenia (Chiu et al., 2001; Papiol et al., 2004; Ni et al., 2005).

D17S22, also known as D17S5 and D17S30 (UniSTS: 146906; Shinya et al., 2004), located at the *C17orf31* gene on chromosome 17p13.3 (GeneID: 23293), and telomeric to *LIS1*, has also been involved in neural tumors (Makos et al., 1993; Saxena et al., 1993).

D17S379: (UniSTS: 146907; Fain, personal communication, 1991) located at the *MNT* gene on chromosome 17p13.3 (GeneID: 4335; MIM: 603039), is telomeric to *LIS1*. This gene belongs to a group of transcription factors that co-interact to regulate gene-specific transcriptional activation or the repression of cell growth (Smith et al., 2004).

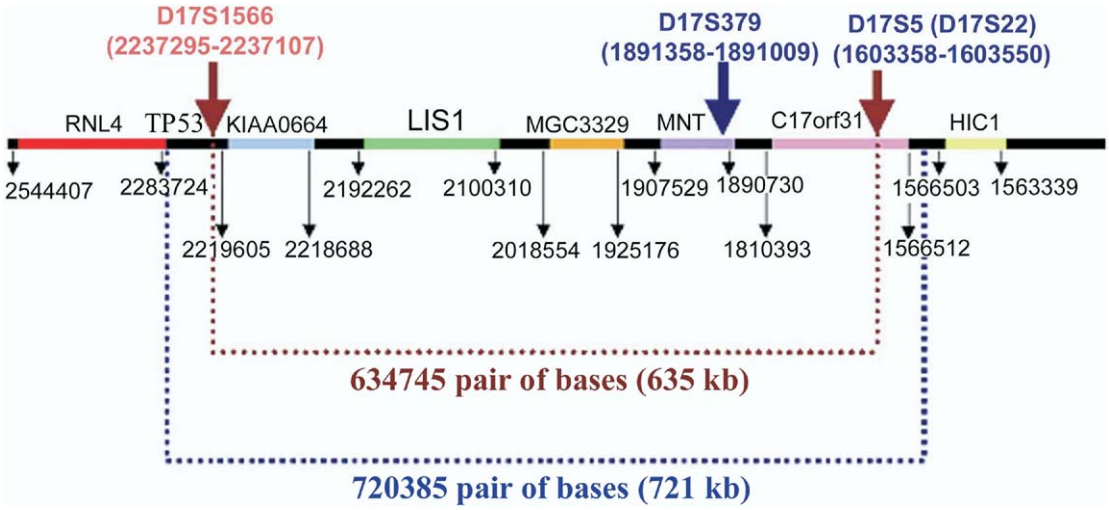

Fig. 1. Scheme of LCR located on chromosome 17p13.3. It represents a 635 kb genomic region located in a critical region for lissencephaly in 17p13.3, including the *LIS1* (NT_010718.15) gene. Localization of markers D17S1566, D17S22 and D17S379 is also represented in this scheme.

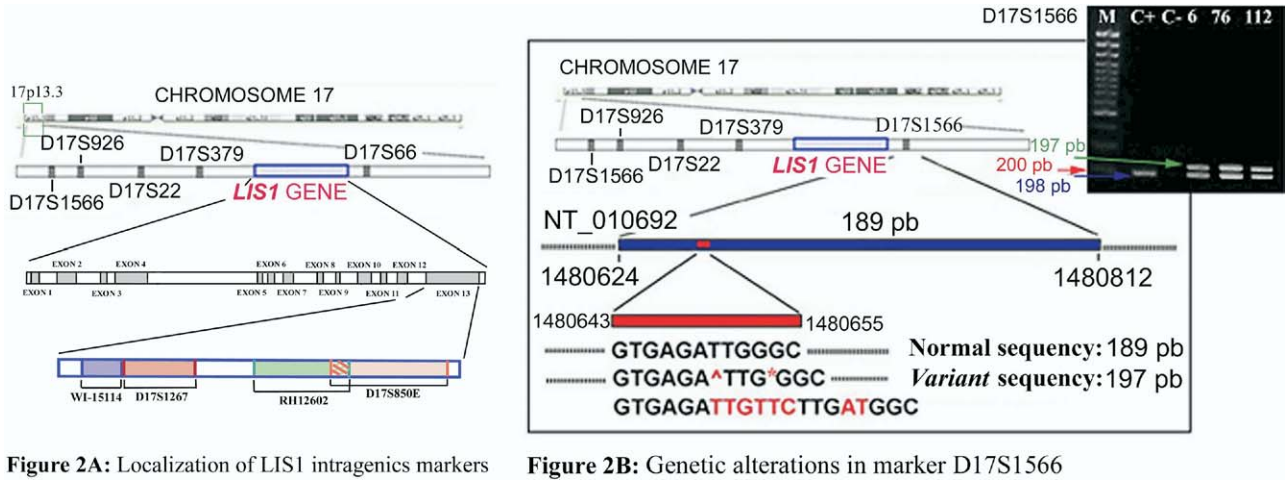

Figure 2A: Localization of LIS1 intragenics markers

Figure 2B: Genetic alterations in marker D17S1566

Fig. 2. Localization of marker D17S1566 on chromosome 17p13.3 (Fig. 2A). Genetic variations detected in patients (Fig. 2B). We detected three bipolar patients (8.3%) with an extra band of 197 bp for marker D17S1566. The fragment sequence contained an insertion of eight bases between bases 166 and 167 (TTGTTCTTGAT).

The *PAFAH1B2* gene (platelet-activating factor acetylhydrolase, isoform Ib, catalytic subunit, 29 kDa, GeneID: 5049 locus tag: HGNC: 8575; MIM: 602508) was located on chromosome 11q23. We analyzed six intragenic markers for its genomic region: RH18271 (UniSTS:17854), RH46116 (UniSTS:42861), A002C39 (UniSTS:60780), G19939 (UniSTS:60781), RH70243 (UniSTS:64354) and RH65106 (UniSTS:91213). The *PAFAH1B3* gene (platelet-activating factor acetylhydrolase, isoform Ib, catalytic subunit, 30 kDa; GeneID: 5050 locus tag: HGNC:8576; MIM: 603074) was located on chromosome 19q13.1, and we studied its genomic region by analyzing three intragenic markers: RH71329 (UniSTS:32311), NIB1825 (UniSTS: 59554) and STS-AA011180 (UniSTS:61055).

The *PTAFR* gene is located on chromosome 1p35-p34.3 and it codifies the PAF receptor, with a seven-transmembrane domain structure belonging to the G-protein-coupled receptor superfamily (GeneID: 5724 locus tag: HGNC:9582; MIM: 173393). The *PTAFR* genomic region was analyzed using five intragenic markers: WI-7771 (UniSTS:19562, G06696 S52624), SHGC-74513 (UniSTS:50081, D31736), RH104513 (UniSTS:98813), GDB:455460 (UniSTS: 157464) and PTAFR (see Table 2 for details).

Coding regions. We proceeded to investigate whether there were mRNA splicing genetic variations in the *LIS1*, *PAFAH1B2*, *PAFAH1B3* and *PTAFR* genes by studying their coding sequences by RT-PCR, using appropriate primers that were designed according to the reported nucleotide sequence (NM_000430, *LIS1* coding region; NM_002572, *PAFAH1B2* coding region; NM_002573, *PAFAH1B3* coding region and NM_000952, *PTAFR* coding region; see Table 2).

Sequencing. The fragments obtained were inserted in pGEM plasmid, and were then cloned. Four clones from each patient and control sample were sequenced (pGEM®-T Easy Vector Systems, Promega Corporation, Madison, WI, USA). In the case of PTAFR, four different amplifications from each patient and control sample were performed, and genomic DNA from the individuals concerned was directly sequenced (without subcloning).

Trained specialists who were blind to both the participant's diagnosis and the research hypothesis conducted the cognitive and molecular analyses.

Statistical analyses

Hardy-Weinberg analyses were performed using the HWE Program (Ott, 2003). Differences in the markers distribution were calculated by the Fisher's exact test (Monte Carlo test). The normality of the continuous variables was verified by the Kolmogorov-Smirnov test. The effect of genetic variations in both the LCR and the PAF system genes on cognitive tests was assessed by four case-control analyses: (*i*) one-way variance analyses (ANOVAs with Bonferroni post hoc correction) on each separate test variable. (*ii*) Covariance analyses (ANCOVAs) were carried out on dependent variables from each neuropsychological test. The factors used were diagnosis, gender and genetic variations groups for both patients and controls, whereas the covariates were age, years of education and IQ. (*iii*) Multiple regressions to test the hypothesis that genetic variations in both the LCR and the PAF system genes were associated with a poor performance in executive prefrontal tests. (*iv*) A logistic regression analysis was performed to investigate which variable was the strongest independent predictor of genetic group membership, by comparing patients with positive genetic variations versus those with negative genetic variations, and by entering all the neuropsychological variables into the equation. The analysis included the following variables to control the influence of other variables: diagnosis, sex, age, years of education, IQ, age of onset, number of admissions, hand dominance, length of illness, PANSS-positive, PANSS-negative, PANSS-general, chlorpromazine equivalents (CPZ-units) and biperiden dose (mg). All collected data were processed by the SPSS statistical package, version 11.5 for Windows.

RESULTS

In comparison with bipolar disorder patients, patients with schizophrenia were significantly younger (mean age$\pm$sd$=$34.8$\pm$7.8; 40.9$\pm$11.1 years, respectively), were younger when the onset of the disorder took place (mean age at onset$\pm$sd$=$25.7$\pm$6.7 and 29.1$\pm$9.9, respectively), and they showed more positive (mean PANSS-positive$\pm$sd$=$13.6$\pm$5.5 and 9.6$\pm$4.7, respectively) and negative (mean PANSS-negative$\pm$sd$=$21.5$\pm$8.6 and 10.3$\pm$4.1, respectively) symptoms. No differences were found in the remaining variables studied: gender, years of education, IQ, number of admissions and hand dominance. None of the bipolar patients showed a rapid-cycling illness.

Molecular genetic analyses

Genetic variations in two markers in the LCR have been detected in the analyzed population of patients but not in

Table 2. *LIS1* and *PTAFR* markers

Marker	GenBank accession number	Position (bp)	Primers	PCR product size (bp)
LIS1 gene				
D17S850E	UniSTS:3456 M78468	2795104–2795301	F-primer: 5′-AACTGTTTTCCTCAATTACCACTTC-3′ R-primer: 5′-GCCTTTTTATCTTCCCACTGTATC-3′	197
RH12602	UniSTS:41394	2794600–2794768	F-primer: 5′-GAAACAGAAAGAGGCGAACG-3′ R-primer: 5′-TGTGACCAATGGTGTGCC-3′	169
WI-15114	UniSTS:25888 G24667 H29572	2792533–2792682	F-primer: 5′-CAGATTCATGTTGAAAAACAAACT-3′ R-primer: 5′-TGTGTCATGGTACAAATCACTATTC-3′	150
D17S1267	UniSTS:149804	2792624–2792747	F-primer: 5′-GCACGCTCCATTAAACCCTG-3′ R-primer: 5′-GTGTGCCCATTTGAAACGAGTG-3′	120
D17S1566	UniSTS:151857	2840324–2840512	F-primer: 5′-AAAGATCCTTATTGCCACTTTACTG-3′ R-primer: 5′-CTCTTACCTTGCTGGTGAGATTG-3′	179
D17S22	UniSTS:146906	2206575–2206767	F-primer: 5′-GCCTACCTTCCACAAATCTTTC-3′ R-primer: 5′-TTGCTGGAGGGATACCTGTGTAC-3′	194
D17S379	UniSTS:146907	2632272–2632430	F-primer: 5′-GTTGGAACAGAACTATGAATAAC-3′ R-primer: 5′-CCTAACTGAATGACATGGAGGAC-3′	160
HLIS1	Coding region	1627–1866	F-primer: 5′-GAT GAC AAG ACC CTA CGC GT-3′ R-primer: 5′-AGA GCT CAA TGG GGT AAC CA-3′	250
HLIS2	Coding region	565–1345	F-primer: 5′-TCC CAG AGA CAA CGA GAT GA-3′ R-primer: 5′-CTA CGA CCC ATA CAC GCA CA-3′	781
HLIS3	Coding region	1015–1760	F-primer: 5′-CAG GAC ATT TCA TTC GAC CA-3′ R-primer: 5′-TGA TCT ACG CTG CCA GTG AC-3′	746
HLIS4	Coding region	1848–2050	F-primer: 5′-GGT TAC CCC ATT GAG CTC TG-3′ R-primer: 5′-TTC TTA GGC CTG GTG TGA CC-3′	203
HLIS5	Coding region	2839–3037	F-primer: 5′-TGG AGC GTG CAT AAA AAT GT-3′ R-primer: 5′-CTA AAG CAT GGC ATT CCA CA-3′	199
HLIS6	Coding region	2945–3131	F-primer: 5′-CAC ATC AAC CTC CAT GTC CTT-3′ R-primer: 5′-GAC GGG GAC ATA ACG GAA GT-3′	190
HLIS7	Coding region	4245–4443	F-primer: 5′-TTG CAA AAT TTC CTG CAC TG-3′ R-primer: 5′-TGA CAA AGC AGC AGT GAA CC-3′	190
HLIS8	Coding region	4343–4548	F-primer: 5′-GCC TGG GAT AAG GAC AAT GA-3′ R-primer: 5′-ATA TTT GGG TGG CAC TGG AA-3′	206
LIS1	Coding region	1335–1866	F-primer: 5′-ATG GGT CGT AGC AAC AAG G-3′ R-primer: 5′-AGA GTC CAA TGG GGT AAC CA-3′	498
PTAFR gene				
WI-7771	UniSTS:19562 G06696 S52624	28080879–28081180	F-primer 5′-ATGCCTGAAGTCTTCTCCTCC-3′ R-primer 5′-AAGTCTTCCCCCAAGGTCTG-3′	302–303
SHGC-74513	UniSTS:50081 D31736	28107861–28108026	F-primer 5′-GCTCTTTGAGAAGCCTCTGT-3′ R-primer 5′-CCTCTATGCTGTCTGGCAAT-3′	164
RH104513	UniSTS:98813	28081354–28082036	F-primer 5′-CGGACATGCTCTTCTTGATCA-3′ R-primer 5′-GTCTAAGACACAGTTGGTGCTA-3′	682
GDB:455460	UniSTS:157464	28080955–28082253	F-primer 5′-ACCAGGACCAGCTGATCATTC-3′ R-primer 5′-CATCAGTCACAGTTACTGTA-3′	1300
PTAFR	Genomic region Coding region	2715214–2714683 632–1164	F-primer 5′-AGC AGG GAC TAA TTT TTG AGG-3′ R-primer 5′-AAC GTC ACT CGC TGC TTT G-3′	533

Markers used in genomic and coding regions of the *LIS1* (and related regions) and *PTAFR* genes, their position on chromosome 17p13.3 and 1p35-34.3, respectively, accession numbers, primers and PCR product sizes.

controls. Specifically, we detected three bipolar patients (8.3%) with an extra band of 197 bp for marker D17S1566. The sequence of the fragment contained an insertion of eight bases between bases 166 and 167 (Fig. 2B). In addition, an amplification of an extra band of 199 bp for marker D17S22 was seen in 4 schizophrenic (7.8%) and two bipolar patients (5.5%). This fragment contained an insertion of five bases (GTTTC or CCTTG or CGTCT) in a region of 33 bp between bases 65–98 (Fig. 3). Therefore, these genetic variations in these two markers were observed in heterozygous state.

The coding regions of the *LIS1*, *PAFAH1B2*, and *PAFAH1B3* genes showed no genetic variation by RT-PCR. However, four schizophrenic patients (7.8%) were negative for the coding sequence amplification of the *PTAFR* gene by RT-PCR. A detailed analysis revealed several genetic variations within this region for each patient (Fig. 4). We analyzed the genomic region with the

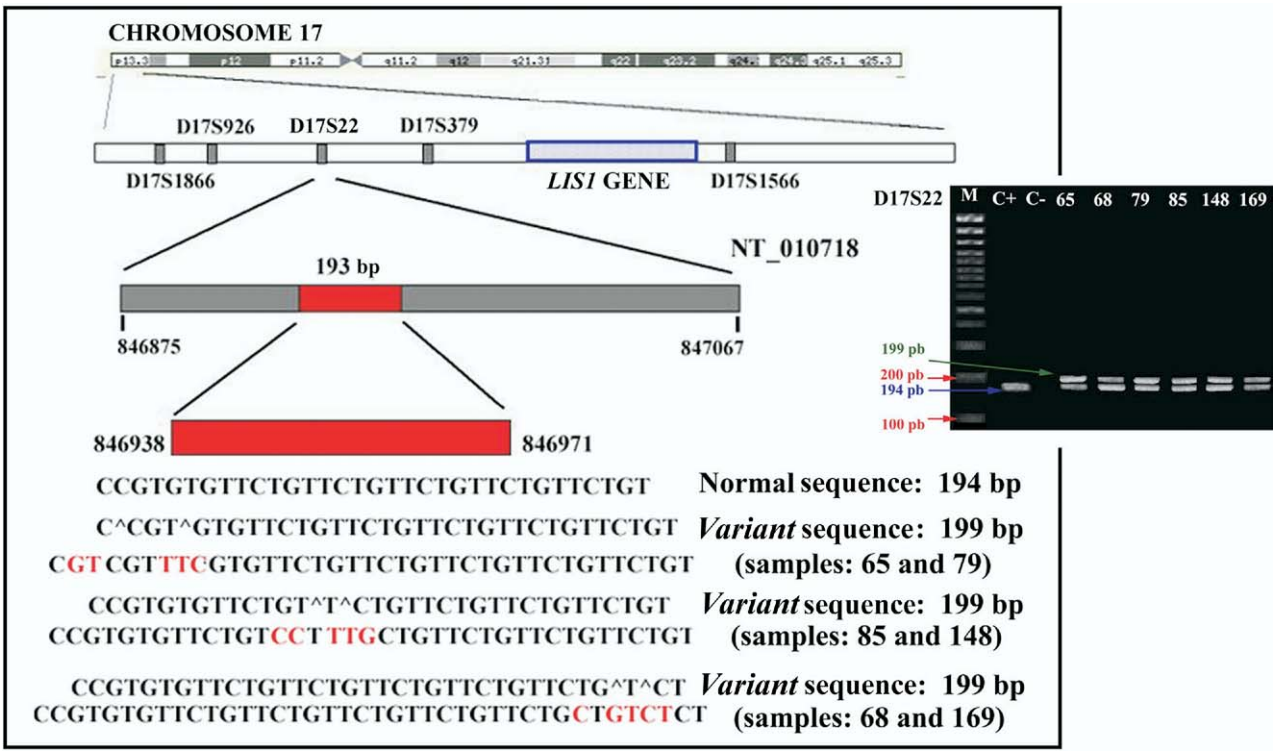

Fig. 3. Localization of marker D17S122 on chromosome 17p13.3. Genetic variations detected in four schizophrenic (7.8%) and two bipolar patients (5.5%). An extra band of 199 bp for marker D17S22 was shown. This fragment contained an insertion of five bases (GTTTC or CCTTG or CGTCT) in a region of 33 bp between bases 65–98.

same primers (we amplified, and directly sequenced, without subcloning, genomic DNA from the individuals concerned), and we detected variations in this genomic sequence (Table 3). All the observed variations resulted in a change of the deduced amino acid sequence (Table 4).

In addition to the genes being directly affected by the observed variations, the modifications in the expression of adjacent genes might possibly generate functional alterations, thus influencing neuronal development.

The control and patient genotypes were at a Hardy-Weinberg equilibrium. A comparison of the schizophrenic and bipolar patients with the normal control subjects revealed significant differences for the marker D17S1566 (Fisher's exact test=6.362; 99% CI: 0.008–0.013; $P<0.011$), and also

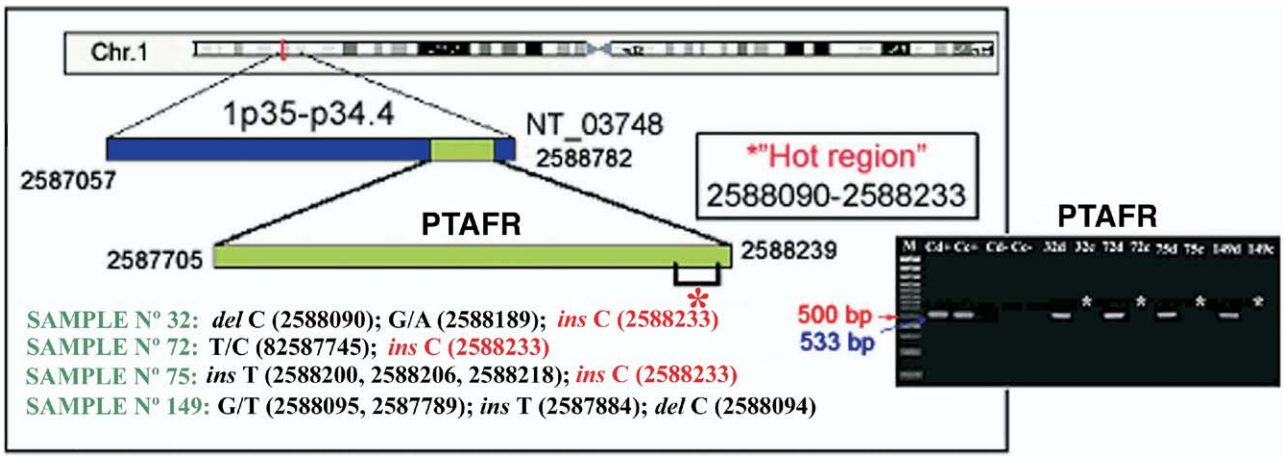

Fig. 4. Localization of marker PTAFR on chromosome 1p35-p34.4. Genetic variations detected in patients 32, 72, 75 and 149. These four schizophrenic patients (7.8%) were negative for the coding sequence amplification of the *PTAFR* by RT-PCR. A detailed analysis revealed several genetic variations in the same region (base 757–1289 of the *PTAFR* gene) for each patient (see Table 2 for details). In the case of samples 32, 72 and 149, genetic variations are located at the *PTAFR* coding region (bases 128–1156), while sample 75 genetic variations are outside this region. Variations in the genomic region change the protein product in each case.

Table 3. Genetic variations in the *PTAFR* genomic sequence

Case	Alteration	Localization on genomic region 1p35-34.4
Case 1 (sample 32)	*del* C	Base 1142
	G/A	1241
	ins C	1285
Case 2 (sample 72)	T/C	797
	ins C	1285
Case 3 (sample 75)	*ins* T	1252
	ins T	1258
	ins T	1270
	ins C	1285
Case 4 (sample 149)	*del* T	819
	G/T	841
	ins T	1046
	del C	1146
	G/T	1147

Insertions detected in genomic *PTAFR* gene sequence of four patients.

for the *PTAFR* gene (Fisher's exact test=5.777; 99% CI: 0.015–0.021; $P<0.019$). Moreover, the patient group showed a greater tendency toward genetic variations in the marker D17S22 (Fisher's exact test=5.302; 99% CI: 0.053–0.065; $P<0.059$) than the control group. A comparison of schizophrenic patients with normal controls revealed significant differences for the marker D17S22 (Fisher's exact test; $P<0.037$), and also for genetic variations in *PTAFR* gene (Fisher's exact test; $P<0.037$). Significant differences were only revealed with the D17S1566 marker (Fisher's exact test; $P<0.018$) when bipolar patients were compared with the control group. No significant differences were found between schizophrenic and bipolar patients.

Effect of LCR/PTAFR variations on cognition

The main demographic and clinical characteristics of the patients grouped either by the presence or absence of genetic variations, and control subjects, are described in Table 5. Briefly, the two groups of patients did not differ in the majority of the variables studied: diagnosis, gender, age of onset, years of education, IQ, length of illness, number of admissions, hand dominance, CPZ-units, biperiden and lithium dose (mg), PANSS-positive and -negative, HRSD and CARS-M factor 1. However, the healthy group had a significantly higher IQ and more years of education than the two groups of patients.

As expected, patients displayed a reduced performance in comparison to control subjects in all the neuropsychological tests (see Table 6). Post hoc and ANCOVA analyses showed that there was a significant difference among control, positive and negative LCR/*PTAFR* subgroups only in the Wisconsin Card Sorting Test-Perseverative Errors (WCST-PE). Perseveration in this test is one of the best indicators of prefrontal-executive dysfunctions. Higher scores from this test indicate reduced performance. Patients with LCR/*PTAFR* genetic variations displayed a significantly reduced functioning in comparison to normal controls, while patients without LCR/*PTAFR* genetic variations were at an intermediate level among these groups (see Table 6 and Fig. 5 for details).

By using a stepwise multiple regression analysis, the presence of LCR/*PTAFR* genetic variations was parametrically related to perseverative errors (adjusted $r^2=0.17$, $t=4.9$, $P<0.0001$). Thus, LCR/*PTAFR* variations accounted for 17% of the variance in the WCST-PE performance. This effect was independent of the other variables used for analysis

Table 4. Sequence of normal and mutant PTAFR proteins

Normal protein has 342 amino acids and its sequence is: MEPHDSSHMD SEFRYTLFPI VYSIIFVLGV IANGYVLWVF ARLYPCKKFN EIKIFMVNLT MADMLFLITL PLWIVYYQNQ GNWILPKFLC NVAGCLFFIN TYCSVAFLGV ITYNRFQAVT RPIKTAQANT RKRGISLSLV IWVAIVGAAS YFLILDSTNT VPDSAGSGNV TRCFEHYEKG SVPVLIIHIF IVFSFFLVFL IILFCNLVII RTLLMQPVQQ QRNAEVKRRA LWMVCTVLAV FIICFVPHHV VQLPWTLAEL GFQDSKFHQA INDAHQVTLC LLSTNCVLDP VIYCFLTKKF RKHLTEKFYS MRSSRKCSRA TTDTVTEVVV PFNQIPGNSL KN

Protein product from sample 32 is a shorter protein, with 312 aa, and Cys (C) at position 173, and it is changed by Trp (W): MEPHDSSHMD SEFRYTLFPI VYSIIFVLGV IANGYVLWVF ARLYPCKKFN EIKIFMVNLT MADMLFLITL PLWIVYYQNQ GNWILPKFLC NVAGCLFFIN TYCSV AFLGV ITYNRFQAVT RPIKTAQANT RKRGISLSLV IWVAIVGAAS YFLILDSTNT VPDSAGSGNV TRWFEHYEKG SVP IRTLLMQ PVQQQRNAEV KRRALWMVCT VLAVFIICFV PHHVVQLPWT LAELGFQDSK FHQAINDAHQ VTLCLLSTNC VLDPVIYCFL TKKFRKHLTE KEYSMRSSRK CSRATTDTVT EVVVPFNQIP GN

Protein product from sample 72 is a shorter protein with 308 aa. Cys (C) at position 173, which is changed by Trp (W). Asn at position 307 is changed by Ser (S): MEPHDSSHMD SEFRYTLEPI VYSIIFVLGV LANGYVLWVF ARLYPCKKFN EIKIFMVNLT MADMLFLITL PLWTVYYQNQ GNWILPKFLC NVAGCLFFIN TYCSVAFLGV ITYNRFQAVT RPIKTAQANT RKRGISLSLV IWVAIVGAAS YFLILDSTNT VPDSAGSGNV TRWFEHYEKG SVPIRTLLMQ PVQQQRNAEV KRRALWMVCT VLAVFIICFV PHHVVQLPWT LAELGPQDSK FHQAINDAHO VTLCLLSTNC VLDPVIYCFLTK KFRKHLTEKF YSMRSSRKCS RATTDTVTEV VVPFS Q

Protein product from sample 75 is a shorter protein with 310 aa. Cys (C) at position 173, and it is changed by Trp (W). His (H) of 176 is changed by Ala (A), while Tyr (Y) at 177 is changed by Leu (L), and Arg (R) appears at position 178: MEPHDSSHMD SEFRYTLFPI VYSIIFVLGV IANGYVLWVF ARLYPCKKFN EIKIFMVNLT MADMLFLITL PLWIVYYQNQ GNWILPKFLC NVAGCLFFIN TYCSVAFLGV ITYNRFQAVT RPIKTAQANT RKRGISLSLV IWVAIVGAAS YFLILDSTNT VPDSAGSGNV TRWFEALRER ASVP–IRTLLM QPVQQQRNAE VKRRALWMVC TVLAVFIICF VPHHVVQLPW TLAELGFQDS KFHQAINDAH QVTLCLLSTN CVLDPVIYCF LTKKFRKHLT EKFYSMRSSR KCSRATTDTV TEVVVPFNQI

Protein product from sample 149 is a shorter protein with 333 aa. Cys (C) at 173 position, and it is changed by Trp (W); His (H) at positions 250 and 251 are changed by Thr (T) and Asn (N), respectively: MEPHDSSHMD SEFRYTLFPI VYSIIFVLGV IANGYVLWVF ARLYPCKKFN EIKIFMVNLT MADMLFLITL PLWTVYYQNQ GNWILPKFLC NVAGCLFFIN TYCSVAFLGV ITYNRFQAVT RPIKTAQANT RKRGISLSLV IWVAIVGAAS YFLILDSTNT VPDSAGSGNV TRWFEHYEKG SVPVLIIHIF IVFSFFL VFL IILFCNLVII RTLLMQPVQQ-RNAEV–RAL WMVCTVLAVF IICFV-HPTN VVQLPWTLAE LGFQDSKFHQ AINDAHQVTL CLLSTNCVLD PVIYCFLTKK FRKHLTEKFY SMRSSRK-SR ATTDTVTEVV VPFNQIP

Underlining indicates the altered sequence of the predicted mutant proteins.

Table 5. Socio-demographic and clinical characteristics of patients grouped by the presence or absence of variations in LCR/*PTAFR* genes, and normal control subjects

	Positive LCR/*PTAFR*	Negative LCR/*PTAFR*	Normal controls		
	n (%)	*n* (%)	*n* (%)	χ^2	*P*
Diagnosis					
Schizophrenia	8 (61.5%)	44 (58.7%)			
Bipolar disorder type 1	5 (38.5%)	31 (41.3%)		0.4	0.5
Gender					
Female	7 (53.8%)	27 (36%)	28 (43.1%)		
Male	6 (46.2%)	48 (64%)	37 (56.9%)	1.8	0.4
	x (s.d.)	*x* (s.d.)	*x* (s.d.)	F^a/t^b	*P*
Age	41.1 (10.3)	37.0 (9.9)	41.4 (11.3)	2.2	0.11
Age of onset (years)	31.2 (9.5)	26.3 (7.7)		−1.4	0.2
Years of education	9.4 (4.3)	10.1 (3.5)	14.3 (4.4)	12.8	0.000
IQ	41.7 (18.1)	46.1 (14.1)	62.6 (10.2)	15.3	0.000
Length of illness (years)	11.4 (10.2)	10.1 (7.8)		−0.4	0.7
Number of admissions	1.5 (1.9)	1.1 (1.7)		−0.9	0.4
Hand dominance	4.5 (2.6)	4.7 (1.4)	4.3 (2.2)	0.3	0.7
Chlorpromazine equivalents (mg)	604.8 (606.3)	611.9 (672.6)		−0.004	0.99
Biperiden (mg)	0.3 (1.1)	0.8 (3.5)		0.5	0.6
Lithium (mg)	246.1 (477.2)	223.4 (399.9)		−0.2	0.8
PANSS-negative	17.2 (9.9)	16.2 (8.8)		−0.3	0.7
PANSS-positive	13.7 (8.5)	11.5 (4.8)		−1.4	0.2
HRSD	5.0 (4.1)	5.7 (5.0)		0.4	0.6
CARS-M factor 1	2.1 (5.1)	1.2 (2.7)		−0.9	0.4

[a] Post hoc Bonferroni tests by three groups.
[b] *t*-Test by two subgroups of patients.

purposes (diagnosis, gender and age). These results suggest that the presence of LCR/*PTAFR* genetic variations exerts its most robust effects on some prefrontal executive functions, and specifically on cognitive inflexibility.

Finally, logistic regression showed that WCST-PE was the strongest independent predictor of LCR/*PTAFR* group membership. Specifically, logistic regression revealed that an increase in the WCST-PE score (β=0.062; E.T.=0.025; Wald=6.1; *df*=1; 95% CI=1.013–1.118; $P<0.014$; Exp (β)=1.064) increased the probability of belonging to the positive LCR/*PTAFR* group (Chi-square model=7.48; *df*=1; $P<0.006$).

In general, the molecular and cognitive results therefore suggest that the LCR/*PTAFR* genetic variations associated with schizophrenia and bipolar I disorder, are also strongly associated with a reduced prefrontal executive function.

DISCUSSION

Our analysis revealed a subgroup of schizophrenic and bipolar disorder type I patients with genetic variations in the LCR region of chromosome 17p, which is implicated in lissencephaly sequence and in the Miller-Dieker syndrome (Toyo-oka et al., 2003), and is a risk for schizophrenia (*p53* gene; Chiu et al., 2001; Papiol et al., 2004; Ni et al., 2005).

Moreover, schizophrenic subjects also showed genetic variations in the *PTAFR* coding region in chromosome 1p. Several authors have hypothesized that the PAF system might be implicated in the etiology of schizophrenia (Oken and Schulzer, 1999). In fact, the possible association of polymorphisms in the LDL-PLA_2 gene (encoding plasma PAF acetylhydrolase, PAFAH) on chromosome 6p12–21.1 with schizophrenia was examined, with no positive results (Bell et al., 1997; Ohtsuki et al., 2002).

Our findings support the existence of a physiopathological relation between genetic alterations of neuronal migration in the cortex and functional psychosis, thus extending the spectrum of clinical manifestations of the lissencephalic sequence. Recently, a molecular complex of Reelin, LIS1, DISC1-1 and Ndel1 was shown to be involved in the neuronal migration (Ross and Walsh, 2001; Ozeki et al., 2003; Assadi et al., 2003; Brandon et al., 2004). The down-regulation of the Reelin expression may contribute to the cortical neuropil hypoplasia found in the cortex of patients with schizophrenia and bipolar disorder (Shu et al., 2004; Tanaka et al., 2004; Ozeki et al., 2003; Tarricone et al., 2004). Although there is still lacking confirmatory genomic data to establish the relation between *Reelin* gene and predisposition for psychotic disorder, several studies suggested that *Reelin* promoter hypermethylation is associated with the etiology of schizophrenia (Grayson et al., 2005).

A synergistic effect on neuronal migration was suggested for the possible genetic interaction between *PTAFR* and *LIS1* (Tokuoka et al., 2003). Moreover, the interactions between LIS1 and the PAF-AH catalytic subunits are mutually exclusive with the interactions of LIS1 and Ndel1,

Table 6. Neuropsychological test results for patients with schizophrenia and bipolar disorder, grouped by the presence or absence of variations in LCR/*PTAFR* genes, and normal control subjects

	Positive LCR/*PTAFR*	Negative LCR/*PTAFR*	Normal controls	ANCOVA results	
	x (s.d.)	*x* (s.d.)	*x* (s.d.)	F^a	P^a
WCST					
WCST-Categories	3.6 (2.2)	4.3 (2.1)	5.8 (1.0)	2.5	0.6[e]
WCST-NPE	19.5 (13.6)	22.8 (20.5)	10.3 (13.3)	1.0	0.3[c]
WCST-PE	33.0 (26.6)	17.9 (13.0)	8.8 (4.8)	8.4	0.005
WCST-TE	52.5 (31.8)	40.7 (29.6)	19.4 (17.5)	0.5	0.5[e,f]
Verbal fluency					
CIG	45.4 (11.0)	50.0 (12.1)	63.9 (12.6)	0.7	0.4[e]
FAS	26.6 (15.8)	31.5 (16.4)	50.9 (14.8)	2.3	0.1[e]
Verbal memory					
WMS immediate	9.0 (3.2)	9.6 (3.4)	12.2 (3.1)	0.0	0.9[d,e]
WMS delayed	9.9 (4.5)	12.9 (4.5)	16.1 (3.4)	0.0	0.9[d,e]
Visual memory					
Figure Rey immediate	11.9 (6.5)	15.0 (7.0)	21.2 (7.9)	0.6	0.4[b,f]
Figure Rey delayed	11.7 (7.2)	14.9 (6.9)	21.8 (7.3)	1.1	0.3[b]
Working memory					
Backward Digit Span	3.8 (0.8)	4.1 (1.3)	4.8 (1.1)	1.0	0.3[e]
Visual-motor processing/attention					
Trail Making A	69.1 (30.4)	53.4 (25.4)	39.4 (14.9)	2.1	0.1[e,f]
Trail Making B	145.2 (66.0)	132.6 (65.4)	92.3 (54.7)	0.7	0.4[d,e,f]
Stroop 1 (Word Reading)	26.8 (9.0)	24.7 (7.9)	18.7 (3.7)	0.1	0.7[e,f]
Stroop 2 (Color Naming)	34.1 (6.9)	32.1 (9.5)	24.1 (4.5)	0.3	0.6[e,f]
Stroop 3 (Word-Color Int.)	88.4 (35.9)	65.3 (23.2)	48.7 (10.1)	2.7	0.1[e,f]
DSST	43.5 (14.7)	47.6 (18.0)	69.8 (16.1)	0.3	0.8[d,e,f]
Sustained attention					
Asarnow CPT	19.7 (4.2)	20.7 (8.7)	23.7 (4.2)	0.0	0.9[e]
Motor activity					
Finger-Tapping unimanual condition					
Right hand	56.2 (22.2)	71.2 (15.4)	89.3 (17.1)	0.1	0.7[b,e,f]
Left hand	45.3 (16.5)	62.2 (16.0)	76.2 (19.6)	0.06	0.8[b,e,f]
Finger-Tapping bimanual condition					
Right hand	50.1 (17.7)	67.6 (16.5)	84.4 (15.8)	0.1	0.7[b,c,f]
Left hand	48.7 (22.2)	60.8 (14.9)	78.6 (15.1)	1.5	0.2[b,e,f]

Higher scores indicate poorer performance, except for: WCST-Categories, CIG, FAS, WMS immediate, WMS delayed, DSST and Finger Tapping Tasks, Asarnow CPT, Figure Rey immediate, Figure Rey delayed, Backward Digit Span Test.

[a] Main effect of LCR/*PTAFR* in ANCOVA.

[b] Main effect of gender ($P<0.05$).

[c] Main effect of diagnosis ($P<0.05$).

[d] Main effect of years of education ($P<0.05$).

[e] Main effect of premorbid IQ ($P<0.05$).

[f] Main effect of age ($P<0.05$).

resulting in striking conformational changes of LIS1 (Tarricone et al., 2004).

Cardoso et al. (2003) have presented evidences of progressive deletions within the LCR, with modifications of the severity in the lissencephalic phenotype. Deletions of the corresponding region in mouse indicated that genes encoded within the *Lis1* flanking regions, particularly in 14-3-3e which interacts with phosphorylated Ndel1, play an important role in neuronal migration (Toyo-oka et al., 2003). DISC1-NUDEL-LIS1 intracellular interaction is necessary to bind the NUDEL-LIS1 complex to the dynein and microtubules (Ozeki et al., 2003; Brandon et al., 2004), thus supporting the relation between neuronal migration disorders and the increasing risk of schizophrenia.

The *p53* gene (*TP53*) was also mapped into the LCR. We have identified new genetic variations of *p53* in bipolar patients, but not in schizophrenics. Since *p53* has been previously reported as being a risk-gene for schizophrenia (Chiu et al., 2001; Papiol et al., 2004; Ni et al., 2005), our data agree with the hypothesis of a common physiopathological mechanism between schizophrenia and bipolar disorders that at least exist in some cases (Crow, 1998).

The second part of our study indicated that genetic variations in both the LCR and *PTAFR* genes affect the efficiency of the prefrontal cognition. This effect on WCST performance was independent of specific psychiatric diagnosis, and it accounted for 17% of the variance in the WCST-PE performance. Perseveration in this test is thought to be one of the best indicators of prefrontal dysfunction (Weinberger et al., 2001). Thus, the present findings suggest possible relations between both the LCR and *PTAFR* genes and the prefrontal cognitive dysfunction. Several authors have supported the

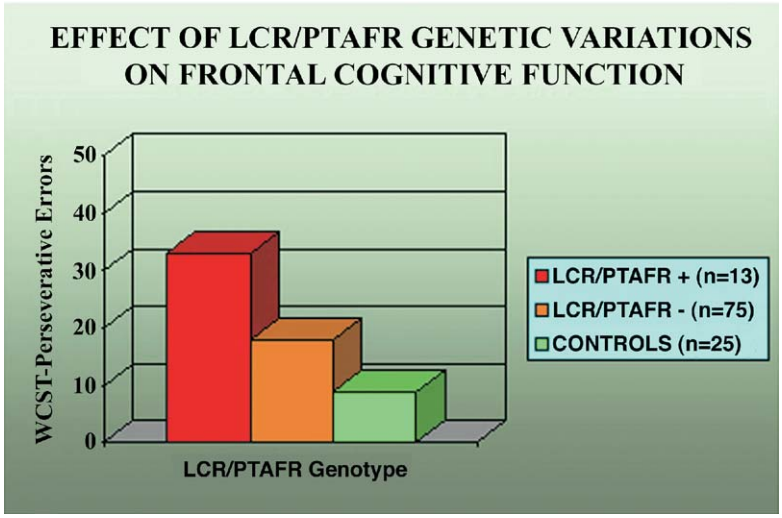

Fig. 5. Effect of LCR/*PTAFR* genetic variations on the prefrontal executive function. WCST-PE for patients with schizophrenia and bipolar disorder, grouped by the presence (LCR/*PTAFR*+) or absence (LCR/*PTAFR*−) of genetic variations in LCR/*PTAFR* genes, and normal control subjects. Higher scores from this test indicate reduced performance. Main effect for the LCR/*PTAFR* group in the WCST-PE ($F=8.4$; $P<0.005$).

view that the GABA-ergic neurons in the prefrontal cortex play a relevant role during executive/working memory tasks, suggesting that reduced GABA neurotransmission is related to executive impairments in schizophrenia (Lewis et al., 2005). Significantly, deficits in neuronal migration observed in lissencephalic cortex may affect interneuron migration, and subsequently the GABAergic function (Pancoast et al., 2005). On the other hand, the prefrontal cognitive dysfunction might also be associated with genetic variations in the *PTAFR* coding region via hypoactive glutamate or/and dopamine neurotransmission (Kato et al., 1994; Xu and Tao, 2004; Chen and Bazan, 1999).

There are two methodological limitations in this study that are worthy of comment. Firstly, the biological function of the LCR genes, *PTAFR* and of other molecules implicated in neuronal migration is currently under investigation. Hence, the complexity of these processes is still far from being entirely understood in the human brain. Secondly, the sample size is relatively small to be absolutely reliable, particularly we recognize this limitation in the number of people with cognitive study in our volunteer control group. Nevertheless, when we performed the same statistical analyses with standardized date from neuropsychological evaluation of the Spanish Speaker population (Ardilla et al, 2004), we also found the same cognitive results.

However, our preliminary results are important as they suggest that heterozygous variations in specific genes for lissencephaly and the *PTAFR* gene, functionally related to neuronal migration, may cause alterations to the cortical development, and by virtue of this mechanism, may affect the efficiency of the prefrontal executive cognition in a limited number of individuals with schizophrenia and bipolar disorder. This study supports some interesting possibilities in developing new animal models of these disorders or, most importantly, it opens novel approaches to treatment (Middleton et al., 2005; Costa et al., 2005). Nevertheless, further studies are required to replicate our findings and to identify their functional consequences.

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