

Further evidence for a possible association between serotonin transporter gene and lithium prophylaxis in mood disorders

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ABSTRACT

We previously reported an association between the functional polymorphism in the upstream regulatory region of the serotonin transporter gene (SERTPR) and the prophylactic efficacy of lithium in a sample of 201 Italian subjects affected by Mood disorders. The aim of the present study was to replicate analyses on an independent sample. In total, 83 subjects affected by Bipolar disorder were recruited in the Mood Disorders Clinic of the Eginition Hospital of the Athens University, Medical School Department of Psychiatry. All patients were administered with lithium as prophylactic therapy and they were prospectively observed for at least 3 years. Subjects were typed for their SERTPR variant using polymerase chain reaction techniques. SERTPR variants were associated with lithium outcome among those subjects who had few manic episodes before lithium treatment and, as a trend, among subjects who received a high daily dose of lithium (≥ 1200 mg/die). In both cases, subjects with the I/I variant showed a higher probability to develop an illness episode within 3 years of prophylactic treatment with lithium. The present study confirmed our previous observation of a better response of SERTPR*I/s carriers, but could not confirm a poor efficacy in subjects with the SERTPR*s/s genotype. Notwithstanding the conflicting results, SERTPR variants are a possible liability factor for lithium long-term efficacy in mood disorders. Further studies on independent and large samples are required to determine the reliability and direction of the possible association between SERTPR variants and lithium outcome.

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INTRODUCTION

Lithium is an effective prophylactic agent in mood disorders but not all patients equally respond to lithium therapy. Clinical predictors account for less than half of the variance,^{1–8} and there are evidences suggesting that genetic factors play a substantial role in lithium prophylaxis effectiveness.^{9–20} A large number of studies reported an association between prophylactic lithium response and a family history of bipolar disorder,^{10,12–14,17} although not unequivocally confirmed.^{21–25} Recently, Grof *et al*²⁶ reported that lithium response in a sample of relatives of responder probands was 67% compared to 30% in the comparison group.

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The mode of action of lithium salts used in the prophylaxis of affective disorders is still unknown. Previous studies^{27–37} evidenced lithium effects on serotonin (5-HT) function at the levels of precursor uptake, synthesis, storage, catabolism, release, receptors and receptor–effector interactions. These actions of lithium may serve to correct 5-HT function abnormalities involved in the pathogenesis of mood disorders.³⁸ However, this view is not univocally accepted because others suggested that the effects of lithium on synaptic transmission and on neuronal excitability appeared independent of changes in endogenous 5-HT.^{39–42}

The serotonin transporter is the major determinant of serotonin inactivation following release at synapses and it is the site of action of most antidepressants. The gene coding for it has been proposed as a possible candidate for involvement in the pathogenesis of major psychoses. A functional polymorphism in the upstream regulatory region of the gene has been associated with both major depressive and bipolar disorders,^{43–45} although subsequent studies did not replicate these results.^{46–58}

The polymorphism in the upstream is a deletion/insertion (SERTPR) located exactly at the 5'-flanking regulatory region of serotonin transporter gene on chromosome 17q11.2. It consists of a 44-bp insertion or deletion involving repeat elements 6 to 8 (from bp –1212 to bp –1255).⁵⁹ In a previous work,⁶⁰ we found SERTPR variants associated with lithium outcome ($P=0.005$) in a sample of 201 patients consecutively admitted to the Lithium Clinic for Mood Disorders of S Raffaele Hospital in Milan. Subjects with the *s/s* variant showed a worse response compared to both *l/s* and *l/l* variants. Interestingly, our results on lithium efficacy were consistent with the observation of a reduced efficacy of antidepressant treatment in subjects with the SERTPR short variant.⁶¹ The only other report was by Del Zompo *et al*,⁶² who found a trend of long allele for higher frequency among lithium nonresponders compared to controls.

The aim of the present work was to replicate analysis on an independent sample from the Mood Disorders Clinic of the Eginition Hospital of the Athens University, Medical School Department of Psychiatry.

MATERIALS AND METHODS

Sample

A total of 83 subjects consecutively admitted to the Mood Disorders Clinic of the Eginition Hospital of the Athens University, Medical School Department of Psychiatry were included to the study.

The present sample composed of 55 females (66.3%) and 28 males (33.7%); they had a mean age of 45.5 ± 14.6 years and a mean age at onset of 19.9 ± 9.7 years. All patients were affected by Bipolar disorder (type I = 64; type II = 19) and, before lithium treatment, they had experienced an average of 8.2 ± 3.2 previous episodes (4.3 ± 1.9 depressive, 4.0 ± 1.6 manic and 3.5 ± 1.7 hypomanic). At the intake, all patients were administered a prophylactic therapy with lithium ($n=64$) as prophylactic treatment, on the basis of a clinical judgement. Other mood stabilizers were added (carbamaze-

pine: $n=11$; sodium valproate: $n=8$) if patients developed an affective episode during lithium treatment, and according to the opinion of their clinician. In this case they were considered nonresponders.

Lithium dosages were adjusted to obtain plasma levels in the range of 0.65–0.75 mMol/l. In the overall sample, lithium dosages ranged between 300 and 1800 mg/die and the mean dosage was 1173 ± 244 mg/die. The majority of patients received lithium at a dosage between 900 and 1500 mg/die ($n=80$). A total of 24 subjects received lithium at a dosage lower than 1200 mg/die (300 mg/die: $n=1$; 600 mg/die: $n=1$; 900 mg/die: $n=22$), while 59 subjects were administered lithium at 1200 mg/die or higher (1200 mg/die: $n=36$; 1350 mg/die: $n=7$; 1500 mg/die: $n=15$; 1800 mg/die: $n=1$).

All patients were observed prospectively for a follow-up period of 3 years after lithium administration with respect to their response to lithium treatment. Responders to lithium treatment were defined as those patients who did not develop any illness phase during the 3 years of lithium treatment. Those patients who developed an illness phase in the 3-year-period were routinely considered nonresponders; information regarding number and characteristics of illness episodes during lithium treatment was not collected. Patients who failed to attend the clinic during the follow-up period were excluded from the study.

Lifetime diagnoses were assigned by two independent psychiatrists on the basis of interviews and medical records, according to DSMIV criteria.⁶³ Information about the illness before contact with the Mood disorder clinic was collected following the best estimate procedure interviewing the subjects, family members, previous health professionals and obtaining records when possible.⁶⁴ The presence of concomitant diagnoses of mental retardation, drug dependence, or other Axis I disorders, together with somatic or neurological illnesses that impaired psychiatric evaluation, represented exclusion criteria. Probands were unrelated and of Greek descent, with antecedents of Greek ethnicity, from all parts of the country. At the intake, none, out of the 83, was affected by a thyroid disorder; 31 developed a thyroid illness during lithium treatment. Informed consent was obtained from participants after the study was explained. The study was carried out in accordance with the principles of the Declaration of Helsinki.⁶⁵

DNA Analysis

Genomic DNA was extracted from leucocytes by NaCl precipitation.⁶⁶ PCR forward primer 5'-GGCGTTGCCGCTCTGAATGC-3' and reverse primer 5'-GAGGGACTGAGCTG GACAACCAC-3' were employed. In total, 35 cycles of 1 min at 95°C, 1 min at 61°C and 1 min at 72°C were performed. The assay mix, in a volume of 30 μ l, contained 50 ng genomic DNA, 2.5 mM dNTPs, 0.1 μ g of sense and antisense primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 5% DMSO and 1 U Taq Polymerase. PCR products were separated on 3% agarose gel supplemented with ethidium bromide allowing differentiation of the long (528 bp) and the short (484 bp) variant.

Statistical Analysis

To investigate the possible association between SERTPR variants and response to lithium treatment, we used the χ^2 test. Alpha levels were considered significant when less than 0.05. Variables considered as possible confounders were: sex, age, age at onset, diagnosis, number of total episodes before lithium administration, presence of psychotic features, thyroid disorders, mood stabilizer treatment, lithium dosages and response to lithium treatment. Possible associations were assessed using Analysis of Variance (ANOVA) for continuous variables and χ^2 test for categorical variables. Logistic regression was performed to control possible confounders (sex, age, age at onset, diagnosis, number of total illness episodes, suicide attempts, psychosis, thyroid disorders).

The power of our sample to detect differences among SERTPR variants was calculated considering an alpha value of 5% two tailed, by the GPOWER programme, version 2.0.⁶⁷ With these parameters in our sample, we had a high power (0.80) to detect a medium effect size ($w=0.31$) that corresponded to a difference of approximately 30% between two major genotypes on response.⁶⁸

RESULTS

In the present sample, SERTPR genotypes were in Hardy–Weinberg equilibrium ($\chi^2=2.0$; $df=1$; $P=0.2$). SERPR long and short allele frequencies were, respectively, 0.51 and

0.49, similar to those of previously published samples in Caucasians^{45,59,69,70} and similar to those observed in our Italian sample (0.57 and 0.43, respectively).⁶⁰ Italian and Greek samples were not different in terms of SERTPR genotypes frequencies ($\chi^2=4.3$; $df=2$; $P=0.1$). On the other hand, Asian frequencies were different from all Caucasian samples.^{71,72}

Demographic and clinical features of the sample are described in Table 1. We observed statistically significant excesses of the SERTPR*s/s genotype among psychotics, of the s/l genotypes among females and a trend for an excess of the l/l genotype among subjects with a younger age at onset. However, those variables were not associated with the response to lithium treatment.

Treatments, dosages and response to prophylactics therapies are described in Table 2. Groups were not different with respect to stabilizing therapy they received (lithium or lithium plus other stabilizers), although a trend towards higher lithium dosages among subjects with the SERTPR*s/l genotype was observed.

SERTPR groups did not differ with respect to their response to lithium treatment; subanalyses by diagnosis (BP I, BP II) did not yield any significant association. However, when considering other subpopulations we found significant associations between SERTPR and lithium outcome. Those subpopulations were defined by the number of manic episodes experienced before lithium administration, and by lithium dosages they were administered too.

Table 1 Clinical variables divided according to SERTPR variants

	SERTPR genotypes			N	χ^2	P
	S/s N (%)	s/l N (%)	l/l N (%)			
Sex						
Males	11 (39.29%)	6 (21.43%)	11 (39.29%)	28	8.7	0.01
Females	12 (21.82%)	30 (54.55%)	13 (23.64%)	55		
Diagnosis					0.2	0.9
BP I	18 (28.13%)	27 (42.19%)	19 (29.69%)	64		
BP II	5 (26.32%)	9 (47.37%)	5 (26.32%)	19		
Psychotic features					7.9	0.02
Present	19 (37.25%)	17 (33.33%)	15 (29.41%)	51		
Absent	4 (12.50%)	19 (59.38%)	9 (28.13%)	32		
Thyroid disorders					0.5	0.8
Absent	15 (28.85%)	21 (40.38%)	16 (30.77%)	52		
Present	8 (25.81%)	15 (48.39%)	8 (25.81%)	31		
	<i>Mean ± SD</i>	<i>Mean ± SD</i>	<i>Mean ± SD</i>			
Age	47.4 ± 17.8	47.8 ± 12.6	40.3 ± 13.2	83	2.2	0.1
Age at onset	22.5 ± 10.0	20.6 ± 10.1	16.3 ± 8.0	83	2.7	0.07
Total episodes (before lithium)	7.4 ± 2.7	8.9 ± 3.7	7.7 ± 2.6	83	1.9	0.1

An excess of the SERTPR*s/l genotype was observed among females; an excess the SERTPR*s/s genotype was observed among psychotics; and an excess of the l/l genotype was observed among subjects with a lower age at onset.

Table 2 SERTPR variants and lithium treatment

	SERTPR genotypes			N	χ^2	P
	s/s N (%)	s/l N (%)	l/l N (%)			
<i>Treatments</i>					0.9	0.6
Lithium	19 (29.69%)	28 (43.75%)	17 (26.56%)	64		
Lithium+other stabilizers	4 (21.05%)	8 (42.11%)	7 (36.84%)	19		
	23	36	24	83		
<i>Response to lithium treatment</i>					3.2	0.2
Nonresponders	8 (22.22%)	14 (38.89%)	14 (38.89%)	36		
Responders	15 (31.91%)	22 (46.81%)	10 (21.28%)	47		
	23	36	24	83		
	<i>Mean ± SD</i>	<i>Mean ± SD</i>	<i>Mean ± SD</i>			
Lithium dosage	1249.6 ± 290.9	1101.7 ± 224.6	1210.0 ± 200.7	83	3.1	0.051

SERTPR genotypes were not associated with response to lithium treatment. A trend for a lower lithium dosage was observed among subjects with the s/l genotype.

In fact, in the present sample, the response to lithium treatment was negatively correlated with the number of previous manic episodes ($R = -0.3$; $P = 0.02$) and a trend for high lithium dosages among l/l SERTPR subjects was found ($F = 3.1$; $df = 80$; $P = 0.051$). We hypothesized that previous illness pattern and dosages of lithium administered could affect the response to lithium treatment. Thus, to control these possible confounders, we grouped patients in terms of the bipolar illness pattern before lithium treatment and for dosages of lithium treatment.

As presented in Figure 1, among subjects who experienced few manic episodes (≤ 4) before lithium administration, those homozygotes for the long SERTPR allele were more likely to develop an affective episode during the 3-years period of follow-up. Conversely, SERTPR short allele-containing genotypes showed a higher incidence of responders (i.e. patients who did not develop an effective episode during the 3 years of lithium treatment). A similar trend was found among subjects with a higher daily dose of lithium (≥ 1200 mg/die) (Figure 2).

Both results were not influenced by the other clinical factors such as sex, age, age at onset, diagnosis, number of total illness episodes, suicide attempts, psychosis and thyroid disorders, except for a marginal decrease of significance for the association between SERTPR and response in the subgroup with higher lithium doses when including suicide attempts, psychosis and thyroid disorders in the logistic regression model.

DISCUSSION

Previous pharmacogenetic studies evidenced the involvement of some candidate genes on the response to lithium prophylactic treatment. Among those candidate genes, the tryptophan hydroxylase C allele (TPH),⁷³ the phospholipase C g-1 gene (PLCG1) repeat,^{74,75} the C973A polymorphism in the inositol polyphosphate 1-phosphatase⁷⁶ and the mito-

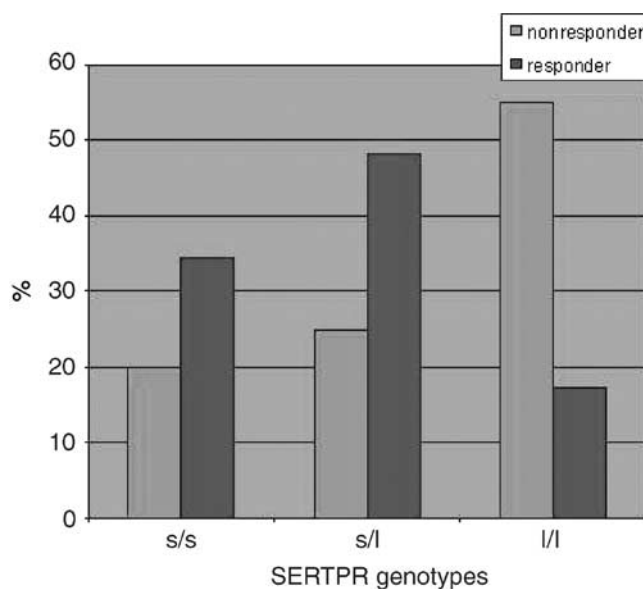


Figure 1 SERTPR genotypes and response to lithium treatment among subjects who experienced a lower number of manic episodes ($n \leq 4$) before lithium. The l/l SERTPR genotype was associated with a higher incidence of non-responders to lithium treatment ($\chi^2 = 7.7$; $df = 2$; $P = 0.02$). The l/l SERTPR genotype was significantly associated with a higher incidence of nonresponders to lithium treatment, compared to short allele-containing genotypes, taken together ($\chi^2 = 7.7$; $df = 1$; $P = 0.005$).

chondrial DNA (mtDNA) 10398 polymorphism,⁷⁷ have been suggested as being associated with positive lithium response.

We previously reported an association between the functional polymorphism in the upstream regulatory region of the serotonin transporter and lithium outcome.⁶⁰ The aim of the present work was to replicate the finding in an independent Greek sample.

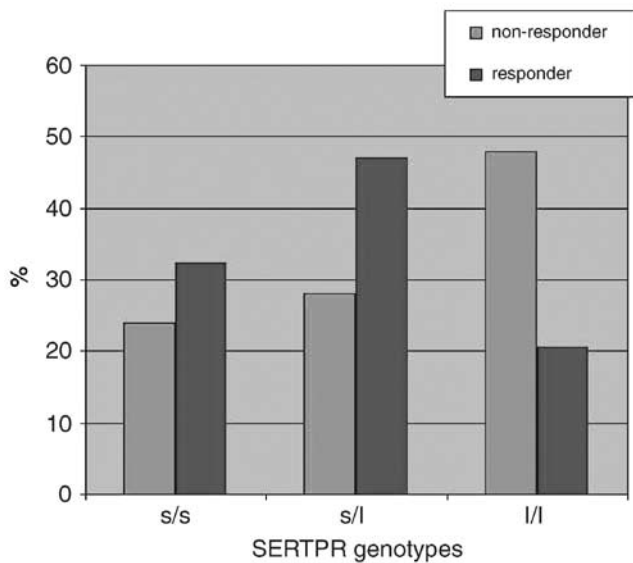


Figure 2 SERTPR genotypes and response to lithium treatment among subjects who received a lithium daily dosage ≥ 1200 mg/die. The SERTPR*1/l genotype showed a trend for a higher incidence of nonresponders to lithium treatment ($\chi^2 = 5.1$; $df = 2$; $P = 0.079$). This difference was statistically significant comparing the SERTPR*1/l genotypes to short allele-containing genotypes, taken together ($\chi^2 = 5.0$; $df = 1$; $P = 0.026$).

In the present sample, we found that homozygosity for the long SERTPR gene was associated with a higher probability of developing an affective episode into 3 years of lithium treatment. This was statistically significant among subjects who experienced few manic episodes (≤ 4) before lithium administration and among subjects who received a high lithium daily dose (≥ 1200 mg/die). No differences were observed between Bipolar disorder sub-diagnoses (BP I, BP II). However, in the whole sample we could observe only a trend towards a poorer response to lithium treatment among subjects carrying the l/l SERTPR genotype. Confounding factors, such as sex, age, age at onset, diagnosis, number of total illness episodes, suicide attempts, psychosis and thyroid disorders, did not affect response to lithium treatment and the observed association.

These results suggest that the relation between SERTPR gene and the response to lithium treatment may also be affected by the natural history of the illness, together with the individual's capacity to retain lithium. In fact, differences among SERTPR genotypes emerged only among those subjects who experienced few previous manic episodes and among those subjects who received high doses of lithium. Since the dosages of lithium were adjusted to obtain adequate plasma levels, subjects who received high lithium dosages may have a low capacity to retain lithium. In fact, in the present sample, l/l SERTPR subjects, the ones with a poorer lithium efficacy, also showed a trend for higher lithium dosages, compared to other subjects. Those subjects may therefore benefit less from lithium. We may hypothe-

size that both illness recurrence and lithium retaining capacity could be affected in a complex interaction by the SERTPR genotype. Moreover, although in the present sample the SERTPR genotype was not associated with the illness time course (data not shown), we previously reported that l/l SERTPR subjects have a higher illness recurrence independent of lithium administration, as compared to subjects carrying other genotypes.^{60,78} However, the total number of episodes was not a significant stratification factor and we should admit that our methodology was not aimed to investigate this issue properly.

Overall, data on the present sample did not confirm our previous finding of a worse outcome of lithium treatment among subjects carrying the s/s SERTPR genotype.⁶⁰ In fact, Greek nonresponders were more likely to be homozygotes for the long SERTPR allele. A similar association between SERTPR*1/l genotype and a poor response to lithium prophylactic therapy was also reported by Zompo *et al.*⁶²

However, our previous study and the present one were different with respect to the definition of 'lithium outcome'. Previously,⁶⁰ we estimated lithium efficacy in terms of *modification of the illness recurrence*, by calculating the difference between the pre- and the on-lithium treatment recurrence rates (continuous evaluation of the outcome).^{79,80} In the Greek sample, the lithium treatment effectiveness was judged by the *absolute absence of recurrences* in the 3 years of observation, independent of the previous illness pattern (categorical evaluation of the outcome).

Results on heterozygotes subjects were instead similar in the two samples. In the Italian sample, we observed a significant reduction of episodes after lithium administration among subjects carrying the SERTPR*s/l genotype. Accordingly, in the Greek sample, s/l SERTPR genotype was associated with a better response to lithium treatment (absence of illness episodes during lithium). Finally, another reason for the conflicting results could be due to the fact that the polymorphism investigated is in linkage disequilibrium with a nearby functional one.⁸¹

Recently, the SERTPR polymorphism has been associated with the antidepressant response to certain selective serotonin reuptake inhibitors, such as fluvoxamine⁸²⁻⁸⁵ and paroxetine.^{86,87} A possible common mechanism may therefore underlie SSRI and lithium efficacy even if the exact mechanism of action of lithium activity is largely unknown.

A limitation of the present study is that a number of clinical variables were not considered, such as number of days of hospitalization, the recurrence rates during lithium treatment, the episodes' sequence type (depression/mania),⁴ the severity of depressive and manic episodes, subthreshold symptomatology,⁸⁸ life events, time course after lithium nonresponse or the time course of plasma lithium levels. Moreover, the sample size did not have the ability to detect small differences, as shown by the power analysis.

Considering these limitations, and the possibility of false-positive findings, the present study supports a possible association between the SERTPR gene and lithium efficacy among patients affected by Bipolar disorders. However, the present data conflict with previous findings; thus further

studies on independent and large samples are required to establish the reliability and the direction of a possible association between SERTPR variants and lithium outcome.

REFERENCES

- Goodwin F, Jamison K. *Manic-depressive Illness*. Oxford University Press: New York 1990.
- Schou M. *Lithium Treatment of Manic Depressive Illness. A Practical Guide*. Karger: Basel 1989.
- Maj M, Arena F, Lovero N, Pirozzi R, Kemali D. Factors associated with response to lithium prophylaxis in DSM III major depression and bipolar disorder. *Pharmacopsychiatry* 1985; **18**: 309–313.
- Maj M, Pirozzi R, Starace F. Previous pattern of course of the illness as a predictor of response to lithium prophylaxis in bipolar patients. *J Affect Disord* 1989; **17**: 237–241.
- O'Connell RA, Mayo JA, Flatow L, Cuthbertson BO, Brien BE. Outcome of bipolar disorder on long-term treatment with lithium. *Br J Psychiatry* 1991; **159**: 123–129.
- Abou-Saleh MT. Who responds to prophylactic lithium therapy? *Br J Psychiatry* 1993; **21**(Suppl 21): 20–26.
- Franchini L, Zanardi R, Smeraldi E, Gasperini M. Early onset of lithium prophylaxis as a predictor of good long-term outcome. *Eur Arch Psychiatry Clin Neurosci* 1999; **249**: 227–230.
- Serretti A, Lattuada E, Franchini L, Smeraldi E. Melancholic features and response to lithium prophylaxis in mood disorders. *Depression Anxiety* 2000; **11**: 73–79.
- Mendlewicz J, Fieve RR, Stallone F, Fleiss JL. Genetic history as a predictor of lithium response in manic-depressive illness. *Lancet* 1972; **1**: 599–600.
- Maj M, Del Vecchio M, Starace F, Pirozzi R, Kemali D. Prediction of affective psychoses response to lithium prophylaxis. The role of socio-demographic, clinical, psychological and biological variables. *Acta Psychiatrica Scand* 1984; **69**: 37–44.
- Grof P, Alda M, Grof E, Zvolzky P, Walsh M. Lithium response and genetics of affective disorders. *J Affect Disord* 1994; **32**: 85–95.
- Abou-Saleh MT, Coppen A. Who responds to prophylactic lithium? *J Affect Disord* 1986; **10**: 115–125.
- Mendlewicz J. Prediction of treatment outcome: family and twin studies in lithium prophylaxis and the question of lithium red blood cell/plasma ratio. In: Cooper TB, Gershon S, Kline NS, Schou M (eds). *Lithium Controversies and Unresolved Issues*. Excerpta Medica: Amsterdam, 1979.
- Nylander PO, Engstrom C, Nordqvist-Karlsson B, Astrom M. Family history of affective disorders and the significance for prophylactic effect of lithium treatment. *Biol Psychiatry* 1999; **45**: 1079–1081.
- Morabito A, Gasperini M, Macciardi F, Smeraldi E. Possible relationship between outcome in primary affective disorders treated with lithium and family history. *Adv Biochem Psychopharmacol* 1982; **32**: 157–163.
- Smeraldi E, Petroccione A, Gasperini M, Macciardi F, Orsini A. The search for genetic homogeneity in affective disorders. *J Affect Disord* 1984; **7**: 99–107.
- Smeraldi E, Petroccione A, Gasperini M, Macciardi F, Orsini A, Kidd KK. Outcomes on lithium treatment as a tool for genetic studies in affective disorders. *J Affect Disord* 1984; **6**: 139–151.
- Cavazzoni P, Alda M, Turecki G, Rouleau G, Grof E, Martin R et al. Lithium-responsive affective disorders: no association with the tyrosine hydroxylase gene. *Psychiatry Res* 1996; **64**: 91–96.
- Turecki G, Alda M, Grof P, Martin R, Cavazzoni PA, Duffy A et al. No association between chromosome-18 markers and lithium-responsive affective disorders. *Psychiatry Res* 1996; **63**: 17–23.
- Alda M. Pharmacogenetics of lithium response in bipolar disorder. *J Psychiatry Neurosci* 1999; **24**: 154–158.
- Dunner DL, Fleiss JL, Fieve RR. Lithium carbonate prophylaxis failure. *Br J Psychiatry* 1976; **129**: 40–44.
- Misra PC, Burns BH. 'Lithium non-responders' in a lithium clinic. *Acta Psychiatrica Scand* 1977; **55**: 32–40.
- Strober M, Morrell W, Burroughs J, Lampert C, Danforth H, Freeman R. A family study of bipolar I disorder in adolescence. Early onset of symptoms linked to increased familial loading and lithium resistance. *J Affect Disord* 1988; **15**: 255–268.
- Engstrom C, Astrom M, Nordqvist-Karlsson B, Adolfsson R, Nylander PO. Relationship between prophylactic effect of lithium therapy and family history of affective disorders. *Biol Psychiatry* 1997; **42**: 425–433.
- Coryell W, Akiskal H, Leon AC, Turvey C, Solomon D, Endicott J. Family history and symptoms levels during treatment for bipolar I affective disorder. *Biol Psychiatry* 2000; **47**: 1034–1042.
- Grof P, Duffy A, Cavazzoni P, Grof E, Garnham J, MacDougall M et al. Is response to prophylactic lithium a familial trait? *Int J Neuropsychopharmacol* 2000; **3**: 339.
- Carli M, Afkhami-Dastjerian S, Reader TA. Effects of a chronic lithium treatment on cortical serotonin uptake sites and 5-HT_{1A} receptors. *Neurochem Res* 1997; **22**: 427–435.
- Carli M, Reader TA. Regulation of central serotonin transporters by chronic lithium: an autoradiographic study. *Synapse* 1997; **27**: 83–89.
- El-Mallakh S. *Lithium: Actions and Mechanisms*. American Psychiatric Press: Washington, DC 1996.
- Pei Q, Leslie RA, Grahame-Smith DG, Zetterstrom TS. 5-HT efflux from rat hippocampus *in vivo* produced by 4-aminopyridine is increased by chronic lithium administration. *Neuroreport* 1995; **6**: 716–720.
- Sharp T, Bramwell SR, Lambert P, Grahame-Smith DG. Effect of short- and long-term administration of lithium on the release of endogenous 5-HT in the hippocampus of the rat *in vivo* and *in vitro*. *Neuropharmacology* 1991; **30**: 977–984.
- Baptista TJ, Hernandez L, Burguera JL, Burguera M, Hoebel BG. Chronic lithium administration enhances serotonin release in the lateral hypothalamus but not in the hippocampus in rats. A microdialysis study. *J Neur Trans—Gen Sect* 1990; **82**: 31–41.
- Odagaki Y, Koyama T, Matsubara S, Matsubara R, Yamashita I. Effects of chronic lithium treatment on serotonin binding sites in rat brain. *J Psychiatric Res* 1990; **24**: 271–277.
- Goodwin GM. The effects of antidepressant treatments and lithium upon 5-HT_{1A} receptor function. *Prog Neuro-Psychopharmacol Biol Psychiatry* 1989; **13**: 445–451.
- Artigas F, Sarrias MJ, Martinez E, Gelpi E, Alvarez E, Udina C. Increased plasma free serotonin but unchanged platelet serotonin in bipolar patients treated chronically with lithium. *Psychopharmacology* 1989; **99**: 328–332.
- Hotta I, Yamawaki S, Segawa T. Long-term lithium treatment causes serotonin receptor down-regulation via serotonergic presynapses in rat brain. *Neuropsychobiology* 1986; **16**: 19–26.
- Treiser SL, Cascio CS, O'Donohue TL, Thoa NB, Jacobowitz DM, Kellar KJ. Lithium increases serotonin release and decreases serotonin receptors in the hippocampus. *Science* 1981; **213**: 1529–1531.
- Price LH, Charney DS, Delgado PL, Heninger GR. Lithium and serotonin function: implications for the serotonin hypothesis of depression. *Psychopharmacology* 1990; **100**: 3–12.
- Cassidy F, Murry E, Carroll BJ. Tryptophan depletion in recently manic patients treated with lithium. *Biol Psychiatry* 1998; **43**: 230–232.
- Chaouloff F, Gunn SH, Young JB. Serotonin does not mediate the adrenal catecholamine-releasing effect of acute lithium administration in rats. *Psychoneuroendocrinology* 1992; **17**: 135–144.
- Lacaille JC, Cloutier S, Reader TA. Lithium reduced synaptic transmission and increased neuronal excitability without altering endogenous serotonin, norepinephrine and dopamine in rat hippocampal slices *in vitro*. *Prog Neuro-Psychopharmacol Biol Psychiatry* 1992; **16**: 397–412.
- Manji HK, Hsiao JK, Risby ED, Oliver J, Rudorfer MV, Potter WZ. The mechanisms of action of lithium. I. Effects on serotonergic and noradrenergic systems in normal subjects. *Arch Gen Psychiatry* 1991; **48**: 505–512.
- Collier G, Stöber G, Li T, Heils A, Catalano M, Di Bella D et al. A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. *Mol Psychiatry* 1996; **1**: 453–460.
- Collier DA, Arranz MJ, Sham P, Battersby S, Vallada H, Gill P et al. The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. *Neuroreport* 1996; **7**: 1675–1679.
- Rees M, Norton N, Jones I, McCandless F, Scourfield J, Holmans P et al. Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). *Mol Psychiatry* 1997; **2**: 398–402.
- Bellivier F, Laplanche JL, Leboyer M, Feingold J, Bottos C, Allilaire JF et al. Serotonin transporter gene and manic depressive illness: an association study. *Biol Psychiatry* 1997; **41**: 750–752.

- 47 Mendes de Oliveira JR, Otto PA, Vallada H, Lauriano V, Elkis H, Lafer B et al. Analysis of a novel functional polymorphism within the promoter region of the serotonin transporter gene (5-HTT) in Brazilian patients affected by bipolar disorder and schizophrenia. *Am J Med Genet* 1998; **81**: 225–227.
- 48 Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES et al. Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. *Am J Med Genet* 1998; **81**: 58–63.
- 49 Hoehe MR, Wendel B, Grunewald I, Chiaroni P, Levy N, Morris-Rosendahl D et al. Serotonin transporter (5-HTT) gene polymorphisms are not associated with susceptibility to mood disorders. *Am J Med Genet* 1998; **81**: 1–3.
- 50 Esterling LE, Yoshikawa T, Turner G, Badner JA, Bengel D, Gershon ES et al. Serotonin transporter (5-HTT) gene and bipolar affective disorder. *Am J Med Genet* 1998; **81**: 37–40.
- 51 Gutierrez B, Arranz MJ, Collier DA, Valles V, Guillamat R, Bertranpetit J et al. Serotonin transporter gene and risk for bipolar affective disorder—an association study in a Spanish population. *Biol Psychiatry* 1998; **43**: 843–847.
- 52 Oruc L, Verheyen GR, Furac I, Jakovljevic M, Ivezic S, Raeymaekers P et al. Association analysis of the 5-HT_{2C} receptor and 5-HT transporter genes in bipolar disorder. *Am J Med Genet* 1997; **74**: 504–506.
- 53 Ewald H, Flint T, Degn B, Mors O, Kruse TA. A functional variant of the serotonin transporter gene in families with bipolar affective disorder. *J Affect Disord* 1998; **48**: 135–144.
- 54 Kelsoe J, Remick R, Sadovnick A, Kristbjarnarson H, Flodman P, Spence M et al. Genetic linkage study of bipolar disorder and the serotonin transporter. *Am J Med Genet* 1996; **67**: 215–217.
- 55 Lenzinger E, Neumeister A, Praschak-Rieder N, Fuchs K, Gerhard E, Willeit M et al. Behavioral effects of tryptophan depletion in seasonal affective disorder associated with the serotonin transporter gene? *Psychiatry Res* 1999; **85**: 241–246.
- 56 Mundo E, Walker M, Tims H, Macciardi F, Kennedy JL. Lack of linkage disequilibrium between serotonin transporter protein gene (SLC6A4) and bipolar disorder. *Am J Med Genet* 2000; **96**: 379–383.
- 57 Ospina-Duque J, Duque C, Carvajal-Carmona L, Ortiz-Barrientos D, Soto I, Pineda N et al. An association study of bipolar mood disorder (type I) with the 5-HTTLPR serotonin transporter polymorphism in a human population isolate from Colombia. *Neurosci Lett* 2000; **292**: 199–202.
- 58 Saleem Q, Ganesh S, Vijaykumar M, Reddy YC, Brahmachari SK, Jain S. Association analysis of 5HT transporter gene in bipolar disorder in the Indian population. *Am J Med Genet* 2000; **96**: 170–172.
- 59 Lesch K, Bengel D, Heils A, Sabol S, Greenberg B, Petri S et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1530.
- 60 Serretti A, Lilli R, Mandelli L, Lorenzi C, Smeraldi E. Serotonin transporter gene associated with lithium prophylaxis in mood disorders. *Pharmacogenom J* 2001; **1**: 71–77.
- 61 Serretti A, Lilli R, Smeraldi E. Pharmacogenetics in affective disorders. *Eur J Pharmacol* 2002; **438**: 117–128.
- 62 Del Zompo M, Ardau R, Palmas MA, Bocchetta A, Reina A, Piccardi MP. Lithium response: association study with two candidate genes. *Mol Psychiatry* 1999; **4**(Suppl 1): s66–s67.
- 63 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. American Psychiatric Association: Washington, DC 1994.
- 64 Leckman JF, Sholomskas D, Thompson WD, Belanger A, Weissman MM. Best estimate of lifetime psychiatric diagnosis: a methodological study. *Arch Gen Psychiatry* 1982; **39**: 879–883.
- 65 W.M.A. World Medical Association declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *JAMA* 1997; **277**: 925–926.
- 66 Lahiri DK, Nurnberger JJ. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucl Acid Res* 1991; **19**: 5444.
- 67 Erdfelder E, Faul F, Buchner A. GPOWER: A general power analysis program. *Behav Res Methods Instrum Comput* 1996; **28**: 1–11.
- 68 Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates: Hillsdale, NJ 1988.
- 69 Rosenthal N, Mazzanti C, Barnett R, Hardin T, Turner E, Lam G et al. Role of serotonin transporter promoter repeat length polymorphism (5-HTTLPR) in seasonality and seasonal affective disorder. *Mol Psychiatry* 1998; **3**: 175–177.
- 70 Flory JD, Manuck SB, Ferrell RE, Dent KM, Peters DG, Muldoon MF. Neuroticism is not associated with the serotonin transporter (5-HTTLPR) polymorphism. *Mol Psychiatry* 1999; **4**: 93–96.
- 71 Ohara K, Nagai M, Suzuki Y, Ochiai M. Association between anxiety disorders and a functional polymorphism in the serotonin transporter gene. *Psychiatry Res* 1998; **81**: 277–279.
- 72 Matsushita S, Muramatsu T, Kimura M, Shirakawa O, Mita T, Nakai T et al. Serotonin transporter gene regulatory region polymorphism and panic disorder. *Mol Psychiatry* 1997; **2**: 390–392.
- 73 Serretti A, Lilli R, Lorenzi C, Gasperini M, Smeraldi E. Tryptophan hydroxylase gene and response to lithium prophylaxis in mood disorders. *J Psychiatric Res* 1999; **33**: 371–377.
- 74 Turecki G, Grof P, Cavazzoni P, Duffy A, Grof E, Ahrens B et al. Evidence for a role of phospholipase C-gamma1 in the pathogenesis of bipolar disorder. *Mol Psychiatry* 1998; **3**: 534–538.
- 75 Lovlie R, Berle JO, Stordal E, Steen VM. The phospholipase C-gamma1 gene (PLCG1) and lithium-responsive bipolar disorder: re-examination of an intronic dinucleotide repeat polymorphism. *Psychiatr Genet* 2001; **11**: 41–43.
- 76 Steen VM, Lovlie R, Osher Y, Belmaker RH, Berle JO, Gulbrandsen AK. The polymorphic inositol polyphosphate 1-phosphatase gene as a candidate for pharmacogenetic prediction of lithium-responsive manic-depressive illness. *Pharmacogenetics* 1998; **8**: 259–268.
- 77 Washizuka S, Ikeda A, Kato N, Kato T. Possible relationship between mitochondrial DNA polymorphisms and lithium response in bipolar disorder. *Int J Neuropsychopharmacol* 2003; **6**: 421–424.
- 78 Smeraldi E, Benedetti F, Zanardi R. Serotonin transporter promoter genotype and illness recurrence in mood disorders. *Eur Neuropsychopharmacol* 2002; **12**: 73–75.
- 79 Gasperini M, Scherillo P, Manfredonia MG, Franchini L, Smeraldi E. A study of relapses in subjects with mood disorder on lithium treatment. *Eur Neuropsychopharmacol* 1993; **3**: 103–110.
- 80 Franchini L, Gasperini M, Smeraldi E. A 24-month follow-up study of unipolar subjects: a comparison between lithium and fluvoxamine. *J Affect Disord* 1994; **32**: 225–231.
- 81 Hamilton S, Slager S, Kraft J, McGrath P, Knowles J. *Genetic Analysis of the Serotonin Transporter in the Treatment of Depression*, Pharmacogenetics in Psychiatry Meeting 2003, Green tab: New York.
- 82 Smeraldi E, Zanardi R, Benedetti F, DiBella D, Perez J, Catalano M. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol Psychiatry* 1998; **3**: 508–511.
- 83 Zanardi R, Serretti A, Rossini D, Franchini L, Cusin C, Lattuada E et al. Factors affecting fluvoxamine antidepressant activity: influence of pindolol and 5-HTTLPR in delusional and nondelusional depression. *Biol Psychiatry* 2001; **50**: 323–330.
- 84 Serretti A, Zanardi R, Rossini D, Cusin C, Lilli R, Smeraldi E. Influence of tryptophan hydroxylase and serotonin transporter genes on fluvoxamine antidepressant activity. *Mol Psychiatry* 2001; **6**: 586–592.
- 85 Yu YW, Tsai SJ, Chen TJ, Lin CH, Hong CJ. Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. *Mol Psychiatry* 2002; **7**: 1115–1119.
- 86 Zanardi R, Benedetti F, DiBella D, Catalano M, Smeraldi E. Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of serotonin transporter gene. *J Clin Psychopharmacol* 2000; **20**: 105–107.
- 87 Pollock BG, Ferrell RE, Mulsant BH, Mazumdar S, Miller M, Sweet RA et al. Allelic variation in the serotonin transporter promoter affects onset of paroxetine treatment response in late-life depression. *Neuropsychopharmacol* 2000; **23**: 587–590.
- 88 Muller-Oerlinghausen B. Does effective lithium prophylaxis result in a symptom-free state of manic-depressive illness? Some thoughts on the fine-tuning of mood stabilization. *Comp Psychiatry* 2000; **41**: 26–31.