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# The World Journal of Biological Psychiatry

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## EDITORIAL

### Could pharmacogenetics play a role as predictor in treatment of depressive disorders?

Whilst the pharmacological treatment of major depressive disorder with antidepressants continues to improve, the responses remain poor in 30–50% of patients, with many patients discontinuing the medication due to distressing side effects and lag periods of more than 4 weeks before efficacy been proven. Highly complex mechanisms underlie this variability in drug responses, which can be attributed to genetic factors and several physiological and environmental factors, including age, renal and liver function, nutritional status, smoking, and alcohol consumption.

The study of genetically determined interindividual variations in the responses to drugs is called pharmacogenetics. Although it has been established for almost half a century that genetic factors also influence both the efficacy of a drug and the likelihood of adverse reactions, efficient clinical predictors remain to be established.

The genetic differences in responses to antidepressants may be due to variants that affect the function of genes involved in both pharmacokinetics and pharmacodynamics. Pharmacokinetics is the study of the bodily absorption, distribution, metabolism, and excretion of drugs. Drug metabolism is a critical determinant of the therapeutic and adverse effects of many antidepressants. CYP2D6 is the best-characterized P450 enzyme that exhibits polymorphism in humans, and many antidepressants are metabolized primarily by CYP2D6. Three phenotypes have been identified to date: slow/poor metabolizers, rapid/extensive metabolizers, and ultrarapid metabolizers. Individuals with a homozygous poor-metabolizer genotype who receive sertraline experience adverse effects (dizziness and nausea) (Wang et al. 2001), which may be due to toxic accumulation of the drug due to the elimination rate being too slow. Thus, individual dose adjustment may be necessary for poor metabolizers to achieve the optimal therapeutic effect and avoid adverse effects.

The term pharmacodynamics encompasses all the processes that influence the relationship between drug concentration and drug effects. Antidepressants have a wide variety of targets within neurotransmitter systems, including neurotransmitter synthesis, degradation of enzymes, storage, receptors, and specific

transporter proteins. There is considerable evidence that imbalances in the neurotransmitter systems contribute to the various symptoms that are associated with depression, and that alterations to neurotransmitter systems are important for optimal antidepressant effects (Delgado et al. 1993). Therefore, neurotransmitter-related genes are important targets of antidepressant medication, with serotonin-related genes being the most widely investigated.

The brain 5-HT transporter (5-HTT) is the principal site of action of many antidepressants. This transporter takes up 5-HT into the presynaptic neuron, thus terminating synaptic actions, and recycles it into the neurotransmitter pool. A functional polymorphism (5-HTTLPR) within the promoter of the 5-HTT gene has been identified.

Several studies have examined the relevance of 5-HTT polymorphisms to therapeutic efficacies in depressive patients. In Caucasian populations, the s allele of 5-HTTLPR is reportedly associated with a poor response to selective serotonin reuptake inhibitors (SSRIs) (Lee 2005). In contrast, Asian depressive patients (e.g., Koreans and Japanese) with the 5-HTTLPR s/s genotype exhibit better responses to acute antidepressant treatments than those with other genotypes (Lee 2005). Therefore, the relationships between 5-HTTLPR genotypes and responses to antidepressant treatments remain controversial.

The frequencies of the s and l alleles among Asians are approximately 86 and 14%, respectively, while the corresponding figures for Caucasians are 43 and 57% (Lee 2005). The discrepancies between the responses to antidepressant treatments in different ethnic groups may therefore be partially due to differences in allele frequencies. However, antidepressants do appear to be effective across ethnicities.

Pharmacogenetic studies exhibit certain limitations. Though a single gene may affect the responses to antidepressants, it would play only a relatively minor role where a complex mechanism is involved. For example, it has been determined that the 5-HTTLPR polymorphism accounts for 5–7% of the variance in SSRI responses (Lee 2005), and that the tryptophan hydroxylase A218C genetic

polymorphism accounts for approximately 5% of the variance in SSRI efficacy, with the effect being independent of that of the 5-HTTLPR polymorphism (Lee 2005). Mossner et al. (2000) have demonstrated that brain-derived neurotrophic factor (BDNF) acts as a neurotransmitter modulator that may influence serotonin uptake in lymphocytes, and preferential regulation of the serotonin transporter has been observed in cells of the l/l genotype of the polymorphism in the serotonin transporter gene promoter. Therefore, it may be of interest to explore the interaction of BDNF and serotonin transporter genetic polymorphisms in responses to SSRI antidepressants. Therefore, pharmacogenetics investigations should consider interactions between many genes. In addition, understanding the frequencies and genetic relationships of haplotypes will improve the selection of suitable intragenic markers for genetic association studies.

Previous studies have had diverse designs, in terms of factors such as antidepressant types, sample size, presence and absence of placebo controls, rating scales, outcome measures, and treatment length, all of which may greatly influence the results. In contrast, successful pharmacogenetic studies may require well-characterized phenotypes, methodologies, and criteria for the selection of candidate genes.

Pharmacogenetic research has revealed that the metabolism, clinical effectiveness, and side-effect profiles differ significantly between patients. The utility and possible applications of these research methodologies in clinical settings remain to be determined. Information concerning the relative efficiencies of drug-metabolizing enzymes obtained through genotyping and phenotyping methodologies could be useful to clinicians who provide pharmacotherapeutic services to patients.

The different genetic make-ups are considered to substantially contribute to the differing responses to antidepressants, for which efficient clinical predictors are not yet available. However, pharmacogenetic

studies have the potential to improve our understanding of how antidepressants operate, and thereby also improve both their safety and efficacy in clinical applications.

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## REVIEW

# Evolving trends in the long-term treatment of bipolar disorder

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### Abstract

The episodic and chronic nature of bipolar disorder usually requires long-term treatment in all patients, yet there is an unmet need for well-tolerated and clinically effective maintenance therapy with enhanced patient adherence. Few well-tolerated treatment options are currently available that are both effective in all phases of bipolar disorder and prevent recurrence of episodes. Lithium has well-established efficacy in the prevention of further manic episodes and may also be effective in the prevention of depression and suicide, but safety is a concern due to narrow therapeutic window. For valproate and carbamazepine, data appear much less compelling. Lamotrigine has shown to be effective for long-term prevention of depressive episodes. Controlled studies suggest that atypical antipsychotics may also have mood-stabilizing properties and might become standard for long-term therapy in the new future. The role of psychoeducation in improving adherence to medication in long-term treatment and overall patient outcomes is also crucial.

**Key words:** *Bipolar disorder, atypical antipsychotics, maintenance, relapse prevention, psychoeducation*

### Introduction

Bipolar disorder is an episodic, chronic, and progressive illness that usually requires long-term treatment in most, if not all patients (Mendlewicz et al. 1999; Bowden et al. 2000b; Judd et al. 2002). Although treatment during episodes may resolve symptoms, impaired functioning may persist for many patients (Tohen et al. 2000; Martinez-Aran et al. 2004). Maintenance therapy is needed to maintain and build upon the initial success of treatment, and aims to prevent relapses and reduce threshold symptoms, risk of suicide, cycle frequency, and mood instability (American Psychiatric Association 2002). Long-term or even lifelong therapy is usually required to improve functioning and maintain quality of life.

Identifying clinically effective treatments for maintenance therapy has been a significant challenge in bipolar disorder. An ideal long-term treatment or mood stabilizer would effectively treat episodes of mania and depression as well as prevent relapses. In addition, such treatment should be well tolerated, with few side effects over the long term. Ideally as well, it should be effective for the most difficult-to-treat patients, including rapid cyclers. In the absence of an ideal mood stabilizer, lithium has been the mainstay of

recommended treatment for maintenance therapy in bipolar disorder (Sachs et al. 2000; Goodwin and Geddes 2003). Overall, some randomized, controlled studies suggest that lithium, carbamazepine, divalproex, and lamotrigine might be clinically useful as maintenance treatment in bipolar disorder (Bowden et al. 2000a,b; Keck and McElroy 2002; Calabrese et al. 2003; Goodwin and Geddes 2003).

Nevertheless, a substantial number of patients with bipolar disorder do not respond, suffer recurrence, or cannot tolerate the side effects of these agents. Intolerance to side effects and inadequate long-term adherence to treatment potentially translates into poor treatment outcomes. Although few adequately designed long-term maintenance studies have been conducted, data is emerging to suggest that some atypicals may be effective maintenance therapy for patients with bipolar disorder. Recent evidence for long-term therapies in bipolar disorder is reviewed in this article.

### Lithium and lamotrigine

Lithium has been a standard choice for long-term treatment for bipolar disorder, based on a few early

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clinical studies. However, these studies have been criticized due to their methodological limitations. Moreover, naturalistic studies, which more closely approximate clinical practice, have shown less activity with lithium maintenance therapy (Rybakowski et al. 2001). Most patients with bipolar disorder also have some residual illness with lithium maintenance treatment (Baldessarini and Tondo 2000), and it is now accepted that abrupt withdrawal from lithium can induce a new manic episode and might increase suicidality (Baldessarini et al. 1999). Thus, the potential advantage of the antisuicidal effects of lithium by means of its prophylactic effects on mania, depression, and suicidal behavior (Baldessarini et al. 2003), is challenged by the risk of increased recurrence rates and suicide after abrupt withdrawal, which is common in bipolar patients due to poor insight (Johnson and McFarland 1996).

In addition to these limitations, some subsets of patients are intolerant to lithium or are unable to achieve adequate efficacy and/or adherence at serum levels necessary for remission of symptomatology (Gelenberg et al. 1989). Results from a double-blind, prospective maintenance trial, in which patients with bipolar disorder were randomly assigned to therapy targeting either standard (0.8–1.0 mmol/l) or low (0.4–0.6 mmol/l) serum lithium levels, found that higher serum lithium levels were associated with a higher rate of side effects and lower rate of adherence (Gelenberg et al. 1989). Moreover, a post-hoc reanalysis of the data, which accounted for baseline lithium levels, showed that patients who entered the study with standard serum lithium levels and were randomized to the low range had the highest risk of recurrence (Sachs and Thase 2000). Ideally, the choice of lithium as long-term prophylactic agent should be driven by predictors of response and good compliance; however, there are no strong and reliable such predictors (Kleindienst et al. 2005).

However, more recent trials have provided further support to the long-term efficacy of lithium. Two 18-month, randomized, double-blind trials compared lamotrigine, lithium, and placebo as maintenance treatment in a total of 1315 recently manic or depressed patients with bipolar I disorder (Bowden et al. 2003; Calabrese et al. 2003). Individual and combined analyses of these studies showed that both lamotrigine and lithium significantly prolonged the time to intervention for any mood episode compared with placebo (Figure 1); in spite of the enriched design for lamotrigine responders, lithium prevented manic episodes more effectively than lamotrigine and placebo (Goodwin et al. 2004).

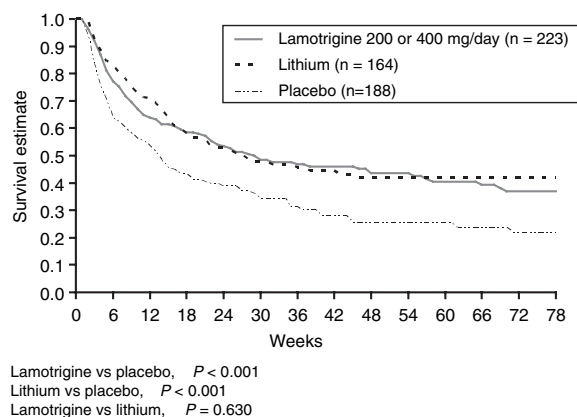


Figure 1. Time to intervention for any episode while using lamotrigine or lithium for 18 months (Goodwin et al. 2004).

### Divalproex

Only one randomized, placebo-controlled study has assessed divalproex in comparison with placebo for maintenance therapy in patients with bipolar I disorder (Bowden et al. 2000a). Patients received divalproex, lithium, or placebo and time to relapse of any mood episode during 1 year of treatment was determined. As shown in Figure 2, there was no significant difference between treatments, although a post hoc analysis showed that patients receiving divalproex had significantly fewer relapses than those receiving placebo if patients had already started divalproex prior to randomization (Gyulai et al. 2003).

### Carbamazepine

Earlier studies of carbamazepine for the long-term treatment of bipolar disorder have not demonstrated good efficacy (Greil et al. 1997). Lithium ( $n=74$ ) and carbamazepine ( $n=70$ ) were compared in a randomized study of 144 patients with bipolar disorder followed for 2.5 years (Greil et al. 1997).

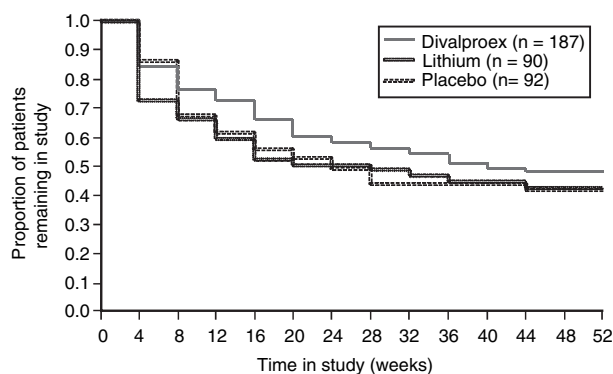


Figure 2. Survival curves for time to any mood episode while using divalproex versus lithium versus placebo for 1 year. Bowden et al. 2000. Arch Gen Psychiatry 57:481–489. Copyright 2000. Adapted with permission.

Patients treated with carbamazepine had a significantly greater number of recurrences and use of concomitant medication ( $P=0.041$ ) and/or adverse events ( $P=0.007$ ) compared with lithium.

In a smaller double-blind study, 52 patients with bipolar disorder were randomly assigned to a year of treatment with lithium or carbamazepine, then treated with the opposite drug for another year and treated with a combination of the two drugs in the final year (Denicoff et al. 1997). More patients treated with carbamazepine (37.1%) failed to complete the first full year of treatment due to lack of efficacy compared with those treated with lithium (31.0%). With the combination treatment, 24.1% of patients withdrew from the study due to lack of efficacy.

Another study randomized 94 patients in remission to double-blind treatment with lithium ( $n=44$ ) or carbamazepine ( $n=50$ ) for 2 years (Hartong et al. 2003). The frequency of mood episodes and the proportion of patients dropping out of the study were higher with carbamazepine treatment than with lithium, with a completion rate of 32 and 36%, respectively.

More recent data suggest, though, that some efficacy in prevention of relapse and recurrence of manic or mixed episodes can be demonstrated for an extended-release formulation of carbamazepine (Ketter et al. 2004). Patients ( $N=92$ ) who had participated in a 3-week double-blind study of carbamazepine or placebo were assigned to 6 months of further open-label treatment with carbamazepine. The estimated mean time to relapse was  $141.8 \pm 5.6$  days and 14.3% patients relapsed during the study. Patients who had previously received carbamazepine in the 3-week treatment period maintained their improvement and those who had received placebo demonstrated significant improvements in manic symptoms. The most common adverse events with carbamazepine were headache, dizziness, and rash. These results indicate that further studies to determine the role of carbamazepine in the long-term treatment of patients with bipolar disorder are warranted.

### **Second-generation antipsychotics as maintenance therapy**

Due to their superior tolerability profile compared with conventional antipsychotics with regards to extrapyramidal symptoms and tardive dyskinesia liability, atypicals are increasingly being used for the treatment of bipolar disorder. Olanzapine, quetiapine, risperidone, ziprasidone and aripiprazole have demonstrated efficacy in the treatment of bipolar mania in 3-week studies, as monotherapy

(Vieta and Goikolea 2005). When used in combination with traditional mood stabilizers, such as lithium or divalproex, olanzapine, risperidone and quetiapine also proved to be more efficacious than lithium or valproate monotherapy (Vieta and Goikolea 2005). Evidence also suggests that some atypicals are effective in the treatment of bipolar depression (Tohen et al. 2003b; Calabrese et al. 2005). Data indicating the efficacy in long-term treatment of bipolar disorder for each of these agents are reviewed below.

#### *Olanzapine*

Several studies have addressed the efficacy of olanzapine as long-term therapy in bipolar disorder (Tohen et al. 2003a, 2004, 2005). Data from a study in which a 6–12-week phase of open-label olanzapine/lithium combination therapy was followed by 52 weeks of double-blind olanzapine or lithium monotherapy found that the prevention of depressive relapse (i.e. maintaining a 21-item Hamilton Depression Rating Scale [HAM-D21] score of 15 or less) was quite similar with both agents, but olanzapine was superior to lithium ( $P<0.001$ ) in reducing the incidence of manic relapse (an increase in the Young Mania Rating Scale [YMRS] score to 15 or more) (Tohen et al. 2005).

Olanzapine's efficacy in manic and depressive episodes and maintenance of remission have also been corroborated in a comparative study against divalproex (Tohen et al. 2003a). In this investigation, 251 adult patients with a bipolar I disorder, manic or mixed (DSM-IV), were randomly assigned to olanzapine (5–20 mg/day) or divalproex (500–2500 mg/day) during a 3-week, randomized, double-blind phase followed by a double-blind continuation phase of 44 weeks (Tohen et al. 2003a).

Olanzapine was significantly superior to divalproex in mean improvement in YMRS total score, although most if not all the benefits were obtained at the beginning of the trial, during the treatment of the manic episode (Tohen et al. 2003a). The median time to symptomatic remission of mania ( $YMRS \leq 12$ ) was significantly shorter for olanzapine (14 days) than for divalproex (62 days) ( $P=0.05$ ), although, importantly, there was no significant difference between groups in mania remission rates and subsequent relapse into mania or depression. Therefore, the potential superiority of olanzapine over divalproex may hold for the short-term effects on manic symptoms (Vieta 2003), but would be much more controversial when long-term and tolerability issues are considered, as treatment with olanzapine was associated with somnolence, increased appetite, and

weight gain more frequently ( $P < 0.05$ ) than divalproex (Tohen et al. 2003a).

In a study conducted in patients with bipolar disorder who had previously received open-label treatment with olanzapine and were then randomized to double-blind treatment for up to 52 weeks, olanzapine was shown to be associated with lower rates of relapse and a longer time to relapse compared with placebo ( $P < 0.001$ ) (Tohen et al. 2006).

Olanzapine added to lithium or valproate has been evaluated in an 18-month study for prevention of relapse (Tohen et al. 2004). Patients achieving syndromic remission after 6 weeks of treatment with olanzapine and lithium or valproate were randomized to further treatment with olanzapine ( $n = 51$ ) or placebo ( $n = 48$ ) in addition to lithium or valproate. At the end of the treatment period, symptomatic (using the total score on the YMRS and the 21-item HAM-D), but not syndromic (meeting DSM-IV criteria for a manic, mixed, or depressive episode) relapse, was significantly different in patients treated with olanzapine and lithium or valproate compared with the group treated with placebo and lithium or valproate ( $P = 0.023$ ). Attrition rates were very high.

The main concerns raised by olanzapine use as long-term therapy in bipolar patients and those related to weight gain and metabolic syndrome.

### Quetiapine

Quetiapine monotherapy has been shown to be superior to placebo in the improvement of mania and associated symptoms as well as maintenance of response and remission rates in two double-blind, 3-month studies of patients with bipolar disorder (Bowden et al. 2005; Vieta et al. 2005; McIntyre et al. 2005). In the first study, patients were randomized to quetiapine ( $n = 107$ ), placebo ( $n = 95$ ), or lithium ( $n = 98$ ). Both quetiapine and lithium decreased YMRS scores significantly more than placebo at 3 weeks ( $P < 0.001$ ), and this difference was maintained to Week 12 ( $P < 0.001$ ). A similar improvement in YMRS scores was observed in quetiapine- or haloperidol-treated patients versus placebo in the second study. The response ( $\geq 50\%$  reduction in YMRS score) and remission ( $YMRS \leq 12$ ) rates in the quetiapine-treated group were significantly greater than placebo at weeks 3 and 12. Haloperidol was more efficacious than quetiapine at week 3 but not at week 12. Similar to results from the individual studies, a combined analysis of these two studies indicated that the majority of patients who responded by week 3 maintained their response through the end of the 3-month study period. Moreover, of the small

proportion of patients who did not respond by week 3 and who had a further assessment, 72% of quetiapine-treated versus 41% of placebo-treated patients responded by the end of the study. Similarly, remission rates in the quetiapine group were maintained to the end of treatment. Compared with placebo, a significantly greater proportion of patients in the quetiapine group met all clinical remission/euthymia criteria ( $YMRS \leq 12$  or  $YMRS \leq 12 + MADRS \leq 10$  or  $YMRS \leq 12 + MADRS \leq 8$ ) by the primary endpoint (Day 21;  $P < 0.01$ ) and rates of remission/euthymia continued to improve to the end of the 3-month treatment period ( $P < 0.001$ ) (Paulsson and Jones 2004). The results from these controlled studies suggest that a significant long-term treatment benefit is likely as the early treatment effect of quetiapine was maintained over a period of 3 months in patients with bipolar mania, but no controlled trial are available for quetiapine beyond 3-month follow-up.

Quetiapine has also been shown to have benefits as long-term therapy in two prospective, open-label studies of patients with rapid cycling (Vieta et al. 2002; Ghaemi et al. 2003). One study prospectively assessed 14 patients with rapid cycling meeting DSM-IV criteria (manic, hypomanic, mixed, depressive, or euthymic) treated with quetiapine (initiated at 50 mg/day and dosed according to tolerability and clinical response) in combination with ongoing psychotropic medication for  $112 \pm 33$  days (Vieta et al. 2002). The Clinical Global Impressions for bipolar disorder (CGI-BP), YMRS, and HAM-D17 rating scales were included in efficacy assessments (Figure 3).

Although controlled maintenance studies are needed, findings from these naturalistic, open-label investigations suggest that quetiapine may be clinically effective in the long-term treatment of rapid-cycling bipolar disorder. The main safety and tolerability concerns with the drug are related to sedation and moderate weight gain liability.

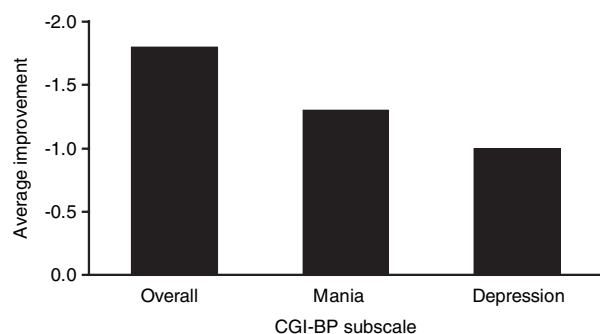


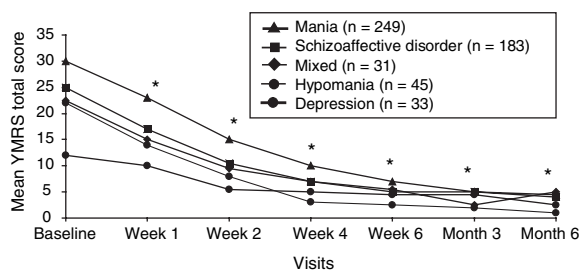
Figure 3. Improvement in the CGI-BP subscales after combination therapy with quetiapine in 14 patients with rapid-cycling bipolar disorder, followed for 112 days (16 weeks) (Vieta et al. 2002).

### Risperidone

No controlled trials are available with risperidone beyond 12-weeks.

A 6-month, open-label study has suggested that monotherapy or combination therapy with risperidone may maintain the improvement of manic and depressive symptoms in patients with bipolar disorder over time (Vieta et al. 2001b). Forty-four bipolar II patients (DSM-IV) with a current hypomanic episode and a YMRS score  $>7$  showed a significant reduction from baseline in YMRS score ( $P < 0.001$ ) by the first week of risperidone treatment (Figure 4) (Vieta et al. 2001b). This improvement in manic symptoms was maintained throughout the 6-month study period. There was no significant difference in the rate of improvement between patients receiving combination therapy or monotherapy with risperidone (Vieta et al. 2001b). A total of 73% of patients were considered responders (those with  $\geq 50\%$  reduction in YMRS score from baseline). Risperidone treatment also significantly reduced HAM-D17 scores by Week 1 ( $P < 0.001$ ) and until the end of the 6-month treatment period ( $P < 0.001$ ) (Vieta et al. 2001b).

Data from this study were included in a larger analysis involving patients with different index episodes (mania, hypomania, mixed, depressive, or schizoaffective disorder, bipolar type) (Vieta et al. 2001a). In this heterogeneous sample ( $n = 541$ ), highly significant reductions in mean YMRS score occurred by week 1 and continued for 6 months ( $P \leq 0.001$  vs. baseline) for all groups except in patients with depression ( $P < 0.05$  vs. baseline) (Vieta et al. 2001a). Risperidone, either in combination with mood stabilizers or as monotherapy, was relatively well tolerated in bipolar II patients, with little evidence of tardive dyskinesia or EPS-related adverse events emerging over a 6-month period (Vieta et al. 2001b). Double-blind controlled studies in patients with bipolar disorder are needed to confirm the findings from these open-label studies.



\* $P < 0.001$  vs baseline for all groups except patients with depression ( $P < 0.05$  vs baseline)

Figure 4. YMRS scores of patients with bipolar disorder (including patients with schizoaffective disorder, bipolar type) treated with risperidone add-on to mood stabilizers (Vieta et al. 2001a).

Safety and tolerability concerns about risperidone in bipolar disorder included some EPS liability, hyperprolactinemia, and moderate weight gain.

### Clozapine

There is some evidence to suggest that clozapine is effective against mood and psychotic symptoms in patients with schizoaffective disorder, bipolar type, and bipolar I disorder. In addition, results from an open study in patients ( $n = 38$ ) with treatment-resistant schizoaffective or bipolar disorder (DSM-IV) suggest that clozapine may have utility as maintenance treatment (Suppes et al. 1999). Patients in this study were randomly assigned to clozapine add-on treatment or treatment as usual (no clozapine) and followed up to 1 year. The CGI, HAM-D24, Brief Psychiatric Rating Scale (BPRS), and Bech-Rafaelson Mania scales were included in the monthly assessments (Suppes et al. 1999).

Significant advantages across all measures except the HAM-D24 were observed with clozapine versus treatment as usual (Suppes et al. 1999). In particular, clozapine was a strong antimanic and anti-mood lability agent. The decrease in the mean rate of change in BPRS score over 1 year (or until last visit) in the clozapine group revealed highly significant improvement, whereas there was worsening in the treatment-as-usual group ( $-3.68$  vs.  $2.51\%$ , respectively;  $P = 0.001$ ). Moreover, 65% of patients taking clozapine met the criteria for response (30% improvement in BPRS from baseline) by 3 months and 82% by 6 months.

Patients who were switched from their treatment-as-usual to clozapine also benefitted. The nine patients (seven with bipolar I disorder) in whom clozapine was substituted for treatment as usual had significant improvement in BPRS score ( $P < 0.05$ ) over 1 year compared with their score at the termination of usual treatment (Suppes et al. 1999). Overall, the findings suggested that clozapine might have a role in patients with bipolar disorder who do not respond to standard treatments, but this needs to be confirmed in larger controlled studies. The safety profile of clozapine appeared to be worse than some other atypical antipsychotics. Side effects were noted throughout the study (Suppes et al. 1999). In particular, somatic complaints increased relative to baseline in clozapine-treated patients, and were more severe than in the treatment as usual group. No patient developed agranulocytosis during the study, but this side effect is a known concern in the use of clozapine (Ertugrul and Meltzer 2003), besides weight gain and metabolic effects.

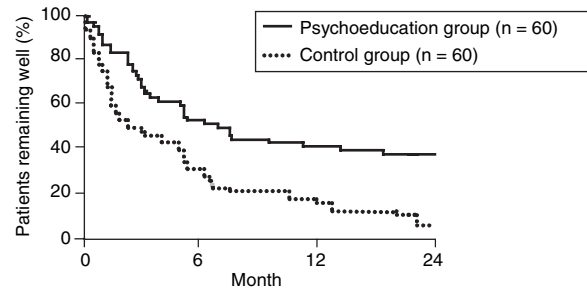
### Aripiprazole

One randomized, double-blind study has compared aripiprazole versus placebo in the maintenance treatment of patients with bipolar disorder (Keck et al. 2006). Patients ( $n=161$ ) who had recently experienced a manic episode or who had just completed an aripiprazole acute mania study were assigned to aripiprazole or placebo for 26 weeks. Time to relapse of symptoms (primary endpoint) and total number of relapses ( $P=0.013$ ) were significantly decreased in patients treated with aripiprazole versus those treated with placebo. No effect was seen in prevention of depression or mixed episodes. Adverse events greater than 10% in the aripiprazole group included anxiety, insomnia, depression, and nervousness.

### Role of psychoeducation in long-term treatment

Concomitant psychosocial intervention during bipolar maintenance treatment is increasingly being recognized as an important tool to enhance treatment adherence and other aspects of illness management (American Psychiatric Association 2002; Goodwin and Vieta 2005). Supporting the benefits of psychoeducation in the maintenance setting are findings from a subanalysis of a study in bipolar I and II outpatients (all receiving a standard pharmacological treatment), which showed that concomitant group psychoeducation produces significantly more stable lithium levels over time ( $P \leq 0.05$  at 6, 16, and 24 months) than nonstructured group meetings (control) (Colom et al. 2005). Such stable lithium levels most likely reflect enhanced long-term adherence to treatment.

Furthermore, intervention with psychoeducation translated into improved outcomes in the parent study, which consisted of a 21-week, single-blind, randomized treatment phase and 2-year follow-up (Colom et al. 2003) (Figure 5). At the end of the follow-up period, group psychoeducation significantly reduced the number of relapsed patients ( $P < 0.001$  psychoeducation vs. control) and increased the time to any recurrence ( $P < 0.001$ ), including depressive (HAM-D17  $\geq 17$ ), mixed (YMRS  $\geq 20$ ; HAM-D17  $\geq 12$ ), and manic (YMRS  $\geq 20$ ) or hypomanic (YMRS  $\geq 12$ ) episodes (Colom et al. 2003). The number and length of hospitalizations per patient were also lower in those who received psychoeducation ( $P < 0.05$ ). Despite the benefits of psychoeducation, however, over half of the patients receiving group psychoeducation (and standard pharmacologic treatment) had relapsed by the end of the 2-year follow-up.



Psychoeducation vs control group,  $P < 0.001$

Figure 5. Psychoeducation versus usual medical care in bipolar disorder over a 2-year period. Data are from a study with a 21-week, randomized, single-blind phase of group psychoeducation and a subsequent 2-year follow-up phase. Recurrence was defined as manic (YMRS  $\geq 20$ ), hypomanic (YMRS  $\geq 12$ ), depressive (HAM-D17  $\geq 17$ ), or mixed (YMRS  $\geq 20$ ; HAM-D  $\geq 12$ ) (Colom et al. 2003b).

### Conclusion

The treatment of patients with bipolar disorder remains a challenge for clinicians. Effective therapeutic approaches are required for the management of acute and chronic symptoms, as well as prophylaxis against future episodes. Controlled studies have shown that atypicals antipsychotics are effective in the treatment of bipolar mania and some also have efficacy in bipolar depression. Some atypicals have also shown promising results in maintenance therapy of bipolar disorder, but concerns about weight gain and metabolic syndrome are increasing. Further research is needed with regards to their potential efficacy in particular conditions, such as bipolar II disorder and rapid cycling. Strategies such as psychoeducation in combination with effective drug therapy may also improve maintenance therapy for bipolar disorder. In the near future, supplementary interventions such as cognitive rehabilitation and interventions focused on physical health may help to reduce the gap between symptomatic recovery and functional recovery.

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### Statement of interest

Dr Vieta has acted as consultant, received grants, or been hired as speaker by the following pharmaceutical companies: Almirall, Astra-Zeneca, Bial, Bristol-Myers-Squibb, Eli Lilly, Glaxo-Smith-Kline, Janssen-Cilag, Lundbeck, Merck-Sharp-Dohme, Novartis, Organon, Otsuka, Pfizer, Sanofi-Aventis, Servier, and UCB pharmaceuticals. Dr Rosa has no

conflict of interest with any commercial or other associations in connection with the submitted article.

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## ORIGINAL INVESTIGATION

# Potential genetic variants in schizophrenia: A Bayesian analysis

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### Abstract

A number of different gene polymorphisms have been found to dispose for the development of schizophrenia. However, no single gene polymorphism is sufficient for the precipitation of schizophrenia. Swedish psychosis patients ( $n=103$ ) and control subjects ( $n=89$ ) were analyzed for 36 single nucleotide polymorphisms in 30 candidate genes for schizophrenia. Evidence of association was analyzed with Bayesian statistical methods. Variants in the genes coding for dopamine-D<sub>2</sub> receptor, brain-derived neurotrophic factor (BDNF), neuropeptide Y (NPY), neuregulin 1, reelin and synapsin 3 showed association with schizophrenia, although few subjects were found in the minority allele for the two latter variants. The six gene variants, all with suspected connection to schizophrenia, were found to be risk factors when considered in combination, but not separately. The results indicate that the Bayesian statistical method gives additional possibilities in the search for risk factors for schizophrenia or other complex disorders.

**Key words:** *BDNF, dopamine-D<sub>2</sub> receptor, neuregulin 1, neuropeptide Y, schizophrenia*

### Introduction

Schizophrenia is a psychiatric disorder that affects approximately 1% of the population and usually leads to functional and social disability for the rest of the life with continuous need for supportive assistance (Andreasen 2000). Schizophrenia typically causes great suffering and loss of quality of life for the ill themselves, their families and society at large. The cost for the society is counted in billions of dollars. This disease afflicts most subjects rather early in life, and at least 10% of individuals with schizophrenia commit suicide (Ösby et al. 2000).

Extensive research during the past four decades has led to the conclusion that schizophrenia's aetiology is heterogeneous, and that both genetic and environmental factors are involved. Heritability for schizophrenia has been estimated to be up to 80%. A number of different risk-increasing genes have been reported, although none of which seems to be sufficient, nor even necessary, for the development of schizophrenia (Sawa and Snyder 2002). Early on, several studies showed association with one gene in specific populations, but lack of independent

association was the rule. However, during the last years promising replications have emerged for a number of candidate genes, such as dysbindin, neuregulin 1 and *RGS4* (Harrison and Owen 2003; Harrison and Weinberger 2005).

In the multidisciplinary research project HUBIN (Human Brain Informatics) at the Karolinska Institutet/Hospital a large number of patients with schizophrenia and healthy control subjects have been systematically characterized with regard to several genotypic and phenotypic variables (Arnborg et al. 2000; Hall et al. 2000). Using sib-pair material, in combination with a cohort in Cardiff, UK, a new chromosomal locus was demonstrated (Williams et al. 2003). A number of association studies have also been performed on the HUBIN population. In a preliminary study we reported a tendency for association between a Ser311Cys variant in the dopamine-D<sub>2</sub> receptor gene and schizophrenia (Arnborg et al. 2002), which was confirmed in a meta-analysis of more than 9000 subjects (Jönsson et al. 2003). A study of the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism between

schizophrenic patients and control subjects did not show association with the diagnosis, although there was an association between the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism and the cerebellar vermis volume (Agartz et al. 2006) in a cohort of 107 individuals, patients and controls.

Despite these findings, research in genetic effects faces several challenges. Establishing genetic effects through standard significance testing would require large samples. Certain potentially relevant genotypes, however, are extremely rare. For research to be effective, it must be focused on those SNPs which are likely to prove fruitful.

The present study was conducted in order to identify such promising SNPs. As this inquiry is a search among a large number of possibilities and not an attempt to establish one (or a few) hypothesis as true effects, a Bayesian analysis offers several appealing features that significance testing (for example, chi-square tests of odds ratios) lack. A Bayesian analysis allows the effect of SNP on the odds of disease to be directly estimated and this estimate is not affected by multiple tests on similar models (Gelman et al. 2004). Significance testing, on the other hand, is specifically designed to prevent mass testing by (rightly) requiring the probability of finding an effect to be lowered when multiple tests are run. Secondly, as just mentioned, genotypes with a potential effect tend to be quite rare. A Bayesian approach allows for inference regardless of sample size, whereas significance testing runs a strong risk of false negatives when the sample is small. This does not, however, make a Bayesian approach immune to sampling error. It should thus be stressed that findings here are merely indications that identified SNPs might be fruitful avenues for future investigation.

## Materials and methods

### Subjects

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Karolinska Hospital and the Swedish Data Inspection Board. All subjects participated after giving informed written consent.

Subjects were Caucasian individuals living in the north-western part of Stockholm County and have been previously described (Jönsson et al. 2003,

2006). The subjects were recruited at the Department of Clinical Neuroscience, Karolinska Hospital, Stockholm, Sweden, and investigated between August 1999 and November 2002. Briefly, subjects were assessed for lifetime psychiatric diagnosis (DSM-III-R and DSM-IV, American Psychiatric Association 1987, 1995) and geographical origin using reviews of hospital case notes and clinical structured interviews (Spitzer et al. 1986, 1988; Wing et al. 1990) performed by psychiatrists trained in Sweden (Ekholm et al. 2005; Vares et al. in press). Control individuals had previously served as healthy comparison subjects in biological psychiatric research at the Karolinska Institute (Farde et al. 1995; Oxenstierna et al. 1996; Damberg et al. 2004) or were drawn from a representative sample of the population in Stockholm County. Routine blood analyses were performed on all subjects, both affected and non-affected.

There were 103 patients (64 men, 39 women, mean age  $\pm$ SD: 42.4  $\pm$  11.5 years) and 89 control subjects (60 men, 29 women, mean age  $\pm$ SD: 39.2  $\pm$  11.5 years). The patients, clinically diagnosed by their treating physician with schizophrenia or schizoaffective disorder, fulfilled research DSM-III-R and DSM-IV diagnoses of schizophrenia ( $n=76$ ), schizoaffective disorder ( $n=15$ ), schizophreniform disorder ( $n=2$ ), psychosis not otherwise specified ( $n=9$ ), or bipolar disorder ( $n=1$ ). None of the control subjects fulfilled diagnostic criteria for any psychosis diagnosis.

### Molecular genetics

DNA was isolated from the blood samples (Geijer et al. 1994). Thirty candidate genes (Table I) were chosen for analysis by the following criteria: either the gene has earlier been reported to be associated with schizophrenia or the corresponding protein may have a role in the mechanism of the disease. In these 30 genes, 36 polymorphisms were picked from different databases (HVBASE, dbSNP, JSNP, CELERA<sup>1</sup>). We selected polymorphisms that are located in the coding part of the gene, reported by at least two groups and with a minor allele frequency of at least 5%, which was used as a definition for being informative.

Published polymerase chain reaction (PCR) primer sequences were used or when needed new PCR primers were designed. Thirty-one of the single nucleotide polymorphisms (SNPs) were analyzed

<sup>1</sup> Electronic web addresses:

HVBASE: <http://hgvbase.cgb.ki.se/>

dbSNP: <http://www.ncbi.nlm.nih.gov/SNP/>

JSNP: <http://snp.ims.u-tokyo.ac.jp>

CELERA/AppliedBiosystem (commercial): <http://myscience.appliedbiosystems.com/>

Table I. Information on the 36 SNPs of the 30 genes studied.

Abbr. gene name	Gene name	SNP name	Locus	Type	SNP location	Result of SNP	Comments
<i>ACHE</i>	Acetylcholinesterase	rs8286	7q22	T/A	CDS exon?	Val333Glu	PS
<i>APOE</i>	Apolipoprotein E	SNP000002314	19q13.2	C/T	CDS exon3	Cys-Arg	PS
<i>BDNF</i>	Brain-derived neurotrophic factor	SNP000006430	11p13	G/A	CDS	Val66Met	PS
<i>BDNF</i>	Brain-derived neurotrophic factor	rs1048221	11p13	G/T	CDS exon?	Arg127Leu	PS
<i>BDNF</i>	Brain-derived neurotrophic factor	rs1048220	11p13	G/T	CDS exon?	Arg125Met	PS
<i>BDNF</i>	Brain-derived neurotrophic factor	rs1048218	11p13	G/T	CDS exon?	Gln75His	PS
<i>CHAT</i>	Choline acetyl transferase	rs868749	10q11.2	C/T			PS
<i>GNTF</i>	Ciliary neurotrophic factor	SNP000006618	11q12.2	A/G	CDS	Arg182His	PS
<i>DBH</i>	Dopamine $\beta$ -hydroxylase	SNP000007898	9q34	G/T	CDS exon1	Ala55Ser	PS
<i>DRD2</i>	Dopamine receptor D2	SNP00000181	11q23.1	G/C	CDS	Ser311Cys	PS
<i>DRD2</i>	Dopamine receptor D2	SNP000003285	11q23.1	C/T	CDS exon1	Ser-Pro	PS
<i>DRD3</i>	Dopamine receptor D3	SNP000000153	3q13.3	G/A	CDS exon5	Ser-Gly	PS
<i>GRIN1</i>	Glutamate receptor, NMDA 1	rs1126448	9q34.3	G/T	CDS nt 3137	Ser682Leu	PS
<i>GRM5</i>	Glutamate receptor, metabotropic 5	rs2941563	11q14.1-14.2	G/T	CDS exon?	Gly1125Gly	PS
<i>HTR1A</i>	5-Hydroxytryptamine receptor 1A	SNP000002381	5q11.2	G/A	CDS exon1	Gly22Ser	PS
<i>HTR1D</i>	5-Hydroxytryptamine receptor 1D	SNP000006081	1p36.3-34.3	C/T	CDS exon1	Ser265Leu	PS
<i>HTR2A</i>	5-Hydroxytryptamine receptor 2A	SNP000006557	13q14-21	A/G	CDS exon1	Ile-Val	PS
<i>HTR5A</i>	5-Hydroxytryptamine receptor 5A	-	7q36.1	C/T	CDS exon1	Pro15Ser	PS
<i>HTR7</i>	5-Hydroxytryptamine receptor 7	-	10q21-24	C/T	CDS exon?	Pro279Leu	PS
<i>MAOA</i>	Monoamine oxidase A	SNP000003322	Xp11.23	A/G	CDS exon3	Arg520Lys	PS
<i>NOTCH4</i>	NOTCH4 <i>Drosophila</i> homologue	rs915894	6p21.3	A/C	CDS exon3?	Gln116Lys	PS
<i>NPY</i>	Neuropeptide Y	SNP000006801	7p15.1	C/A	CDS exon5?	Leu22Met	PS
<i>NPY</i>	Neuropeptide Y	SNP000003321	7p15.1	T/C	CDS exon5?	Leu7Pro	PS
<i>NR4A2</i>	Nuclear receptor, subfamily 2 group A, member 2	rs12803	2q22-23	G/T	3'UT		PS
<i>NRG1</i>	Neuregulin 1	CV2870393	8	C/T		Thr294Met	PS
<i>PDXN</i>	Prodynorphin	48 bp repeat		1-4x repeat			PCR
<i>PENK</i>	Preproenkephalin	SNP000003457	8q23-24	A/G	CDS	Gly 247Asp	PS
<i>PNOC</i>	Prepronociceptin	-	8p21	C/G	CDS exon3	Ala118Gly	PS
<i>PNOC</i>	Prepronociceptin	-	8p21	A/G	CDS exon3	Gln172Arg	PS
<i>RELN</i>	Reelin	rs2229860	7q22	C/G	CDS	Pro1703Arg	PS
<i>RGS4</i>	Regulator of G-protein signalling 4	SNP000020757	1p36.13-31.3	G/T	CDS	Ala195Ser	PS
<i>SLC6A4</i>	Serotonin transporter	Promoter del/ins	17q11.1-12	del/ins 44 bp	Promoter		PCR
<i>SYN2</i>	Synapsin II	-	3p25	A/G	CDS exon3?	Gln296Arg	PS
<i>SYN3</i>	Synapsin III	-	22	AAA-GAG	CDS exon 12	S470N	RFLP, <i>Ban</i> II
<i>TNFR<math>\alpha</math></i>	Tumour necrosis factor $\alpha$	-	6p21.3	G/A	5'UT		RFLP, <i>Nco</i> I
<i>TPH</i>	Tryptophan hydroxylase	SNP000002367	11p15.3	A/C	Intron	splice site change	

Abbreviations: CDS, coding sequence, PCR, analysed with PCR and agarose gel; PS, analysed with pyrosequencing; RFLP, *Ban* II, change of restriction site with *Ban* II; RFLP, *Nco* I, change of restriction site with *Nco* I; UT, untranslated.

by PCR for 50 cycles in a total volume of 25  $\mu$ l with 5–7 pmol primer and 0.5 unit of Hot Star Taq (VWR International, West Chester, PA, USA), followed by pyrosequencing (Ahmadian et al. 2000) according to the manufacturers protocol (Pyrosequencing AB, Uppsala, Sweden). A sequencing primer were also designed for those analyses, see Table II. The two variable number tandem repeats (VNTR) were investigated using only PCR and agarose gel electrophoresis, and another two SNPs were analyzed by PCR followed by restriction enzyme digestion and agarose gel electrophoresis, see Table III. Only the informative polymorphisms were included in the statistical analysis.

### Statistical theory

The goal of the statistical analysis was to identify those of candidate SNPs which showed the greatest promise of having a real relationship with a diagnosis of schizophrenia. The relationship between risk of schizophrenia and genotype was expressed in a logit linear model where the log of the odds ratio  $\eta$  was modeled as a linear regression of the genotype  $X$  with regression coefficients  $\beta$  and error  $\varepsilon$ .

$$\eta = X\beta + \varepsilon \quad (1)$$

where  $\eta$  was related to the risk factor. The regression coefficients  $\beta$  reflect the influence of each polymorphism in the genotype on the risk to develop schizophrenia. The regression was made to the logit of the odds ratio:

$$\eta = g(\Pi) = \ln[\Pi/(1-\Pi)] \quad (2)$$

where  $\Pi$  was the probability of being affected. It was not possible to model  $\Pi$  directly, since  $\eta$  in the linear model has an infinite range while probabilities must be between 0 and 1. The logit function corrects this by mapping  $(-\infty, +\infty)$  to the interval  $[0,1]$ . The value of  $\Pi$  was estimated by the ratio of affected to non-affected subjects in each genotype.

The genotype was represented by a binary code as follows. Each test returns one of three results, say CC, CT or TC, or TT. A two-bit code was used. The first bit indicated if the genotype contained C (0 indicates C, 1 indicates no C). The second bit indicated if the genotype contained T (1 indicates T, 0 indicates no T). Thus CC  $\rightarrow$  00, CT and TC  $\rightarrow$  01, and TT  $\rightarrow$  11. For SNPs where only two genotypes are represented in the data, a one-bit code was used. The regression coefficients  $\beta$  then reflect the influence each allele had on the risk factor. If, for a given polymorphism, having a genotype containing C (that is CC or CT/TC) modified the risk of being affected, the  $\beta_i$  associated with that column of  $X$  should be non-zero. If it had no effect,  $\beta_i$  should be zero.

The true value of  $\beta$  is impossible to determine. Its probability distribution, however, can be estimated using Markov Chain Monte Carlo (MCMC). The credible intervals of the distribution were used to identify SNPs that show an effect. If the probability that  $\beta_i = 0$  was less than 5% (i.e. that the 95% credible interval does not include zero), it can be suggested that the SNP variation was a risk factor for developing schizophrenia (Gelman et al. 2004).

MCMC was implemented using the Bayesian inference Using Gibbs Sampling (BUGS) software (Gilks et al. 1994). A sample of BUGS code can be seen in Scheme 1. The distribution of  $\beta$  was estimated from a 10 000-iteration sample taken after the Markov chain had converged, and averaged over 10 runs.

The technique rests on the estimate of the odds of being affected given genotype. This estimate in turn depends on the number of individuals who have the given genotype. In this study 36 polymorphisms were included, of which 15 were informative in this population. The three possible outcomes for each informative polymorphism give a theoretical potential of  $3^{15}$  genotypes. As this number overwhelms the 192 individuals for whom genetic data were available, results from a full model were not considered reliable.

For our study, the polymorphisms were modelled in groups of three by taking the first to third polymorphisms in the list, then the second to fourth, and so on, wrapping around at the end. Each model had a mathematical potential of 27 ( $=3^3$ ) genotypes, though the number that are biological possible could be lower. Our data contained on average 12 genotypes for each combination of three. Most of these were quite rare. In the present population, for a given combination, two or three genotypes accounted for between a half and one-third of the subjects, with the remaining subjects spread over seven to 11 rare genotypes.

Given the rarity of certain genotypes, the primary study's patient group included a few cases who did not fully meet the DSM-III or DSM-IV criteria for schizophrenia. A second analysis was conducted on the smaller group in which the patient group contained only patients who fulfilled DSM-III or DSM-IV criteria for schizophrenia. The selection of models tested was also increased, by forming random pairs of SNPs to test and with each SNP included in approximately 20 models.

## Results

Models were searched for by MCMC sampling. The technique associated the following six SNPs with increased risk for schizophrenia and related

Table II. PCR primer sequences (5'-) for pyrosequencing analysis.

Gene abbr	PCR primer sequences	Pyrosequencing primer	Informative
<i>ACHE</i>	F: CCTCCAGGCGGCACTG R: b-AGGGCCTCTGGGGTGTCACT	GGTTCTCCTTCGTGCC	No
<i>APOE</i>	F: CCTCCACCTGCGCAAGCTG R: ACGCGGCCCTGTTCCAC	GCCGATGACCTGCAGAAG	Yes
<i>BDNF</i> SNP000006430	F: AAGGCAGTTCAAGAGGCTT R: b-TTGACTACTGAGCATCACCC	CTGTTGGATGAGGACC	Yes
<i>BDNF</i> rs1048218	Same as for SNP000006430	CTGTTGGATGAGGACC	No
<i>BDNF</i> rs1048220	F: CGCAGACTTGACACGTCCA R: b-CTGCTGCCGTTACCCACTCA	TGCAAACATGTCCATGA	No
<i>BDNF</i> rs1048221	Same as for SNP rs1048220	Same as for SNP rs1048220	No
<i>CHAT</i>	F: b-CTATGGGCTCTTCTCCTCCTA R: TGTGCCCTCCCTCACCAAGAC	CGATGACGTGCTCAGGCTC	No
<i>CNTF</i>	F: TCTCTTTGAGAAGAAGCTGTG R: b-CATTTTCTTGTTGTTAGCAAT	CCTTCGTTTCATTTCTTCTC	No
<i>DBH</i>	F: b-TTGCCCCTGCCAGACCTG R: GCAGCAGAGCCCGCTACCT	AGGACCCTGGACCCCCGAAG	Yes
<i>DRD2</i> SNP000000181	F: GCACCAGCCCACCCGAGAG R: b-GCAATCTTGGGGTGGTCTTT	CCAGCTGACTCTCCCCGAC	Yes
<i>DRD2</i> SNP000003285	Same as for SNP000000181	Same as for SNP000000181	No
<i>DRD3</i>	F: CAAGCCCCAAAGAGTCTGAT R: b-CGCAGTAGGAGAGGGCATAG	TGGCATCTCTGAGTCAGC	Yes
<i>GRIN1</i>	F: GCATCACGGGCATCAACGAC R: b-CGCCGCACTCTCGTAGTTGT	CCCCTCGACAAGTTTATCT	No
<i>GRM5</i>	F: b-CCCGAGTCCACCGAGTCTCT R: ACGACCTTTGCCGAAATCCA	TCTGCCGGCCATCGAAGTCA	Yes
<i>HTR1A</i>	F: CAGGGCAACAACACCACATC R: b-CCGCCAAAGAGCCAATAAGAT	Same as forward primer eller CAACACCACATCACCA	No
<i>HTR1D</i>	F: CCACCTCATCACAGGCTCTG R: b-GAAATCCTCTTGCGTTCCAG	CAGCCTCCATGAGGGGCACT	No
<i>HTR2A</i>	F: AACTCCAGAATAAGGCATT R: b-ACCACCCAAAACAGTAGATT	Same as forward primer	No
<i>HTR5A</i>	F: CTCTTGAACACCCCTTCTGC R: b-AGCCCAGCAAGGTGAGAATA	CTCCTTTTCCCTCTCCAC	Yes
<i>HTR7</i>	F: b-CAGGACTTTGGCTATACGAT R: TCCTTCTGGAGCTTCACTAT	CGCTGTCTGGCTCCACTCG	No
<i>MAOA</i>	F: b-CCTTCATCTAGGACGTTCCAG R: GAACAGAACTTCAAGACCGTG	AGACCGTGGCAGGAGCTTGT	No
<i>NOTCH4</i>	F: b-TGAGACGTGCCAGTTTCCCTGAC R: GAGGGAGGACAAGGGTCTTCAA	Same as reverse PCR primer	Yes
<i>NPY</i> SNP000006801	F: b-AGCCCGTCCGTTGAGCC R: CGCAGCGCCGAGTAGTATCTG	GCCTCGGCCAGCGCACCC	No
<i>NPY</i> SNP000003321	Same as for SNP000006801	AGGGTCAGTCCGGACAGC	Yes
<i>NR4A2</i>	F: GCATCCTGGATTTAGAACAT R: b-TTGTCTGAACTGCAACAACC	TGTTCAGAAGAAAGATTGCT	Yes
<i>NRG1</i>	F: AAAGCTGCATGACCGTCTTC R: b-ATAAACTTCAAGCGTCTGA	TCGGTCTGAACGAAAC	Yes
<i>PENK</i>	F: b-CCAGAGTGGTGGATGGACTA R:GCCACTAGTGGGAAAAGATAT	CTTCTTTGGAGTAACTTTTCG	No
<i>PNOC</i>	F: b-CGAGTCCGGAGCTTGTTTC R: CCCTTCCGGCTACACATT	TGCTTCTGCTCCATCTCACC	No
<i>PNOC</i>	Same as for SNP Ala118Gly	Same as reverse primer	No
<i>RELN</i>	F: b-AATGGCAAGGACTGGCATCT R: TGACAGGCACCCAAAGTTCT	CAGCCAATGGTTGGA	Yes
<i>RGS4</i>	F: ATTCTACCGCCGCTTCCTC R: b-TCTTGGCATTTTCATCCCTCT	AGAAAGGAGCCAAGAGTTCA	No
<i>SYN2</i>	F: b-AGAGCGGAACACTTAAGGAT R: GAAAAATTAACCACCAATCA	AATGCCATTACAGTCGAGAA	No
<i>TPH</i>	F: ATTGGATTTGATTTGATTG R: b- GGCAAAACTAGGTTTCAGC	CAGCGTGACAACTTGTACC	Yes

Abbreviations: b, 5'-biotinylated.

Table III. PCR primers used for analyzing SNPs by RFLP analysis.

Gene abbr.	SNP name	PCR primer ssequences	Restriction enzyme for RFLP	Informative
<i>PDYN</i>	48-bp repeat	F: AGCAATCAGAGGTTGAAGTTGGCAGC R: GCACCAGGCGGTTAGGTAGAGTTGTC	–	Yes
<i>PDYN</i>	48-bp repeat	F: AGCAATCAGAGGTTGAAGTTGGCAGC R: GCACCAGGCGGTTAGGTAGAGTTGTC	–	Yes
<i>SLC6A4</i>	long/short – ins/del 44-bp promoter	F: ATGCCAGCACCTAACCCTAATGT R: GGACCGCAAGGTGGGCGGGA	–	Yes
<i>SYN3</i>	Ser470Asn	F: TTTTCTGGCCCCTTAGGAG R: GGATGCCCCGGGATAGCTG	BanII	Yes
<i>TNF<math>\alpha</math></i>	Pro205Leu	F: AGGCAATAGGTTTGTAGGGCCAT R: TCCTCCCTGCTCCGATTCCG	NcoI	Yes

psychosis: *DRD2* (SNP00000181, Ser311Cys), *BDNF* (SNP00006430, Val66Met), Neuropeptide Y (*NPY*) (SNP00003321, Leu7Pro), neuregulin 1 (*NRG1*) (CV2870393, Thr294Met), reelin (*RELN*) (rs2229860, Pro1703Arg) and synapsin-3 (*SYN3*) (Gln296Arg). Eight of ten runs selected *DRD2*, and *BDNF* was selected in seven out of 10 runs. The remaining four appeared in all 10 runs. No other SNPs were selected. The numbers of affected or nonaffected subjects per allele combination in the six final SNPs are shown in Table IV.

These results were further analyzed by testing all pair-wise combinations of these six SNPs. Each SNP can be paired five ways. Each resulting model was simulated 5 times, giving 25 simulations per SNP. The SNPs were found to have an effect with the following frequencies:

*DRD2* 13/25  
*BDNF* 13/25  
*NPY* 19/25  
*RELN* 24/25  
*SYN* 24/25  
*NRG1* 25/25

All SNPs were selected more than half the time. The lower selection rates for *DRD2* and *BDNF* could be due to correlations within the pair. When paired with each other, neither *BDNF* nor *NPY* showed an effect. In all other pairings, however, *NPY* showed an effect in all but one simulation. *BDNF* had a more varied response, showing a response about half the time with *DRD2*, *NRG1*, and *SYN3*, and an effect in all five runs when paired with *RELN*. The full results for all pairings are shown in Table V.

A further investigation of all models with three polymorphisms produced results in line with the pairwise search shown above. Two models are presented in the combined Figure 1 and Table VI.

As a contrast, we also performed standard case–control genetic statistic analyses, i.e. Fisher's exact test for the allelic comparisons and chi-square testing using a Monte Carlo approach for genotype comparisons (Sham & Curtis 1995). When each of these gene variants were analyzed separately the following results emerged: *DRD2*:  $p=0.13$  and  $p=0.37$  for allele and genotype comparisons, respectively; *BDNF*:  $p=0.26$ ,  $p=0.20$ ; *RELN*:  $p=0.22$ ,  $p=0.21$ ; *NPY*:  $p=0.43$ ,  $p=0.78$ ; *NRG1*:

```

model logit;
const
  N = 19,      # number of observations
  C = 2;      # number of covariates
var
  alpha, beta[C], pi[N], aff[N], tot[N], x[N,C];
data in "s_genetic.dat";
{ # begin model
  alpha ~ dnorm(0,0.001);          # prior on alpha
  for (c in 1:C){
    beta[c] ~ dnorm(0,0.001);     # prior on beta
  }
  for (i in 1:N){
    logit(pi[i]) <- alpha + beta[1] * x[i,1]
                          + beta[2] * x[i,2];
    aff[i] ~ dbin(pi[i],tot[i]);
  }
} # end model

```

Scheme 1. Sample BUGS code. This template is used for all of the genetic data, modified for the actual number of observations and covariates in the data file.

Table IV. Number of affected or nonaffected subjects in the six final SNPs.

Gene name	Total number of subjects per allele combination	Number of affected subjects per allele combination	Number of non-affected subjects per allele combination
Dopamine receptor D2	C/C: 186 C/G: 5 G/G: 1	C/C: 98 C/G: 4 G/G: 1	C/C: 88 C/G: 1 G/G: 0
Brain-derived neurotrophic factor	G/G: 121 G/A: 62 A/A: 9	G/G: 67 G/A: 34 A/A: 2	G/G: 54 G/A: 28 A/A: 7
Reelin	G/G: 190 C/G: 2 C/C: 0	G/G: 103 C/G: 0 C/C: 0	G/G: 87 C/G: 2 C/C: 0
Neuropeptide Y	T/T: 179 T/C: 12 C/C: 1	T/T: 97 T/C: 6 C/C: 0	T/T: 82 T/C: 6 C/C: 1
Neuregulin 1	T/T: 117 C/T: 67 C/C: 8	T/T: 64 C/T: 38 C/C: 1	T/T: 53 C/T: 29 C/C: 7
Synapsin 3	GAG/GAG: 190 GAG/AAA: 2 AAA/AAA: 0	GAG/GAG: 101 GAG/AAA: 2 AAA/AAA: 0	GAG/GAG: 89 GAG/AAA: 0 AAA/AAA: 0

$p=0.27$ ,  $p=0.69$ ; SYN3:  $p=0.50$ ,  $p=0.50$ . Thus, in this small material there were no significantly statistical findings. So far, three of these gene variants (*DRD2* Ser311Cys, *BDNF* Val66Met, *NRG1* Thr294Met) have been analyzed in an enlarged sample (173–187 schizophrenic patients) from the same population with evidence for association between the *DRD2* (Jönsson et al. 2003) and *NRG1* (unpublished data), but not *BDNF* variants and schizophrenia. However, the *BDNF* results were in the same direction as other studies reporting association between this gene variant and schizophrenia and as a preliminary meta-analysis of more than 6000 subjects (Jönsson et al. 2003).

Table V. Results of pairing over 5 runs.

First element	Second element	First has effect	Second has effect
<i>DRD2</i>	<i>BDNF</i>	3	3
	<i>RELN</i>	3	5
	<i>NPY</i>	1	4
	<i>NRG1</i>	3	5
	<i>SYN3</i>	3	5
<i>BDNF</i>	<i>RELN</i>	5	5
	<i>NPY</i>	0	0
	<i>NRG1</i>	2	5
	<i>SYN3</i>	3	4
<i>RELN</i>	<i>NPY</i>	4	5
	<i>NRG1</i>	5	5
	<i>SYN3</i>	5	5
<i>NPY</i>	<i>NRG1</i>	5	5
	<i>SYN3</i>	5	5
<i>NRG1</i>	<i>SYN3</i>	5	4

Results from the second study on the reduced patient group (containing only patients who fulfilled DSM-III-R or DSM-IV criteria for schizophrenia) were largely consistent with the first study, with the same six SNPs identified as having an effect. The study gave one additional finding, as the 5-HT<sub>5A</sub> receptor gene (*HTR5A*) showed an effect. It should be noted that the study contained only two subjects with type T/T for this gene, one healthy and one with a schizoaffective diagnosis. Eliminating the schizoaffective patient meant that *HTR5A* T/T variant was only found in the control group in the second study. The resulting reduction in odds of diagnosis given a T allele at this SNP is most likely a sampling error.

## Discussion

The 36 SNPs included in the study were selected from molecular, aetiological, pathophysiological or pharmacological reasons to be involved in schizophrenia (“candidate genes”). Statistical links, however, have proved elusive. Other work, such as by Arnborg et al. (2002), has found the data insufficient to reject the hypothesis that the gene has no effect.

The results of this Bayesian data analysis show that six identified genes may have some involvement in the development of schizophrenia. The number of subjects is low in this pilot study, restricting this analysis to identifying genes that might be important risk factors. It should be noted that for four of these six, the evidence comes from only one or two individuals. The remaining two, *BDNF* and

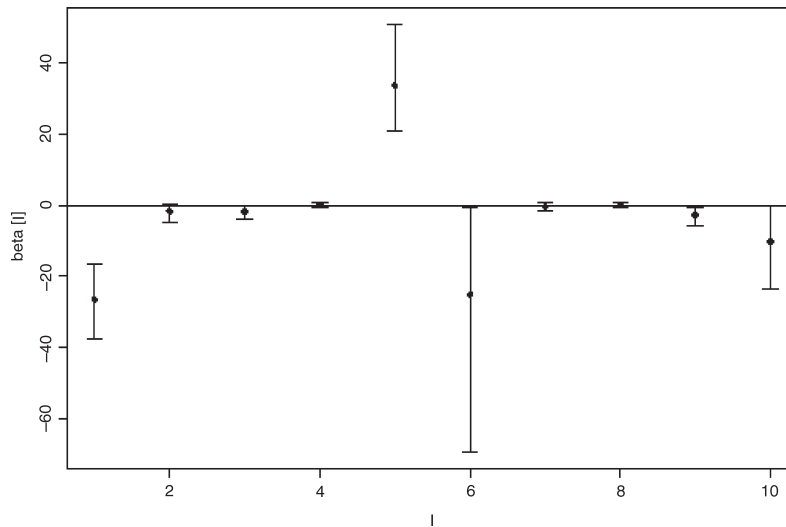


Figure 1. The 95% credible interval for  $\beta_i$ . Table VI indicates which index refers to which SNP.

*NRG1*, have more than eight subjects with the rarest genotype. The proteins arising from these six genes have very different functions. All have previously been discussed to be associated to schizophrenia, although this has not been widely accepted for some of the genes, due to lack of replication in other studies or populations.

The *dopamine-D<sub>2</sub> receptor* has been considered to be involved in schizophrenia, mainly because of the affinity for this receptor among all antipsychotic drugs. In a preliminary study using a HUBIN sample, there was a tendency for association between a Ser311Cys variant in the dopamine-D<sub>2</sub> receptor gene and schizophrenia (Arnborg et al. 2002). In an enlarged material the association was manifest and

Table VI. The mean and 95% credible interval of  $\beta_i$  from one run of the MCMC sampler.

I	SNP	Mean	Bottom	Top
1	<i>DRD2</i>	-26.44	-37.76	-16.48
2		-1.76	-5.03	0.51
3	<i>BDNF</i>	-1.71	-3.71	-0.09
4		0.10	-0.50	0.75
5	<i>RELN</i>	33.49	20.69	50.55
6	<i>NPY</i>	-25.07	-69.27	-0.61
7		-0.43	-1.73	0.83
8	<i>NRG1</i>	0.16	-0.46	0.78
9		-2.71	-5.77	-0.70
10	<i>SYN3</i>	-10.23	-23.62	-0.31

Estimates from runs of the MCMC sampler on models combining three SNPs are shown. One model contained *DRD2*, *BDNF*, and *RELN*, while the second contained *NPY*, *NRG1*, and *SYN3*. The SNPs were encoded using two bits except *RELN* and *SYN3*, which were represented only two genotypes each. Each SNP was therefore represented by two covariates in the model. For each of the SNPs in the table, one of the associated covariates has a credible interval that excludes zero, whereas the second covariate did not. These data are also presented graphically in Figure 1.

supported by several meta-analyses of more than 9000 subjects (Glatt et al. 2003; Jönsson et al. 2003; Glatt and Jönsson 2006). In a previous study we also detected an association between another dopamine-D<sub>2</sub> receptor variant (-141C Ins/Del) and schizophrenia (Jönsson et al. 1999), although a meta-analysis has so far not been able to support this finding (Glatt et al. 2004). It is thus not surprising that the dopamine-D<sub>2</sub> receptor falls out as one of the six genes suggested in the present study to be risk factors for schizophrenia.

*BDNF* belongs to a group of growth factors, which are highly relevant or essential during the early growth and development. BDNF is also relevant in the adult brain where it is being released in the striatum and the hippocampus as a consequence of brain activity. The polymorphism Val66-Met, which stood out in our study, was recently found by Egan et al. (2003) to affect human memory and activation in the hippocampus in a learning task using fMRI. Furthermore, this study (Egan et al. 2003) showed that the polymorphism, which occurs in *pro-BDNF*, a part of the precursor to *BDNF* but not in the molecule itself, affects the intracellular handling of BDNF, which is not properly secreted. Thus, the amount of message (RNA) and protein are unchanged but it is the activity-dependent release that is affected. This study also illustrates the need for a differential search for correlation between various markers of the phenotype and genetics. A more recent study has shown that the Val66Met polymorphism was associated with schizophrenia, an association even more prominent in a haplotype analysis including the Val66Met polymorphism (Neves-Pereira et al. 2005).

Another recent study (Sklar et al. 2002) indicates the relevance of the Val66Met polymorphism in

pro-BDNF for human psychopathology. Of altogether over 60 markers studied in bipolar disorder, this marker was the only one identified as a risk locus (Sklar et al. 2002). A microsatellite marker within 1 kb of the *BDNF* gene was previously associated with parietal lobe volume in schizophrenia (Wassink et al. 1999) and may well have identified the same polymorphic locus.

Another protein that has been given much recent attention is neuregulin 1 (*NRG1*), a growth factor affecting neuronal migration and differentiation. In the adult brain it is a modulator of synaptic plasticity. A polymorphism in the *NRG1* gene has been found to be associated with schizophrenia in an Icelandic cohort (Stefansson et al. 2002).

*NRG1* interacts with several erbB receptors. One of the *NRG1* receptors, erbB3, has been shown to be lower in the prefrontal cortex of schizophrenic patients than in controls (Corfas et al. 2004). Moreover, the erbB4-receptor is co-localized with the glutamate NMDA-receptor and regulates its kinetic properties and as there is substantial evidence that glutamate neurotransmission is abnormal in schizophrenia this further strengthens the indications of a connection between *NRG1* and schizophrenia.

*Reelin* is a protein, expressed in GABAergic interneurons, that regulates neuronal growth and positioning during brain development (Impagnatiello et al. 1998). Several studies have shown that reelin and mRNA coding for reelin are reduced up to 50% in several brain areas of post-mortem brains of patients with schizophrenia compared to controls (Impagnatiello et al. 1998; Costa et al. 2002). Variations in genes encoding reelin have been suggested to be involved in the cytoarchitectural abnormalities observed in post-mortem brains of schizophrenics. However, no association of reelin and schizophrenia was found in a study of 150 schizophrenic patients (Akahane et al. 2002). This study is therefore to our knowledge the first demonstration of an association of reelin with schizophrenia. It should, however, be noted that only two subjects (nonaffected controls) out of 192 had the C/G variation, and this SNP only stands out in combination with the other SNPs. This finding must be verified in a larger sample of subjects.

*Neuropeptide Y*. Some studies have show alterations in *NPY* in schizophrenia (Caberlotto and Hurd 2001). Genetic analyses of the *NPY* gene, assigned to chromosome 7p15.1, in schizophrenia have revealed that the -485T allele is more common in schizophrenia (Itokawa et al. 2003). The SNP that shows up in the present study, Leu7Pro has to our knowledge not previously been associated with schizophrenia. This polymorphism appears to be

associated with lipid metabolism (Karvonen et al. 1998; Schwab et al. 2002). Studies showing association to alcoholism have also been published (Kauhanen et al. 2000; Ilveskoski et al. 2001; Lappalainen et al. 2002).

*Synapsin 3*. The synapsins are synaptic vesicle-associated phosphoproteins which are involved in synaptogenesis and neurotransmitter release (Ohmori et al. 2000). The synapsin 3 gene has been located at chromosome 22q12-13, which has been suggested as a potential susceptibility locus for schizophrenia. However, no significant positive association between synapsin 3 polymorphisms and schizophrenia has been observed (Ohmori et al. 2000). Previous results have therefore suggested that the synapsin 3 gene polymorphisms do not confer increased susceptibility to schizophrenia. As with reelin, it should be noted that only two subjects (schizophrenic patients) out of 192 had the C/G variant, and the SNP does only show significance in combination with the other SNPs. Also this finding must be verified in a larger sample of subjects.

One difficulty with genetic studies is the rarity of certain genotypes. It should be noted that the procedure rests on estimating the odds of being affected given a certain genotype or combination of genotypes. If one of the genotypes in the model has just one or two members, the estimate of the odds could be unreliable. When the more common genotype was evenly divided between the control and subject groups (indicating no association between the SNP and the disease), any finding of effect is highly dubious. The number of subjects per allele combination for all selected SNPs is shown in Table IV, where it is shown that four of the six SNPs identified suffer this problem: *DRD2*, *NPY*, *RELN* and *SYN3*. For *DRD2*, the only G/G subject was affected, as were four of the five C/G subjects. The C/Cs were evenly split. This suggests that the G allele may increase the odds. The suggestion was not supported by our approach. When the G/G subject was removed from the data, *DRD2* was no longer identified as having an effect. For *NPY*, there was only one subject with the C/C genotype, who was not affected. The T/C and T/T groups were evenly divided between affected and non-affected subjects. For *RELN*, there were two subjects with C/G, none of whom were affected. The G/G genotype was evenly split while the C/C genotype was not represented in the data. For *SYN3*, the two subjects with GAG/AAA were both affected, the GAG/GAG were evenly split, and the AAA/AAA were not represented.

The two remaining SNPs, *BDNF* and *NRG1*, have more supporting evidence. There are nine subjects with the A/A genotype for *BDNF*, only

two of whom are affected. The other two alleles are evenly split between affected subjects and nonaffected subjects. For *NRG1*, the C/C genotype has only one out of eight affected subjects, with the other two alleles evenly split. In the HUBIN database, there is data for *NRG1* for 256 subjects. The additional data on *NRG1* was excluded from the analysis above, as data were not available for the other SNPs. If we include these additional subjects, the number of affected C/Cs drops to one out of 10.

## Conclusions

This study demonstrates one approach for identifying SNPs which might show a biochemical link to schizophrenia. A Bayesian approach resolves some of the difficulties which challenge a significance-test based investigation. Thirty-six SNPs were originally selected on solid biological grounds. Despite a minor allele frequency of at least 5% in the general population, only 15 of these proved informative in a population of 192 subjects. Of these 15, six showed some evidence of effect, though sample sizes were too small to establish significance. Quite obviously these tests must be verified and validated in larger samples, especially including more representatives of the uncommon genotypes. However, the number of candidate SNPs deserving such study has been reduced 6-fold.

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## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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ORIGINAL INVESTIGATION

## Brain activation in elderly people with and without dementia: Influences of gender and medication

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### Abstract

Patients suffering from dementia show altered functional brain activation patterns especially in prefrontal brain regions, as research suggests. The present study follows three aims: to replicate these findings, to investigate treatment effects when administering galantamine, and to put gender differences in focus. We compared 12 patients with dementia to 12 age- and gender-matched healthy subjects regarding changes in haemoglobin concentration in brain tissue while performing a verbal fluency task (VFT). Concentration changes of oxygenated ( $O_2Hb$ ) and deoxygenated (HHb) haemoglobin were measured by multi-channel near-infrared spectroscopy (NIRS), an easily applicable and non-invasive method of optical topography. In the patient group, measurement was repeated 4 and 8 weeks after starting treatment with galantamine. The results showed a reduced increase in  $O_2Hb$  during task performance for patients compared to healthy controls. Furthermore, female subjects showed more pronounced activation in  $O_2Hb$  as well as HHb compared to male subjects. Regarding treatment effects, no clear results could be obtained. In HHb, evidence for an entrainment effect was found. In the light of existing literature, the present study suggests an interaction of gender and age regarding brain activation patterns which should be aimed at in future investigations.

**Key words:** Near-infrared spectroscopy, dementia, verbal fluency task, galantamine

### Introduction

Near-infrared spectroscopy is a non-invasive method to continuously measure changes in cerebral haemoglobin concentration. During the last few years it has become a frequently used technique, especially in psychophysiological research (Obrig et al. 2000; Strangman et al. 2002) because it is easily applicable and less restrictive for patients compared to fMRI or PET. With NIRS it is possible to detect BOLD-like responses in certain brain areas during the performance of neuropsychological tests. In several studies it could be shown that during the active phase of cognitive tasks, such as the Wisconsin Card Sorting Test or a verbal fluency task, the concentration of oxygenated haemoglobin increased while deoxygenated haemoglobin decreased (Villringer et al. 1993; Fallgatter and Strik 1998; Schroeter et al. 2002). Based on a seminal PET study of Fox and Raichle (1986), such a pattern of increasing oxygenated

haemoglobin and decreasing deoxygenated haemoglobin is considered to reflect brain activation.

Using a verbal fluency task (VFT), activation patterns showing increase of  $O_2Hb$  and decrease of HHb were found with most distinct activation in frontal and temporal cortical regions (Kameyama et al. 2004; Herrmann et al. 2003), which is in accordance with results gained with other imaging techniques (Cuenod et al. 1995; Dickins et al. 2001). Furthermore, it could be shown that increases in  $O_2Hb$  were larger for males than for females (Kameyama et al. 2004).

A dominance of the left hemisphere during verbal tasks could be found in several studies (e.g. Henriques and Davidson 1997; Herrmann et al. 2006). Hemispheric asymmetry was also reported for other cognitive tasks (Cabeza et al. 2004) mainly in younger subjects; studies with elderly people showed a reduction or loss of lateralization

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(El-Yagoubi et al. 2005; Herrmann et al. 2006; Cabeza 2002), which holds true also for dementia patients (Fallgatter et al. 1997).

In several neurophysiological and neuroimaging studies, patients with dementia showed activation deficits, particularly in the hippocampus and frontal and parietal cortical regions (For a review see Prvulovic et al. 2005). A marked reduction of oxygenation in these degenerating cortical areas during a VFT could be shown in an NIRS study with Alzheimer's disease patients (Hock et al. 1997).

The purpose of the present study was to investigate differences in cerebral haemoglobin concentration between dementia patients and healthy controls, while also considering possible gender differences. Furthermore, we examined to what extent NIRS activation patterns in the dementia group changed when administering galantamine, a substance used for dementia treatment.

## Methods

### Subjects

Twelve patients suffering from dementia and 12 healthy subjects matched for gender, handedness, and age (see Table I) with women tending to be older than men ( $M_{\text{female}} = 69.6$ ,  $M_{\text{male}} = 65.0$ ;  $p = 0.057$ ) were investigated in a non-blind manner. The patients were consecutively recruited within a larger study on dementia investigating 37 dementia patients with different neuropsychological tests. Thirteen patients completed all three NIRS measurements, one of them had to be excluded due to measurement artefacts. All were in- or outpatients of the University Hospital of Psychiatry and Psychotherapy in Wuerzburg diagnosed as having probable mild to moderate dementia according to the ICD-10 criteria (Dilling et al. 2005). All subjects were naïve to galantamine medication at the beginning of the study, but some subjects (six patients and five healthy controls) took medication for high cholesterol (two patients, one control), high blood pressure (four controls) or mild depression (five

patients, one control). Two patients took risperidon (Risperdal® tablets, 1 mg/2 mg) which was stopped when entering the study. All subjects gave written informed consent. The study was approved by the local ethics committee and designed in accordance with the Declaration of Helsinki.

All subjects had to perform the DemTect (Kalbe et al. 2004), a measurement of dementia severity that includes different tasks for testing, e.g. verbal learning, retrieval or attention. Furthermore, all dementia patients were measured three times: at T1 without medication, at 4 weeks (T2) and at 8 weeks (T3) later under medication with galantamine. Galantamine was administered as Reminyl® tablets with most patients taking 8 mg/day (4-0-4). Galantamine doses were adjusted following individual treatment needs. In one patient, the dose had to be lowered to 2 mg/day (2-0-0) after 1 week because of diarrhoea which then vanished. This was the only reported side effect.

As expected, at T1 the patients showed a significantly worse performance in the DemTect compared to the control group. The performance did not change in a significant way over time (For DemTect scores, see Table I). Furthermore, no gender differences regarding performance were found. The data at T1 of 15 subjects (eight patients, seven controls) will also be published elsewhere.

### Measurement

All subjects had to perform a verbal fluency task (VFT) consisting of two versions: a letter version where three different letters (A, F, and S) were presented and the subjects had to produce as many nouns as possible starting with these letters, and a category version where nouns should be given belonging to a certain category (animals, fruits, and flowers). A block design was used where each condition of the respective version lasted 30 s followed by 30 s resting period.

The dementia group had a worse performance in the letter version as well as in the category version of the VFT compared to the healthy controls (see

Table I. Sample description and performance data (DemTect, VFT) for the patient and control group, respectively.

	Healthy controls <sup>a</sup>	Dementia patients		
		T1	T2	T3
Mean age	66.3 ± 3.2 ( $t[14.63] = 1.1$ ; n.s.)	69.1 ± 7.7		
males/females	5/7	5/7		
lefthanders	1	1		
DemTect	17.2 ± 1.3 ( $t[22] = 7.0$ ; $p < 0.001$ )	8.1 ± 4.3	9.1 ± 3.6	9.1 ± 5.1
VFT letter	21.3 ± 6.6 ( $t[22] = 2.6$ ; $p = 0.018$ )	14.0 ± 7.5	13.3 ± 8.5	13.0 ± 9.2
VFT category	33.9 ± 7.0 ( $t[22] = 4.8$ ; $p < 0.001$ )	19.5 ± 7.6	18.6 ± 5.7	18.4 ± 7.6

<sup>a</sup>Data given in brackets refer to the comparison between healthy controls and dementia patients at T1.

Table I). There was no significant change in performance over time in the dementia group (letter version:  $F[1.7; 21.2] = 0.389$ , n.s.; category version:  $F[2.0; 23.8] = 0.539$ , n.s.). Again, no gender differences were detected.

During the VFT we acquired data of the cerebral tissue oxygenation in all subjects using 24-channel NIRS equipment (ETG-100 Optical Topography System, Hitachi Medical Co., Japan). The exact functioning of this NIRS device has already been described elsewhere (Ehlis et al. 2005). In the present study, two arrays of  $3 \times 3$  light detectors and emitters were used, which enabled us to measure 12 points in two areas of  $6 \times 6$  cm<sup>2</sup>. Each of the 12-channel probes was placed over one hemisphere with the most posterior and inferior channel (10) over electrode positions T3 and T4, respectively. With this set-up, we are able to measure the dorsolateral prefrontal cortex (DLPFC) assumed to be involved in VFT processing and already found to be less activated in dementia (see Hock et al. 1997). Integral mode was used to obtain averaged data of HHb- and O<sub>2</sub>Hb-concentration over the three blocks of each VFT version.

#### Statistical analysis

Statistical analyses were performed using SPSS for Windows, version 12.0.

For analysis, HHb and O<sub>2</sub>Hb were averaged over the baseline and task phase, respectively. These mean values were used when comparing patients and healthy controls. Each probe set was divided into three areas consisting of four channels: an anterior area covering only DLPFC structures (channels 1, 3, 6, 8), a posterior area mainly covering more central cortical structures (channels 2, 5, 7, 10) and one intermediate area (channels 4, 9, 11, 12). A projection of the channel locations on a standard brain is displayed in Figure 4. ANOVAs with the within-factors 'hemisphere' (left vs. right), 'condition' (baseline vs. activation period) and 'area' (anterior, middle, posterior) and the between-factors 'diagnosis' (dementia vs. healthy controls) and 'gender' (male vs. female) were calculated.

To assess treatment effects in the dementia group, ANOVAs with the above within-factors and an additional factor 'time' (T1, T2, T3) were calculated while keeping the between-factor 'gender'. Whenever necessary, Greenhouse–Geisser correction was used and post-hoc tests (*T*-tests as well as post-hoc ANOVAs) were performed. Explorative correlations (Pearson) between each baseline-corrected area and the performance in the VFT have been performed to see if there are certain kinds of correlation patterns.

## Results

### Dementia patients at T1 and control group

*HHb.* In the category version, there were significant main effects of 'condition' ( $F[1; 20] = 13.4$ ;  $p = 0.002$ ) and 'hemisphere' ( $F[1; 20] = 10.9$ ;  $p = 0.004$ ), and interaction effects of 'condition  $\times$  area' ( $F[1.6; 33.3] = 3.5$ ;  $p = 0.050$ ) 'condition  $\times$  hemisphere' ( $F[1; 20] = 11.1$ ;  $p = 0.003$ ), 'gender  $\times$  hemisphere' ( $F[1; 20] = 5.2$ ;  $p = 0.034$ ), 'condition  $\times$  gender  $\times$  hemisphere' ( $F[1; 20] = 5.5$ ;  $p = 0.029$ ), 'diagnosis  $\times$  gender  $\times$  hemisphere' ( $F[1; 20] = 4.6$ ;  $p = 0.044$ ) and 'diagnosis  $\times$  gender  $\times$  condition  $\times$  hemisphere' ( $F[1; 20] = 4.7$ ;  $p = 0.042$ ).

Only female dementia patients showed significant HHb decreases in both hemispheres (left:  $T[6] = 2.5$ ;  $p = 0.049$ ; right:  $T[6] = 3.5$ ;  $p = 0.014$ ), in all other groups no significant changes in deoxygenated haemoglobin could be detected between baseline and activation period. These results are displayed in Figure 1.

In the letter version, there were results comparable to the category version with a significant main effect of 'condition' ( $F[1; 20] = 26.0$ ;  $p < 0.001$ ) and interaction effects of 'gender  $\times$  hemisphere' ( $F[1; 20] = 6.1$ ;  $p = 0.022$ ) and 'gender  $\times$  condition  $\times$  hemisphere' ( $F[1; 20] = 5.8$ ;  $p = 0.026$ ).

Post hoc tests showed that during activation, the HHb concentration decreases in both hemispheres in female subjects (left:  $T[13] = 5.9$ ;  $p < 0.001$ ; right:  $T[13] = 4.1$ ;  $p = 0.001$ ) whereas the decrease in male subjects is only significant in the right hemisphere ( $T[9] = 2.4$ ;  $p = 0.039$ ).

*O<sub>2</sub>Hb.* In the category version, significant main effects of 'condition' ( $F[1; 20] = 23.1$ ;  $p < 0.001$ ), 'diagnosis' ( $F[1; 20] = 6.6$ ;  $p = 0.019$ ) and 'gender' ( $F[1; 20] = 6.7$ ;  $p = 0.017$ ) as well as interaction effects of 'diagnosis  $\times$  condition' ( $F[1; 20] = 5.9$ ;  $p = 0.024$ ; see Figure 1), 'gender  $\times$  condition' ( $F[1; 20] = 6.0$ ;  $p = 0.024$ ), 'diagnosis  $\times$  gender  $\times$  area' ( $F[1.6; 32.5] = 5.8$ ;  $p = 0.011$ ), 'gender  $\times$  hemisphere  $\times$  area' ( $F[1.9; 38.6] = 4.9$ ;  $p = 0.014$ ), 'gender  $\times$  hemisphere  $\times$  area  $\times$  condition' ( $F[1.9; 38.7] = 5.0$ ;  $p = 0.013$ ) and 'diagnosis  $\times$  gender  $\times$  area  $\times$  condition' ( $F[1.7; 33.1] = 6.5$ ;  $p = 0.006$ ) were detected.

No significant O<sub>2</sub>Hb increase during activation was found for male healthy controls and patients in any area. For female controls all areas showed O<sub>2</sub>Hb increase:  $T[6] = 6.3$ ;  $p = 0.001$  (anterior area),  $T[6] = 9.9$ ;  $p < 0.001$  (middle area) and  $T[6] = 8.5$ ;  $p < 0.001$  (posterior area). For the female patient group significant increase was detected in the anterior ( $T[6] = 3.9$ ;  $p = 0.008$ ) and middle area ( $T[6] = 2.6$ ;  $p = 0.039$ ). Female controls showed significantly more increase than patients (anterior:  $T[12] = 4.0$ ;

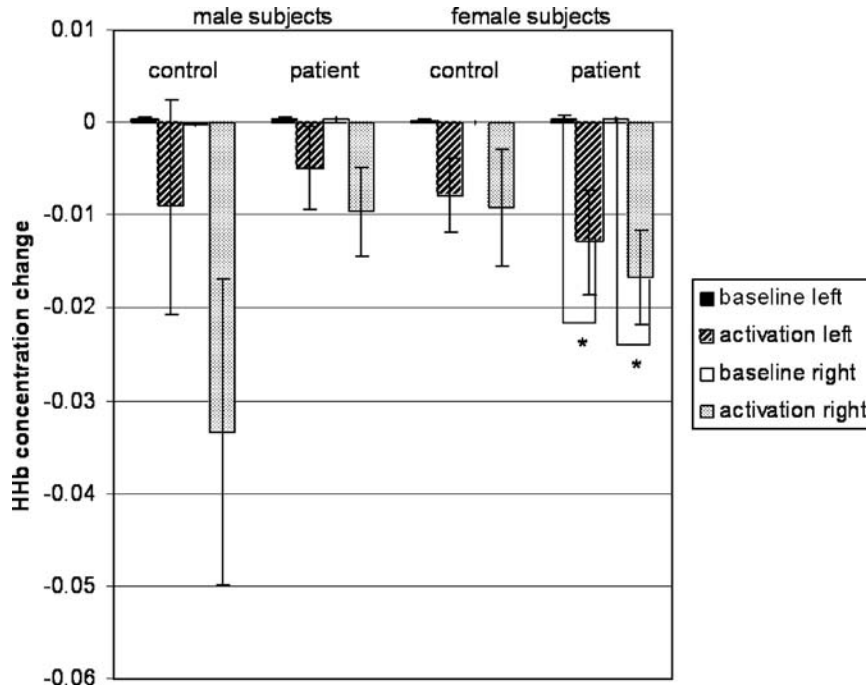


Figure 1. HHb concentration change in the category version of the VFT at T1. Changes in haemoglobin concentration are demonstrated in both hemispheres for male and female patients and healthy controls, separately. Significant HHb decreases between baseline and activation period are flagged (\* $p < 0.05$ ). Bars are standard errors.

$p = 0.002$ ; middle:  $T[12] = 4.5$ ;  $p = 0.001$ ; posterior:  $T[12] = 3.6$ ;  $p = 0.003$ ). Figure 2 demonstrates these findings.

In the letter version, there only was a significant main effect of 'condition' ( $F[1; 20] = 12.4$ ;  $p = 0.002$ ) with  $O_2Hb$  increasing during activation.

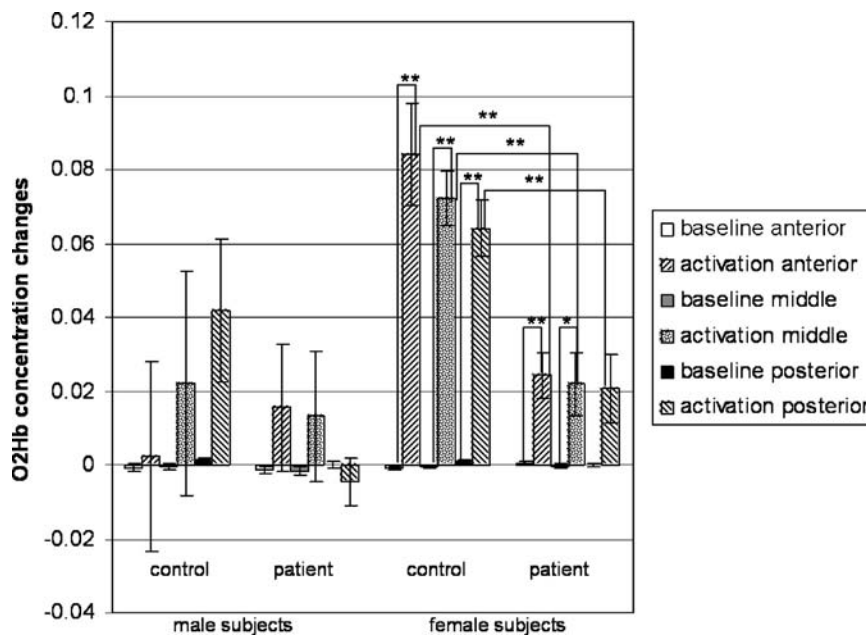


Figure 2.  $O_2Hb$  concentration change in the category version of the VFT at T1. Changes in haemoglobin concentration are demonstrated in all three areas of the probe sets for male and female patients and healthy controls, separately. Significant  $O_2Hb$  increases between baseline and activation period are flagged as well as differences between female patients and healthy controls (\* $p < 0.05$ ; \*\* $p < 0.01$ ). Bars are standard errors.

*Dementia patients' treatment effects*

**HHb.** In the category version, a significant main effect of 'condition' ( $F[1;10] = 10.1$ ;  $p = 0.010$ ) and interaction effects of 'time  $\times$  hemisphere' ( $F[1.3; 12.9] = 4.6$ ;  $p = 0.044$ ), 'time  $\times$  area  $\times$  gender' ( $F[2.7; 26.7] = 3.8$ ;  $p = 0.026$ ) and 'time  $\times$  condition  $\times$  area  $\times$  gender' ( $F[2.8; 28.5] = 3.7$ ;  $p = 0.025$ ) were detected. To investigate treatment influence, post hoc ANOVAs with the factor 'time' were performed for each area and split by gender using baseline-corrected HHb concentration. None of these comparisons reached significance, so further post hoc *t*-tests were performed that revealed that male patients had significant HHb decrease at T2 in the anterior ( $T[4] = 3.6$ ;  $p = 0.022$ ) and middle ( $T[4] = 3.0$ ;  $p = 0.041$ ) area, whereas females showed decrease at T1 (middle:  $T[6] = 2.9$ ;  $p = 0.029$ , posterior:  $T[6] = 3.1$ ;  $p = 0.020$ ) and T3 (anterior:  $T[6] = 4.1$ ;  $p = 0.006$ , middle:  $T[6] = 3.4$ ;  $p = 0.014$ ). These results are shown in Figure 3.

In the letter version, there were significant main effects of 'area' ( $F[1.9; 18.8] = 6.7$ ;  $p = 0.007$ ) and 'condition' ( $F[1;10] = 20.0$ ;  $p = 0.001$ ) as well as an interaction effect of 'area  $\times$  condition' ( $F[1.9; 19.0] = 7.7$ ;  $p = 0.004$ ): a post hoc ANOVA revealed a significant decrease of HHb during the active phase ( $F[1; 11] = 22.9$ ;  $p = 0.001$ ) as well as an effect of 'area' ( $F[1.9; 21.3] = 6.4$ ;  $p = 0.007$ ) with a more pronounced decrease in the anterior ( $p = 0.013$ ) and middle ( $p = 0.012$ ) area compared to the posterior area.

**O<sub>2</sub>Hb.** In the letter version, a significant main effect of 'area' ( $F[1.5; 15.1] = 7.3$ ;  $p = 0.009$ ) and interaction effects of 'gender  $\times$  area' ( $F[1.5; 15.1] = 4.5$ ;  $p = 0.038$ ), 'area  $\times$  condition' ( $F[1.5; 14.8] = 7.8$ ;  $p = 0.008$ ) and 'gender  $\times$  area  $\times$  condition' ( $F[1.5; 14.8] = 4.7$ ;  $p = 0.036$ ) could be found showing that O<sub>2</sub>Hb increases during activation only in female subjects in the anterior area ( $T[6] = 2.7$ ;  $p = 0.034$ ).

In the category version, there were no significant results.

*Correlations of brain activity with performance in the VFT*

In the letter version, no significant correlations in any subject group could be found after applying Bonferroni correction neither with HHb nor with O<sub>2</sub>Hb.

In the category version, three correlations between HHb concentration change and VFT performance in the female patient group withstood a Bonferroni correction: At T1, negative correlations in the left and right anterior area were found (left:  $r = -0.908$ ,  $p = 0.005$ ; right:  $r = -0.883$ ,  $p = 0.008$ ), and at T2 a negative correlation in the left middle area ( $r = -0.879$ ,  $p = 0.009$ ). No correlations were found in male patients or healthy controls of both genders as well as for O<sub>2</sub>Hb concentration change.

Figure 4 shows the significant areas in the category version for female patients at T1 and T2.

Looking at the data, it seems that the standard deviations in the male subgroups are larger than in

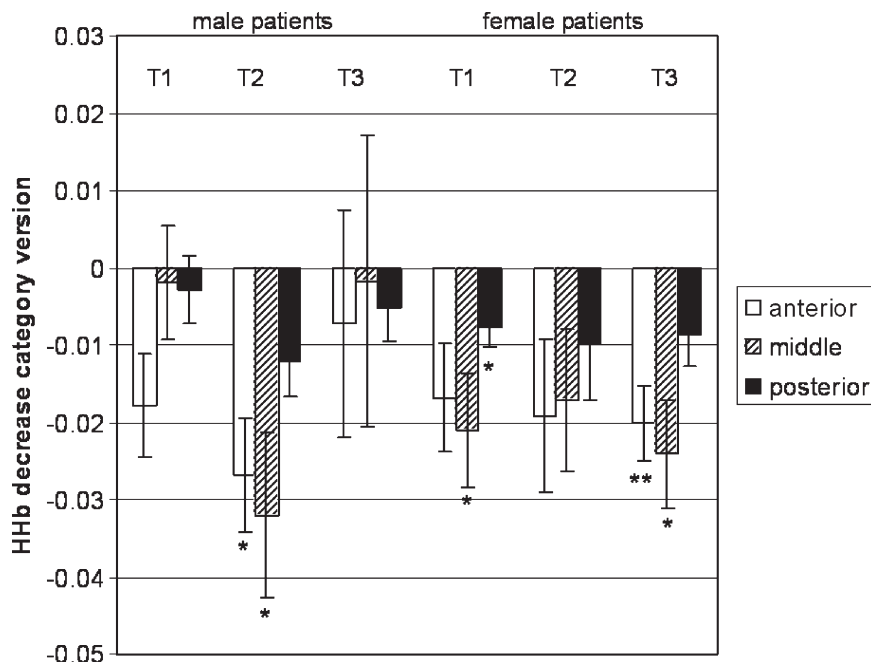


Figure 3. HHb decrease in the category version over time in the patient group. The baseline-corrected HHb decrease over time in the patient group is displayed, separated by gender. Significant HHb decreases are flagged (\* $p < 0.05$ ; \*\* $p < 0.01$ ). Bars are standard errors.

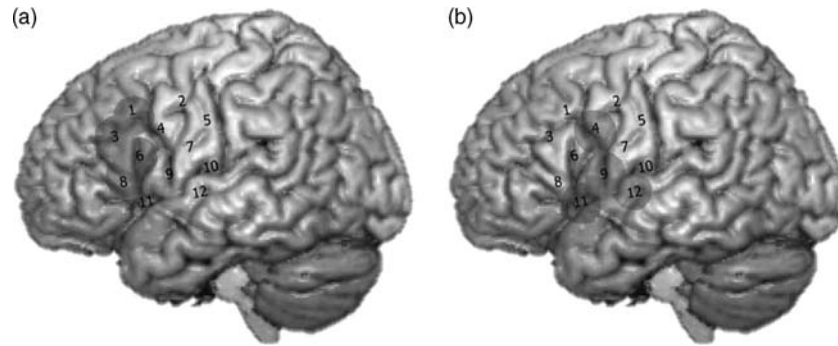


Figure 4. Areas significantly correlating in the category version for HHb in female patients at T1 (left) and T2 (right). (a) the significantly correlated area at T1. A similar correlation was found on the right hemisphere (not in picture). (b) the correlation at T2.

female subgroups. To further explore this,  $F$ -tests were conducted. Because of the problem of multiple testing, Bonferroni correction was applied and the corrected significance threshold was  $p < 0.0017$  ( $0.05/29$  because of 29 comparisons). None of the comparisons reached significance level. Without correction seven comparisons reach significance, mainly in contrasts between male and female healthy participants.

## Discussion

As expected, patients showed a worse performance than controls in the DemTect as well as in both versions of the VFT. It can be stated that there was a distinct cognitive impairment of the dementia patients accompanying their disease. Furthermore, no lateralization effect was found in any analysis, which was expected for the old age subjects we investigated (see introduction).

The comparison of the dementia patients with healthy controls showed interactions pointing to a more pronounced  $O_2Hb$  increase in healthy controls compared to patients, just as Hock and colleagues (1997) found. This result may in part be explained by the worse VFT performance in the patient group which could lead to a shift of the centre of activation to frontopolar regions that were not measurable with our device (see Papousek and Schuster 2004). For HHb there was a decrease mainly in female subjects with female patients being the only ones to show significant decrease in the category version of the VFT. The fact that female patients exceeded all other subject groups may come from a principally low effect size for all effects found in this study which results from generally poor activation in elderly people (e.g. see Johnson et al. 2004).

Interestingly, both activation effects were accounted for by female subjects. Following Kameyama and colleagues (2004), more activation would have been expected in male subjects than in females. This discrepancy may be explained by

different age groups that were investigated with the Kameyama subjects being younger. So there is evidence that during aging, prefrontal brain activation changes in a more pronounced way in men than in women. This possible explanation needs to be confirmed in further studies.

Looking at treatment effects in the patient group, no clear results were found. Regarding HHb, there were differences in that female patients showed significant decrease at T1 and T3, whereas male patients showed this decrease at T2, but no main effect for time could be identified. This pattern may be the consequence of entrainment effects by the administration of galantamine, but is too irregular to be a convincing medication effect. A study with longer intervals between measurements would help to clarify that.

Regarding the correlation between VFT performance and haemoglobin concentration change, results were only found for HHb in female patients in anterior and middle areas which is in line with findings showing the involvement of the DLPFC in VFT. Female patients who showed more HHb decrease performed better than patients with less decrease during the VFT. This is a weak effect because it only holds true for female patients and not for any other investigated group, which could be explained by the finding of little activation in male subjects in this study. This result may also be explained by less overall activation in elderly subjects.

Looking at standard deviations and error variances, it seemed that male and female subjects differ in that regard, but statistical analyses showed no significant differences. Thus it can be concluded that non-significant effects in the male subgroup are not due to different variances. Nevertheless, the phenomenon of large variances should be kept in mind when conducting further studies.

It has to be mentioned that in the present study, no placebo group has been established in the study design which would have given us the possibility to

compare the dementia progression between patients with and without medication. This would have given us more insight into a treatment effect of galantamine. For ethical reasons, we decided not to administer placebos to dementia patients thus putting up with the mentioned shortage of our investigation.

The present study showed different brain activation patterns in elderly healthy subjects compared to dementia patients depending on gender. It could also be demonstrated that the strongest activation was found in probe set areas covering the dorsolateral prefrontal cortex, as research suggests (see introduction). Furthermore, we failed to demonstrate an impact of galantamine on cognitive functioning or brain activation in the patient group in a time interval of 8 weeks, which should be reassessed in further studies with longer intervals. There possibly are gender effects in prefrontal brain activation depending on age that should be followed up in next studies.

### Acknowledgements/Statement of interest

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## ORIGINAL INVESTIGATION

# The ultrastructure of lymphocytes in schizophrenia

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### Abstract

**Objective:** Replicated abnormalities in schizophrenia include decreased cellular immunity. The aim of the study was to verify whether there are some abnormalities in the ultrastructure of lymphocytes in drug-free schizophrenic patients. **Method:** Fifty-nine in-patients with paranoid schizophrenia (DSM-IV 295.30) and 31 normal controls were used. Psychosis severity was assessed by the PANSS psychotic cluster. Electron microscopy and morphometric methods were applied to estimate the frequency and ultrastructural parameters of small, large, large activated lymphocytes (LAL) (containing 10 and more mitochondria) and of atypical lymphocytes (lymphoblasts, LB). **Results:** The frequency of small lymphocytes in schizophrenic patients was lower and that of large lymphocytes, LAL and LB was higher than in controls (all  $p < 0.01$ ). The volume density (Vv) of mitochondria in LAL in individuals with schizophrenia was lower than in controls ( $p < 0.05$ ), correlated negatively with the frequency of LB, Vv and number of lysosomes in LB (all  $p < 0.01$ ) and with the psychosis severity ( $p < 0.05$ ). In schizophrenic patients a trend towards positive correlations between the frequency of LB and psychosis severity were found ( $p < 0.07$ ). **Conclusion:** The data suggest that the excess of LB in schizophrenic patients is associated with the dysfunction of energy metabolism in LAL, and these abnormalities are related to schizophrenia.

**Key words:** Lymphocytes, electron microscopy, schizophrenia, ultrastructure, morphometry

### Introduction

The presence of immunological abnormalities in schizophrenic patients has been widely reported (Ganguli et al. 1993, 1994; Kolyaskina et al. 1998, 1999, 2004; Muller et al. 2000). Replicated immune abnormalities in schizophrenic patients include a decrease of cellular branch and prevalence of humoral branch of immunity, especially the increased production of antinuclear antibodies, antiphospholipid antibodies, antibodies to cardiolipin, DNA, and myelin basic protein (Ganguli et al. 1992, 1994; Sirota et al. 1993; Spivak et al. 1995; Kolyaskina et al. 2004). These data suggest an autoimmune component in schizophrenia, and they are consistent with brain abnormalities of oligodendrocytes and myelinated fibers reported in schizophrenia: decreased size of nuclei and increased volume fraction of heterochromatin, decreased volume fraction of mitochondria, damage of myelin sheath lamellae in the prefrontal cortex and caudate nucleus in schizo-

phrenia (Uranova et al. 2001). However, the ultrastructural basis for the immune abnormalities in schizophrenic patients remains uncertain.

Interest in atypical lymphocytes in schizophrenia is associated with the autoimmune and viral hypotheses of schizophrenia, because the prevalence of atypical lymphocytes has been described in autoimmune diseases, such as rheumatoid arthritis or systemic lupus, and in infectious mononucleosis (Hirata-Hibi et al. 1987). Morphological studies have demonstrated blast-type atypical lymphocytes (BTAL) (Kamp et al. 1962; Fessel and Hirata-Hibi 1963; Kokai et al. 1998) or P-cells (Hirata-Hibi 1992) in peripheral blood of schizophrenic patients. No significant difference in the frequency of atypical lymphocytes was reported in schizophrenic patients with and without medication (Torrey et al. 1989; Kokai et al. 1998) or lower relative numbers of atypical lymphocytes ('P-cells') was found in schizophrenia after drug treatment (Hirata-Hibi et al. 1982). An increased frequency of activated lymphocytes,

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including atypical lymphocytes similar to 'P cells,' has been reported in the cerebrospinal fluid of patients with acute schizophrenia (Nikkila et al. 2001). However, Rudolf et al. (2004) found no difference in lymphocyte morphology in schizophrenic patients. The authors explained the presence of atypical lymphocytes as artifacts of venepuncture and staining.

Electron microscopy of lymphocytes and of atypical lymphocytes, or 'P cells' have been described (Hirata-Hibi et al. 1987; Zucker-Franklin et al. 1988). We performed electron microscopic morphometric study of lymphocytes, including atypical lymphocytes, and of their relationships with the psychosis severity in drug-free schizophrenic patients. The present study is a part of the program of the study of immunological abnormalities in schizophrenia.

## Methods

### *Clinical*

After receiving approval for the study from the Ethics Committee, patients were selected. Patients were included if they met the following inclusion criteria: (1) in-patients at entry who satisfied Research Diagnostic Criteria (RDC) (Spitzer et al. 1977) for chronic schizophrenia, paranoid type (DSM-IV 295.30); (2) no less than two relapses of schizophrenia in the past 2 years; (3) male patients, 18–60 years of age; (4) each patient had a level of understanding sufficient to communicate intelligently with the investigator and nurse; (5) patients were reliable and agreed to cooperate with all tests and examinations required by the protocol; (6) each patient (or patient's authorized legal representative) understood the nature of the study and signed an informed consent document; (7) patients had scores on the BPRS, extracted from the PANNS, of at least 36; (8) patients had scores on the Clinical Global Impression Severity of at least 3.

Patients were excluded for any of the following reasons: (1) uncorrected hypothyroidism or hyperthyroidism; (2) myasthenia gravis; (3) narrow-angle glaucoma; (4) chronic urinary retention and/or clinically significant prostate hypertrophy; (5) one or more seizures without clear and resolved aetiology; (6) leucopenia; (7) history of severe allergies or multiple adverse drug reactions; (8) DSM-IV substance (alcohol or other drugs) abuse or dependence within the past 3 months, (9) inflammatory or autoimmune disorders.

In order to form the most homogeneous group of patients, we selected only one type of schizophrenia and one type of course. Eligible study subjects were

inpatients at entry. The selection of patient groups and all clinical examinations were done in the Laboratory of Psychopharmacology (Head –M.A. Morozova, MD) of the Mental Health Research Center, Russian Academy of Medical Sciences (MHRC of RAMS), Moscow, Russia. The severity measure was the Positive and Negative Syndrome Scale (PANSS: Kay et al. 1986). The patients received traditional antipsychotic therapy before hospitalization. The drug washout period between entering the study and the clinical and laboratory examinations was between 2 and 9 days.

For the purposes of this study, patient psychosis severity was assessed by the PANSS psychotic cluster (PPC). The PPC consists of the eight PANSS-derived psychotic items: delusions, conceptual disorganization, hallucinatory behaviour, grandiosity, suspiciousness, somatic concern, feelings of guilt, and unusual thought content (Breier and Berg 1999).

Normal controls ( $n=31$ ) were 19 males and 12 females, age-matched (22–56 years old). Thirty-one cases were used for the estimation of frequency of different subtypes of lymphocytes, and 21 cases from this group were used for the estimation of the ultrastructural parameters of lymphocytes. The control group was recruited among mentally healthy volunteers without inflammatory disorders – students or research workers of Russian State Medical University (Moscow).

### *Electron microscopy*

Venepuncture was performed between 08:00 and 10:00 h, and 10-ml of venous blood were taken into sterile heparinised tubes. After 60 min of sedimentation, the upper leukocyte layer was extracted and centrifuged (1000/min) for 10 min. Plasma was then extracted, and the cells in tubes (blood samples) were fixed with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (PB) (pH 7.2–7.4). After fixation for 24 h, blood samples were rinsed with PB, post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer for 60 min. Blood samples were then rinsed with 0.1 M PB, embedded in 4% agar in 0.1 M PB, kept in a thermostat 50°C for 30 min, cooled, and the excised cells were embedded in agar. Samples with agar were cut into small pieces, dehydrated in a series of graded alcohol solutions, and embedded into Araldite epoxy resin. Ultrathin sections were cut using the ultra microtome LKB (Sweden), counterstained with uranyl-acetate and lead citrate and viewed in an electron microscope (Philips EM-210, Holland). Blood samples contained all blood cells: lymphocytes, red cells, granulocytes, monocytes, platelets.

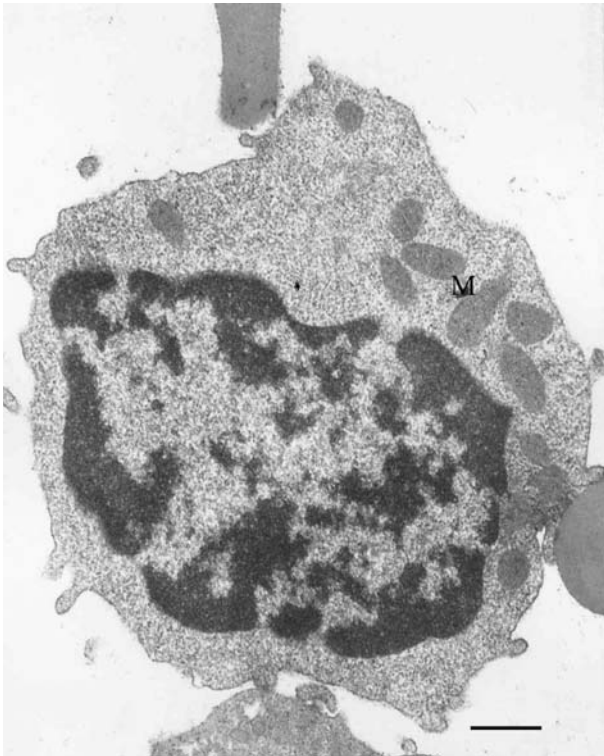


Figure 1. The ultrastructure of large activated lymphocyte from control case. M, mitochondria. Bar: 0.5  $\mu$ m.

*Identification of lymphocytes.* Lymphocytes were divided on the basis of their size into small and large lymphocytes. Small lymphocytes had a high nucleus-to-cytoplasm ratio and contained a small rim of cytoplasm. Large lymphocytes were subdivided into inactivated, activated and atypical lymphocytes. Large lymphocytes were characterized by their big size, low nucleus-to-cytoplasm ratio, and well-developed cytoplasm containing different organelles. Large activated lymphocytes contain 10 and more mitochondria per cross-sectional cell area, vacuoles, smooth and/or granular reticulum, and Golgi complex (Figures 1 and 2). Large atypical lymphocytes (lymphoblasts) were identified as large lymphocytes with specific finely dispersed nuclear chromatin structure (very euchromatic) and few nucleoli. Lymphoblasts have a nucleus of irregular form, and their cytoplasm contains many free ribosomes and polysomes, Golgi apparatus and mitochondria (Figures 3 and 4).

Cases were coded for a blind study. The frequency of each subtype of lymphocytes and of lymphoblasts was estimated by electron microscope as a percentage of lymphocytes (number per 100 lymphocytes counts). The following parameters of lymphocytes and lymphoblasts were estimated: areas of cell, nucleus, cytoplasm, volume fraction ( $V_v$ ) of heterochromatin of nucleus,  $V_v$  and number of mitochon-

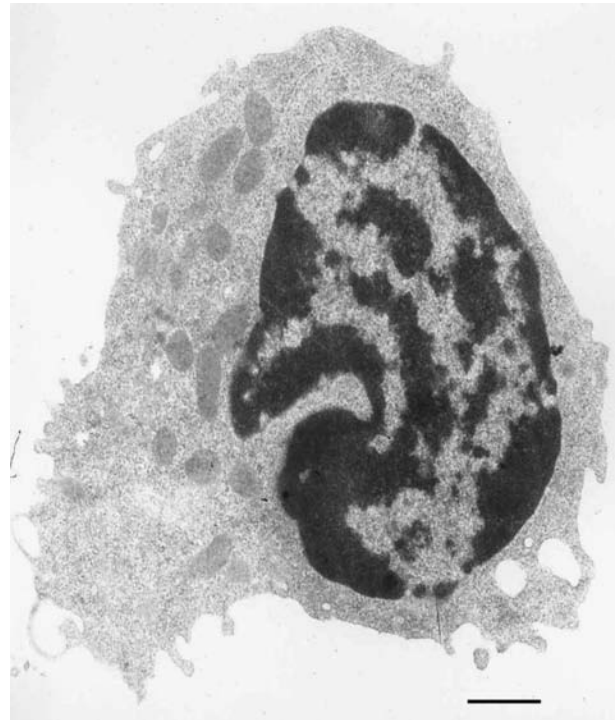


Figure 2. The ultrastructure of large activated lymphocyte from schizophrenic case. Bar: 0.5  $\mu$ m.

dria, lysosomes and vacuoles (as indicators of cell metabolism and activation) using unbiased stereological test-grids (Gundersen et al. 1988).

The data were analysed using the software package STATISTICA, release 6 (StatSoft Inc, Tulsa, OK, USA). Significant differences of morphometric data between patient and control groups were examined using one-way analysis of variance (ANOVA). Relationships between ultrastructural parameters and PPC scores were assessed using Spearman  $R$  correlations.

## Results

### *Patient population*

The total population included 59 patients (38 males and 21 females). Mean age of the population ( $M \pm SD$ ) was  $33.4 \pm 10.2$  years (minimum, 19 years; maximum, 56 years); illness duration ( $M \pm SD$ ) was  $7.8 \pm 8.1$  years (min, 0.2 year; max, 43 years); age of onset ( $M \pm SD$ ) was  $25.9 \pm 9.5$  years (min, 11; max, 52).

The most prominent clinical features of the patients were acute persecutory delusions with psychotic tension, auditory hallucinations, and disorganized cognition, along with marked signs of emotional deficit. Insight was poor in all patients.

PANSS total scale score ( $M \pm SD$ ) was equal to  $76.3 \pm 13.8$  (min, 53; max, 127); PANSS positive

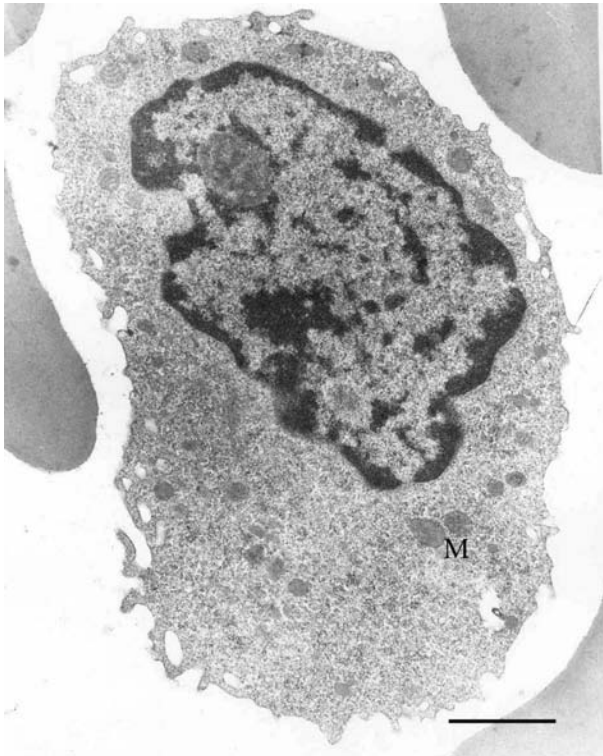


Figure 3. The ultrastructure of lymphoblast from control case. M, mitochondria. Bar: 1  $\mu$ m.

subscale score,  $17.1 \pm 4.5$  (min, 10; max, 29); PANSS negative subscale score,  $20.4 \pm 5.5$  (min, 9; max, 40); PANSS psychopathological subscale score,  $38.8 \pm 6.9$  (min, 26; max, 58). CGI-severity scale was equal to  $5.0 \pm 0.8$  (min, 3; max, 7). PPC score was equal to  $19.5 \pm 4.9$  (min, 10; max, 30).

#### Laboratory data

The frequency of different subtypes of lymphocytes in healthy controls and in schizophrenic patients is given in Table I. The percentage of small lymphocytes was significantly lower in the schizophrenic group as compared to the control group ( $-24\%$ ):  $F(1,88) = 46.15$ ,  $p < 0.001$ . There was a significant increase of the percentage of large lymphocytes ( $+29\%$ ,  $p < 0.001$ ), including large activated lymphocytes ( $+21\%$ ,  $p = 0.01$ ) and lymphoblasts ( $+152\%$ ,  $p < 0.001$ ) in schizophrenic patients as compared to controls (see Table I).

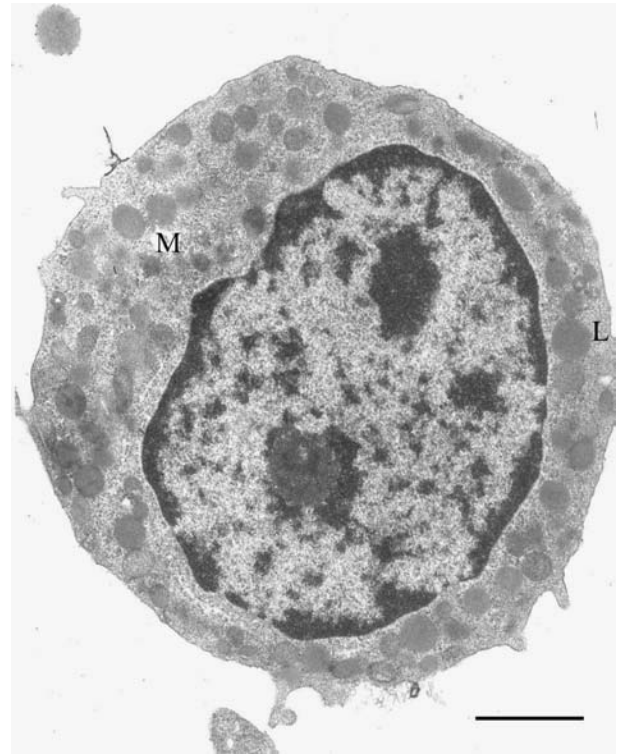


Figure 4. The ultrastructure of lymphoblast from schizophrenic case. M, mitochondria; L, lysosomes. Bar: 1  $\mu$ m.

The ultrastructural parameters of small lymphocytes and lymphoblasts were not changed in the schizophrenic group as compared to the controls (Table II). However, the areas of cell and nucleus in both large and large activated lymphocytes increased significantly, and the volume density of heterochromatin in the nuclei of these cells decreased significantly in the schizophrenic group as compared to the control group. By contrast, the volume density of mitochondria in large activated lymphocytes was significantly lower in the schizophrenic patients:  $F(1,78) = 5.57$ ,  $p = 0.02$  (Table II).

There were significant correlations between lymphoblast parameters' values in patients and their psychosis severity assessed with PANSS Psychotic Cluster (PPC) score. PPC score positively correlates with volume density and number and of lysosomes in lymphoblasts ( $r = 0.34$ ;  $t = 2.7$ ;  $p = 0.008$  and  $r = 0.3$ ;  $t = 2.4$ ;  $p = 0.02$ , respectively). PPC also negatively correlates with the volume density of mitochondria ( $r = -0.31$ ;  $t = -2.45$ ;  $p = 0.019$ ) and

Table I. Frequency of different subtypes of lymphocytes in healthy controls and in patients (mean  $\pm$  SD).

Frequency of lymphocyte's subtypes (%)	Small lymphocytes	Large lymphocytes	Large activated lymphocytes	Lymphoblasts
Controls ( $n = 31$ )	$58.8 \pm 10.1$	$39.44 \pm 9.01$	$18.54 \pm 6.6$	$1.74 \pm 2.08$
Individuals with schizophrenia ( $n = 59$ )	$44.47 \pm 9.21$	$51.1 \pm 0.20$	$22.43 \pm 7.3$	$4.38 \pm 2.7$
$p$ value	$< 0.001$	$< 0.001$	$= 0.01$	$< 0.001$

Table II. Ultrastructural parameters of lymphocytes in controls and in schizophrenic patients (mean  $\pm$  SD).

Parameters	Small lymphocytes		Large lymphocytes		Large activated lymphocytes		Lymphoblasts	
	Controls (n = 21)	Individuals with schizophrenia (n = 59)	Controls (n = 21)	Individuals with schizophrenia (n = 59)	Controls (n = 21)	Individuals with schizophrenia (n = 59)	Controls (n = 21)	Individuals with schizophrenia (n = 59)
Area of cell ( $\mu\text{m}^2$ )	16.11 $\pm$ 2.22	16.28 $\pm$ 1.78	19.20 $\pm$ 2.28	20.40 $\pm$ 1.88*	20.37 $\pm$ 2.45	22.30 $\pm$ 2.37**	31.42 $\pm$ 5.88	26.71 $\pm$ 8.21
Area of cytoplasm ( $\mu\text{m}^2$ )	6.60 $\pm$ 1.12	6.54 $\pm$ 1.04	11.09 $\pm$ 1.43	11.68 $\pm$ 1.43	11.86 $\pm$ 1.68	12.94 $\pm$ 1.88*	16.77 $\pm$ 4.21	14.47 $\pm$ 5.30
Area of nucleus ( $\mu\text{m}^2$ )	9.51 $\pm$ 1.24	9.74 $\pm$ 1.01	8.11 $\pm$ 1.00	8.72 $\pm$ 0.71**	8.51 $\pm$ 1.05	9.36 $\pm$ 0.9***	14.66 $\pm$ 3.77	12.24 $\pm$ 3.90
Vv of heterochromatin	51.92 $\pm$ 3.84	50.58 $\pm$ 4.13	51.24 $\pm$ 2.71	47.50 $\pm$ 3.60***	49.53 $\pm$ 3.76	44.42 $\pm$ 4.31***	31.04 $\pm$ 2.84	27.48 $\pm$ 8.34
Vv of mitochondria	5.28 $\pm$ 1.42	4.98 $\pm$ 1.43	7.88 $\pm$ 1.38	7.48 $\pm$ 1.27	9.62 $\pm$ 2.04	8.58 $\pm$ 1.61*	4.07 $\pm$ 3.34	5.46 $\pm$ 2.50
Vv of lysosomes	0.53 $\pm$ 0.52	0.36 $\pm$ 0.55	1.38 $\pm$ 0.65	1.24 $\pm$ 0.99	1.72 $\pm$ 0.92	1.77 $\pm$ 1.97	2.34 $\pm$ 3.85	1.64 $\pm$ 2.67
Vv of vacuoles	0.79 $\pm$ 0.52	0.69 $\pm$ 0.64	0.96 $\pm$ 0.66	0.92 $\pm$ 0.56	1.03 $\pm$ 0.65	0.99 $\pm$ 0.68	0.43 $\pm$ 0.70	1.21 $\pm$ 1.61

Vv, volume density.

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p$ 

volume density of heterochromatin in large activated lymphocytes. However, there was only a trend level positive correlation ( $p < 0.1$ ) between PPC score lymphoblast count ( $r = 0.23$ ;  $t = 1.9$ ;  $p = 0.07$ ).

There were not significant correlations between other parameters measured and PPC scores. There were no correlations of the ultrastructural parameters measured with age, gender, duration of the disease, and age of the disease manifestation.

## Discussion

Our findings of increased frequency of large activated lymphocytes and lymphoblasts as well as of increased size of nucleus and cytoplasm of large activated lymphocytes and decreased content of their heterochromatin provide a morphological basis for activation of the immune system in schizophrenic patients. These data are in line with the results of an immunological study of the same 59 schizophrenic patients and 38 healthy controls performed at the laboratory of Clinical Neuroimmunology, Mental Health Research Center (Kolyaskina et al. 2004). In this study, circulating immune complexes and auto-antibodies to cardiolipin were significantly elevated in patients in the acute stage before treatment. The data support the thesis that the immune system of acute schizophrenic patients is activated (Muller et al. 2000; Nikkila et al. 2001; Theodoropoulou et al. 2001).

However, cellular immunity shows signs of immunodeficiency, i.e. phagocyte activity of neutrophils and monocytes, cytotoxic activity of lymphocytes natural killers, IL-2, IL-10 and INF- $\gamma$  production was significantly lower before the treatment (Kolyaskina et al. 2004). These data are consistent with our data showing a deficit of mitochondria in large activated lymphocytes that might be the ultrastructural basis for decreased cellular immunity of T-cells reported in schizophrenic patients (Zucker-Franklin et al. 1988; Muller et al. 2000; Kolyaskina et al. 2004). Though the sample size of the control group was rather small as compared to schizophrenic group, the interpersonal variability in the control group was not very big that allowed to compare both groups studied.

The deficit of mitochondria in lymphocytes is consistent with other evidence of mitochondrial dysfunction in schizophrenia, including reduced levels of enzymes of energy metabolism in lymphocytes and in postmortem brains (Whatley et al. 1998), dysfunction of the oxidative phosphorylation system (Maurer et al. 2001), and altered mitochondrial-related gene expression in postmortem brain in schizophrenia (Karry et al. 2004; Prabakaran et al. 2004). The data are also in agreement with the

results of morphometric studies of postmortem schizophrenic brains demonstrating mitochondrial hypoplasia (Kung and Roberts 1999), decreased volume fraction of mitochondria in astrocytes (Uranova et al. 1996), in oligodendroglial cells (Uranova et al. 2001) in myelinated fibers (Uranova et al. 2003), and in the neuropil (Kung and Roberts 2000). The data suggest that lymphocytes might be a peripheral model of altered energy metabolism in schizophrenia. Lymphocyte could reflect the metabolism of brain cells (Gladkevich et al. 2004).

Our finding of an increased frequency of lymphoblasts compared to healthy controls is in line with the excess of blast-type atypical lymphocytes (Kamp et al. 1962; Fessel and Hirata-Hibi 1963; Kokai et al. 1998; Torrey et al. 1989) or P-cells (Hirata-Hibi 1992) in the peripheral blood of schizophrenic patients and with increased frequency of activated 'P cells' found in the cerebrospinal fluid of patients with acute schizophrenia (Nikkila et al. 2001). However, Rudolf et al. (2004) did not find any correlations of immunological alterations in schizophrenia with morphological characteristics of lymphocytes observable by light microscopy. We focused on the ultrastructural characteristics of different lymphocyte subgroups, using morphometry, and found clinical correlates of lymphoblasts and large activated lymphocytes. We believe that electron microscopy allows the identification of atypical lymphoblasts more precisely than light microscopy, because it is possible to view the ultrastructure of the nucleus and cytoplasmic organelles. Thus, our data provide evidence that the increased frequency of atypical lymphoblasts is related to schizophrenia.

Little is known about the reasons for the increased frequency of lymphoblasts in schizophrenia. Our study demonstrated that the volume fraction of mitochondria in large activated lymphocytes is inversely and highly significantly ( $p < 0.01$ ) correlated with the percentage of lymphoblasts, with volume fraction, and with the number of lysosomes in lymphoblasts. These data suggest that the prevalence and activation of lymphoblasts in acute schizophrenic patients might be a compensatory response to a cellular immunodeficiency of lymphocytes, especially of mitochondria deficiency and is not associated with the effects of drug therapy. The excess of lymphoblasts in schizophrenic patients might represent an anomalous plasticity of immune system in schizophrenia. The latter finding provides some new evidence in support of autoimmune hypothesis of schizophrenia. Antinuclear antibodies unrelated to drug treatment have been reported in schizophrenic patients (Spivak et al. 1995) and antiphospholipid antibodies in unmedicated individuals with schizophrenia (Schwartz and Silver

2000). Lowered IFN- $\gamma$  and IL-2 production in schizophrenic patients, irrespective of drug therapy, indicate that schizophrenia resembles autoimmune disorders (Arolt et al. 2000; Androsova et al. 2004; Kolyaskina et al. 2004). An inverse relationship between IL-2 level and the PANSS positive subscale P in schizophrenic patients has been reported (Zhang et al. 2002), and platelet-associated antibodies correlated with the BPRS psychometric scores have been reported in schizophrenic patients irrespective of medication and gender (Sinyakov et al. 2003). Recently, antibodies to neurotransmitter receptors including 5-HT, cholinergic and opioid receptors have been reported that might be associated with the induction of psychiatric symptoms (Tanaka et al. 2003). Taken together, the data suggest that schizophrenia might be an active autoimmune disease with lymphocyte dysfunction. Viral agents might trigger the autoimmune process (Karlsson et al. 2001, 2004; Dickerson et al. 2003; Yolken et al. 2004). Since we could not differentiate between different functional types of lymphocytes due to the lack of estimation of surface molecules, future studies of the ultrastructural changes of B-lymphocytes, T-lymphocytes in schizophrenia are needed to support the view that schizophrenia might be an autoimmune disorder and to explain the functional implications of the changes of lymphocytes.

The finding of significant negative correlations between the volume density of mitochondria in large activated lymphocytes and the frequency of lymphoblasts, volume density and number of lysosomes in lymphoblasts supports the notion that immunological defects are associated with autoimmunity in schizophrenic patients (Sleasman 1996). However, we cannot exclude the effects of drug therapy on the parameters studied because of a short drug washout period. Further study of cellular immunological defects and their association with autoimmunity in acute unmedicated individuals with schizophrenia and of drug treatment effects are needed to develop new drug therapies for the disease.

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## BRIEF REPORT

# Quetiapine in the treatment of psychotic adolescents: A case series of 23 patients with severe early onset psychosis

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### Abstract

Clinical efficacy, safety and tolerability of quetiapine in the treatment of 23 hospitalized psychotic adolescents were evaluated retrospectively. Twelve patients were changed to quetiapine from another antipsychotic medication during their hospital stay. In these patients, CGI-S improved from  $4.75 \pm 0.87$  to  $2.92 \pm 0.67$  (observation period  $3.7 \pm 1.6$  months). The most common adverse events were transient tachycardia and sedation. Mild EPS occurred only in one patient under quetiapine monotherapy. Transaminase increases more than threefold above norm were observed in two patients. fT4 values were slightly below the norm in 67% of the cases. In 11 patients, quetiapine was initiated using a rapid titration schedule with high dosages in the acute phase. Receiving a mean maximum daily dose of  $927 \pm 300$  mg, CGI-S improved from  $6.00 \pm 0.63$  to  $3.18 \pm 1.25$  (observation period  $2.9 \pm 1.8$  months). Severe adverse events did not occur. Besides applying lorazepam temporarily in nine of the 11 patients, antipsychotic co-medication was not necessary in this group. In line with other studies, quetiapine may be considered as an effective treatment for adolescents with a severe psychotic disorder showing a favourable side-effect profile. Our preliminary data suggest that a rapid initiation with high doses could be a promising approach in acute psychotic adolescents.

**Key words:** Psychosis, schizophrenia, quetiapine, adolescents, acute setting

### Introduction

Early onset schizophrenia interferes strongly with patients' and their families' life. Its treatment represents a challenge to child and adolescent psychiatrists and health facilities. Schizophrenia has a chronic outcome with persisting symptoms in 93%, which indicates the importance of an effective medication (Marneros et al. 1991). In the acute setting, a fast reduction of positive psychotic symptoms, agitation, anxiety as well as aggressive and disruptive behaviour are of particular importance (Zimbhoff 2003).

Side-effects and potential risks significantly determine the compliance with the antipsychotic medication. There is a high susceptibility to extrapyramidal symptoms (EPS) in adolescent populations (Schulz et al. 1998). Therefore, the application of traditional antipsychotics is limited. Other side effects strongly influencing patients' quality of life are weight gain and complications induced by increased hormone levels. The latter are strongly associated with certain

atypical antipsychotics, while EPS are less common in this substance group. Most of the atypical antipsychotics, in Germany all except from clozapine, are not approved in adolescents and underlay an off-label use. There are only a few trials in this age group, mainly on clozapine, olanzapine and risperidone (Freisleder et al. 1997; Findling and McNamara 2004; Dittmann et al. submitted). Nevertheless, atypical antipsychotics are regarded as first line treatment in psychotic disorders in adolescents, especially in long-term treatment.

In contrast, the management of severe psychotic adolescents in the acute setting is difficult and often leads to polypharmaceutical strategies and use of typical antipsychotics in clinical practice.

Quetiapine, one of the newer atypical antipsychotics, has been demonstrated to be efficacious for adult psychotic patients and also to have a favourable side-effect profile (Cheer and Wagstaff 2004). First open trials suggest similar findings in adolescents (McConville et al. 2000, 2003; Shaw

et al. 2001). There is growing evidence that a rapid initiation schedule with high dosages is well tolerated and provides benefits in the treatment of acute exacerbations in adult patients (Arrango and Bobes 2004). So far, no data are available for this medication schedule in adolescents.

The aim of this investigation was to evaluate clinical efficacy, safety and tolerability of quetiapine in adolescents with a severe psychotic disorder, retrospectively. Analyses addressed two different aspects:

- change-over to quetiapine from another anti-psychotic medication;
- quetiapine in the acute setting using a rapid titration schedule with high dosages.

**Material and methods**

*Subjects*

Twenty-three psychotic adolescents (13–17 years; 16 males and seven females) treated with quetiapine while being hospitalized in a primary care hospital for child and adolescent psychiatry from 2001 to June 2005 were included. All patients were admitted to an acute locked unit of the clinic because of their predominant positive psychotic symptoms, agitation and desorganization. After stabilization, they were transferred to an open unit.

Patients were stratified according to whether they were changed to quetiapine from another antipsychotic medication during their stay (change-over group: *N* = 12) or directly treated with quetiapine in the acute setting using a rapid titration schedule with high dosages (acute–high dosage group: *N* = 11)<sup>1</sup>. Sample characteristics of the two groups are summarized in Table I.

Patients fulfilled ICD-10 criteria for schizophrenia (F20), acute schizophrenia-like psychotic disorder (F23.2) or schizoaffective disorder (F25.0). Assessment by board certified child and adolescent psychiatrists included clinical interviews with parents and patients. Severe neurological or organic disorders were excluded by a physical and neurological examination in all subjects, supplemented by a cranial MRI (*N* = 18) and lumbar puncture (*N* = 10) in part of the subjects. Nine patients reported THC consumption, two of them also had experiences with other illegal drugs. None of the subjects fulfilled criteria of a drug addiction according to ICD-10.

Table I. Sample characteristics.

	Change-over group ( <i>N</i> = 12)	Acute-high dosage group ( <i>N</i> = 11)
Age (years; months)	16;2 ± 1;0 [14;0–17;4]	15;10 ± 1;5 [13;6–17;8]
Sex (male/female)	9/3	7/4
ICD-10 diagnoses at discharge:		
schizophrenia (F20)	12	8
acute schizophrenia like disorder (F23.2)	0	1
schizoaffective disorder (F25.0)	0	2
First episode, first hospitalization	10	10
THC consumption	6	3
Previous antipsychotic medication	atypical: 9; typical 3; stopped due to inefficacy: 7, weight gain: 6 and/or EPS: 7	–

*Treatment*

Quetiapine was given as an off-label use allowed by German law after obtaining consent from the patients and their parents. It was always given twice daily, exceeding the manufacturer’s recommendation for the maximum dosage of 750 mg in both groups when clinically indicated. In the acute–high dosage group a more rapid titration was initiated for the management of acute symptoms. Psychiatric comedication was given when clinically indicated.

Besides medication all patients received an individual continuous multimodal therapeutic setting (e.g. psycho-education, family interventions, single and group psychotherapies, functional and physically oriented therapies and cognitive behavioural interventions) which basically started after improvement of acute symptoms.

*Parameters and statistics*

Clinical efficacy was assessed using the Clinical Global Impression–Severity of Illness (CGI-S) scale (Guy 1976). Psychiatric interviews and explorations were performed regularly, at least once a week. Safety and tolerability were controlled regularly (physical examination, weight, laboratory tests, blood pressure, heart rate, temperature, ECG and EEG). The observation period for this investigation lasted from the begin of quetiapine medication until discharge. Wilcoxon tests were computed to analyse clinical (CGI-S) effects.

<sup>1</sup> This rapid-titration, high-dosage medication schedule was applied from 2004 on.

## Results

### Change-over group

Seven of the 12 patients of the change-over group had already been transferred to an open unit before they were changed to quetiapine. From the begin of quetiapine medication until discharge (observation period:  $3.7 \pm 1.6$  months), their CGI-S score improved from  $4.75 \pm 0.87$  to  $2.92 \pm 0.67$  ( $p = 0.002$ ). At discharge 10 patients were receiving quetiapine, nine as monotherapy (see Table II). The final average daily treatment dose was  $595 \pm 207$  mg (range 400–1000 mg), the average daily maximum dose was  $617 \pm 227$  mg (range 300–1000 mg). Plasma levels were  $113 \pm 46$  ng/ml (range 52–169 ng/ml; dose:  $700 \pm 105$  mg).

The average uncorrected weight gain in the change-over group was  $5.9 \pm 6.5$  kg (BMI at baseline:  $22.9 \pm 3.4$ ). Sedation ( $N = 4$ ) and moderate tachycardia ( $N = 4$ ) were observed transiently. A mild rigor and hypokinesia occurred in one patient under quetiapine monotherapy and subsided completely after reducing the dose from 600 to 500 mg. There were no pathological ECG and EEG changes under quetiapine. In eight patients, transaminases were raised temporarily, in two patients by more than threefold above norm. In one patient, a GOT of 383 U/l and a GPT of 177 U/l were measured under 700 mg quetiapine monotherapy. After reducing the dose to 550 mg and adding amisulpride, GOT and GPT reduced to 40 and 54 U/l, respectively, at discharge 6 weeks later. Including this patient, three subjects showed mildly elevated transaminases at discharge (maximum: GOT 47 U/l; GPT 63 U/l). Increased eosinophils (maximum: 8%) occurred temporarily in two subjects, and a decreased number of leucocytes (minimum: 3100 cells/ $\mu$ l) in three subjects. fT4 values were slightly below the norm in 67% of the cases. No significant changes for TSH and fT3 were observed.

### Acute-high dosage group

In the acute-high dosage group, a mean CGI-S score of  $6.00 \pm 0.63$  was assessed at baseline. When

transferred to an open unit (after  $42 \pm 22$  days), CGI-S had decreased to  $4.00 \pm 0.77$  ( $p = 0.001$ ). At discharge ( $2.9 \pm 1.8$  months after starting quetiapine medication), it was  $3.18 \pm 1.25$ .

Quetiapine was titrated to a mean dosage of 600 mg by day 5 in these patients (in single cases 600 mg by day 2). The mean maximum dose was  $927 \pm 300$  mg/day (range 400–1400 mg/day), with eight patients receiving more than the manufacturer's recommendation for the maximum dosage of 750 mg. At discharge, patients received an average daily dose of  $791 \pm 298$  mg (range 400–1200 mg). Plasma levels were  $100 \pm 74$  ng/ml (range 36–235 ng/ml; dose:  $1000 \pm 245$  mg).

Nine patients of the acute-high dosage group were comedicated with lorazepam temporarily, with three subjects receiving 4 mg/day or more (maximum 7 mg/day). The use of other antipsychotics was not necessary in these patients. One patient with a schizoaffective disorder was comedicated with lamotrigine and pipamperone.

The average uncorrected weight gain in the acute-high dosage group was  $5.3 \pm 3.1$  kg (BMI at baseline:  $19.7 \pm 2.4$ ). Sedation ( $N = 3$ ), vertigo ( $N = 2$ ), moderate tachycardia ( $N = 7$ ), constipation ( $N = 3$ ) and xerostomia ( $N = 3$ ) were observed transiently. EPS did not occur in patients of the acute-high dosage group. There were no pathological ECG and EEG changes under quetiapine. In seven patients, transaminases were raised temporarily (maximum: GOT 75 U/l; GPT 72 U/l). Increased eosinophils (maximum: 8%) and a decreased number of leucocytes (minimum: 3000 cells/ $\mu$ l) occurred temporarily in two subjects each. fT4 values were slightly below the norm in 33% of the cases. No significant changes for TSH and fT3 were observed.

## Discussion

Efficacy, safety and tolerability of quetiapine in 23 hospitalised adolescents with severe psychotic disorders were analysed retrospectively.

Ten out of 12 patients of the change-over group and all 11 patients of the acute-high dosage group were discharged on quetiapine, which underlines the

Table II. Psychiatric comedication and medication at discharge.

	Change-over group ( $N = 12$ )	Acute-high dosage group ( $N = 11$ )
Psychiatric comedication (mainly temporarily)	high potency antipsychotics: 1 Low potency antipsychotics: 2 Lorazepam: 3	Low potency antipsychotics and mood stabilizer: 1 Lorazepam: 9
Medication at discharge	QUE monotherapy: 9 QUE + amisulpride: 1 change-over to clozapine: 1 discontinue (low compliance): 1	QUE monotherapy: 10 QUE + lamotrigine + pipamperone: 1

high responder rate and good safety and tolerability profile. Considering that seven patients had shown EPS under a different antipsychotic medication during their hospital stay, the low incidence of EPS under quetiapine seems to be remarkable. Thus, results of other open-label trials in adolescents were confirmed (McConville et al. 2000, 2003; Shaw et al. 2001).

The treatment of psychotic patients in the acute setting requires an efficient management of positive psychotic symptoms, anxiety, agitation, aggressive and disruptive behaviour. For the first time, quetiapine was applied in adolescents using a rapid initiation with high dosages in this setting. Rapid initiation and high dosages were well tolerated by the youth patients. Antipsychotic effects were convincing with sufficient clinical stabilization achieved in all subjects. Results also indicate that benzodiazepines are (still) essential for a satisfactory reduction of agitation and anxiety in the major part of the patients.

Our investigation has several limitations which are mainly due to the fact that data were collected under non-study (naturalistic) conditions. Patients received an individual multimodal treatment program which basically started after improvement of acute symptoms. So, other interventions than quetiapine medication may also have contributed to the clinical improvement. However, it is very likely that quetiapine medication contributed most, particularly in the acute, high-dosage group during their stay in the acute-locked unit.

No standardized instruments were applied during treatment. So, *inter alia* medication effects could not be related to symptom domains.

Prolactin and cholesterol were not measured. Though quetiapine does not seem to increase these metabolic parameters in adolescents (Findling and McNamara 2004), prolactin and cholesterol should also be determined to exclude a possible influence of quetiapine, particularly when applied with a rapid initiation and high dosages.

But despite these limitations, our preliminary data suggest that quetiapine using a rapid initiation with high dosages could be a promising approach in the treatment of acute psychotic adolescents. They could serve as an impetus to conduct controlled studies with larger samples to confirm the potential benefit of this medication schedule.

## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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## CASE REPORT

# Neuroleptic malignant syndrome induced by ziprasidone on the second day of treatment

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### Abstract

Neuroleptic malignant syndrome (NMS) is the rarest and most serious of the neuroleptic-induced movement disorders. We describe a case of neuroleptic malignant syndrome (NMS) associated with the use of ziprasidone. Although conventional neuroleptics are more frequently associated with NMS, atypical antipsychotic drugs like ziprasidone may also be a cause. The patient is a 24-year-old male with a history of schizophrenia who developed signs and symptoms of NMS after 2 days of treatment with an 80-mg/day dose of orally administered ziprasidone. This case is the earliest (second day of treatment) NMS due to ziprasidone reported in the literature.

**Key words:** Neuroleptic malignant syndrome, schizophrenia, treatment, ziprasidone

### Introduction

Neuroleptic malignant syndrome (NMS) is a rare but potentially fatal disorder characterized by fever, muscular rigidity, delirium and autonomic instability. Although the classical presentation of NMS has been most commonly associated with the typical neuroleptic medications, sporadic cases in association with atypical neuroleptic medications have been reported. Estimates of the 1-year prevalence of this syndrome in individuals exposed to neuroleptic medications range from 0.02 to 2.4%. Mortality rate of cases without specific treatment was reported to reach 21% (Leibold et al. 2004; Sadock and Sadock 2000). NMS is characterized by muscular rigidity, hyperpyrexia, altered consciousness, and autonomic dysfunction (Mahmood and Warren 1989; Sadock and Sadock 2000).

Ziprasidone is a relatively new atypical antipsychotic agent, and differs from typical anti-psychotic agents by its lower affinity for dopaminergic receptors and binding antagonistically to serotonin receptors in the nigrostriatal pathway (Rasmussen 1998; Shad 1998). Ziprasidone does not increase serum prolactin levels and is virtually devoid of extrapyramidal side effects and weight gain. Its major side

effects are nasal congestion and somnolence (Collaborative Working Group 1998).

The following case report describes a male patient with schizophrenia who developed NMS after the second day of orally administered ziprasidone, and who was successfully treated with anticholinergic therapy.

### Case report

A 24-year-old man who had never previously received antipsychotics, or other psychotropics, was admitted to the outpatient unit of a hospital with persecutory delusions and auditory hallucinations continuing for 8 months. The patient did not seem to be aggressive or harmful to the environment. On psychiatric examination, the patient spoke normally and was in a euthymic mood. Family history did not reveal any psychiatric disease. Substance use and any other organic disorders were excluded through laboratory analysis and his history. He was diagnosed as having schizophrenia according to DSM-IV-TR. Ziprasidone was administered at an oral dose of 80 mg/day. On the second day of the treatment, the patient presented with a tactile fever;

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severe muscle rigidity, was diaphoretic, tremulous, and difficult to arouse; and had persistent urinary incontinence, elevated temperature, labile blood pressure, mutism and altered mental status. The Parkinsonism subscale of the Extrapyrimal Symptoms Rating Scale (ESRS) (Chouinard and Ross-Chouinard 1984) score was 28. The results of laboratory blood tests have shown normal serum iron level (72 µg/dl, normal range 59–158) and total iron binding capacity (269 µg/dl, normal range 228–428). Laboratory evaluation included an initial serum creatinine phosphokinase (CK) level of 5110 U/l which is a very high level for reported NMS cases. On complete blood count (CBC) analysis, leukocyte count was found to be 11.430/µl, hemoglobin 14.3 g% and hematocrit 42.7%, respectively. Serum sodium level was 142 mmol/l, potassium 3.85 mmol/l, chloride 105 mmol/l and creatinine 0.74 mg/dl, respectively. To exclude any intracranial cause of altered consciousness, computed tomography (CT) of the cranium was performed and reported as normal. Urine drug screening failed to show any evidence of amphetamine, cocaine, tricyclic antidepressant, opiate, cannabis, barbiturate, or benzodiazepine exposure.

Initial vital signs were: 145 beats/min, blood pressure 110/80 mmHg and rectal temperature 39.0°C (104°F). The patient's overall muscle tone was initially rigid. He had dry mucous membranes. Lung was clear on auscultation and he had a sinus tachycardia with a regular rhythm. Abdominal examination was unremarkable for acute findings of any infection. The patient had normal pulses in all four extremities. No infectious source was detected for fever. Neurological consultation also failed to show any neurological cause for the altered consciousness and rigidity. Also internal medicine consultation found no nephrological or infectious cause.

Lethal catatonia and malignant hyperthermia, included in hyperthermic syndromes related to use of medication, were excluded because of diaphoresis, muscle rigidity, elevated CK, leukocytosis and history of antipsychotic use (Sadock and Sadock 2003).

After the evaluation of these findings, 2 days after the first application of ziprasidone, NMS was diagnosed according to DSM-IV-TR criteria (American Psychiatric Association 2000). Daily biperiden and diazepam were administered via total of 3000 cc/day fluid (1500 cc/day 0.9% NaCl and 1500 cc/day 5% dextrose) was applied. The patient was chilled for the high fever. During the first 24 h of admission, the patient's temperature was refractory to treatment and his blood pressure oscillated from 50/30 to 200/110 mmHg. On the second day of hospitalization, the patient's CK levels decreased to

1300 U/l, and his temperature had been normalized. By the third day of hospitalization, muscle rigidity began to decrease. On the seventh day, his temperature, autonomic symptoms and laboratory measures returned to the normal range. The ESRS Parkinsonism subscale score was 18 on the fifth day of admission. By the 11th day of admission, ESRS parkinsonism subscale score decreased to 0, and patient was discharged. Olanzapine 5 mg/daily was applied in the follow-up period.

## Discussion

NMS is an uncommon but potentially life-threatening adverse effect associated mostly with typical antipsychotic agents. However, with the introduction and common use of atypical antipsychotics in schizophrenia and mood disorders, some cases of NMS due to these drugs were reported previously (Bora 2003; Leibold et al. 2004; Sing 2002). NMS is thought to be caused by the blockade of dopaminergic receptors in the nigrostriatal pathway. Dopaminergic receptor blockade is the source of extrapyramidal symptoms, such as tremor, shuffled gait, and bradykinesia, often encountered with the use of antipsychotics. Ziprasidone has higher affinity for human 5HT<sub>2A</sub> receptors than dopamine D<sub>2</sub> receptors. The D<sub>2</sub> receptor affinity of ziprasidone for human receptors and rat transporters is relatively lower than for haloperidol and risperidone, but higher than for olanzapine and quetiapine (Anne et al. 2001). So this kind of D<sub>2</sub> receptor affinity may cause higher frequency of extrapyramidal syndromes, including NMS, than other atypical antipsychotics.

Medical treatment of NMS cases have also been reported before (Elechi 1992; Lew and Tollefson 1983). The efficacy of medication is probably related to its facilitatory effect upon dopamine activity in the central nervous system. Discontinuation of the antipsychotic agent and administration of anticholinergic agents is known to be effective in the first steps of the treatment of NMS. In this case report, NMS was treated successfully with daily biperiden, an anticholinergic agent, and diazepam, through daily fluid replacement. Application of this protocol is commonly used and is still the best known effective way of coping with such a highly lethal state as NMS in a short time period. The beneficial effect of anticholinergic therapy upon motor symptoms may explain its usefulness in management of patients with severe forms of NMS.

Several cases of ziprasidone-induced NMS have been reported (Gray 2004; Hasan and Buckley 1998). However, in our case, the appearance of

NMS was seen earlier than that of the cases studied in the related literature.

Ziprasidone is a new generation antipsychotic with a more favourable side effect profile than its predecessors. It is important to be aware that NMS may also be associated with ziprasidone therapy. Although extrapyramidal syndrome rates with ziprasidone have been reported to be lower or similar to placebo, it may cause NMS. We recommend caution when starting to use even an atypical antipsychotic such as ziprasidone in order to avoid unwanted events such as NMS.

### Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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## CASE REPORT

# Risperidone-induced priapism

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### Abstract

A 31-year-old patient suffering from schizophrenic psychosis was treated with risperidone, and developed priapism which required surgical intervention and resulted in long-term erectile dysfunction.

**Key words:** Adverse effects, antipsychotic drug, atypical, psychopharmacology, schizophrenia

### Introduction

Psychotropic agents, particularly antipsychotics, have been known as occasionally causing severe adverse effects such as priapism, a prolonged, persistent and often painful penile erection (Thompson et al. 1990). Although modern antipsychotic drugs are often considered to be relatively safe, they also have been implicated in this severe side effect.

Here, we report a case of priapism in a physically healthy patient after ingestion of a high dosage of risperidone.

### Case report

A 31-year-old man who was diagnosed with schizophrenia at the age of 20, had been treated with risperidone 4 mg/day for more than 1 year. Prior to this therapy, he had received, over a period of 10 years, several other antipsychotic and antidepressant medications (haloperidol, flupentixol, olanzapine, amitriptyline and lofepramine) without any major side effects. There was no prior history of pelvic or spinal injury, leukemia, blood dyscrasia, lymphoma or sickle cell disease. There was also no history of sexual dysfunction or priapism.

Whilst on risperidone, compliance was inconsistent and psychotic symptoms were not fully suppressed. During a paranoid episode, the patient took a single dose of 16 mg risperidone in an attempt to control his psychotic symptoms. Within 24 h, he developed priapism and was admitted to the local

emergency department. He underwent drainage of the cavernous sinuses which proved to be ineffective. He then had to undergo major corrective surgery which resulted in a loss of erectile function.

### Discussion

Penile erection depends (among others) on  $\alpha$ -adrenergic neural activity (Lue et al. 1986; O'Brien et al. 1989).

The mechanism of priapism associated with psychotropic drugs is thought to be related to blockade of  $\alpha$ -adrenergic receptors in the corpora cavernosa; the blockade favours parasympathetic mediated erection and inhibits sympathetic mediated detumescence (Segraves 1989). The ratio of  $\alpha$ -adrenergic blockade to anticholinergic activity may thus be a deciding factor (Fishbain 1985; Greenberg and Lee 1987; Patel et al. 1996). Many psychotropic drugs have some level of  $\alpha$ -adrenergic antagonism, which may upset this regulatory balance.

Since it is currently believed that psychotropic-induced priapism is correlated with  $\alpha$ 1-adrenergic antagonism within or close to the cavernous space (Dorman and Schmidt 1976; Kogeorgos and De Alwis 1986), the propensity of individual antipsychotics to induce priapism can thus be presumably estimated based on their  $\alpha$ 1-adrenergic blockade affinity (Table I). Interestingly, risperidone has a relatively higher  $\alpha$ 1-adrenergic affinity than many other antipsychotics.

## Conclusions

During recent years, there have been a few case reports of risperidone-induced priapism (Emes and Millson 1994; Tekell et al. 1995; Nicholson and McCurley 1997; Madhusoodanan et al. 2002; Reeves and Mack 2002; Bourgeois and Mundh 2003; Relan et al. 2003; Slauson and LoVecchio 2004; Yang and Tsai 2004). Cases of priapism have also been described in patients who underwent a risperidone treatment in combination with other psychotropic drugs (Owley et al. 2001; Seger and Lamberti 2001). Furthermore, there have been cases which required surgical intervention or in which idiopathic priapism was exaggerated (Ankem et al. 2002; Freudenreich 2002). And priapism has not only been described as an adverse effect of risperidone, but also of most conventional antipsychotics as well as clozapine and olanzapine (review: Compton and Miller 2001). However, this severe adverse effect can usually be managed and does normally not lead to long-term or permanent deficiencies. However, much less desirable outcomes such as chronic erectile dysfunction can occur, as described in this case.

Regarding the management of this rather rare, but very serious side effect, an immediate referral to an urologist is key. Meanwhile, conservative measures can be initiated, including pain control, vigorous hydration, and cold compress, while the patient awaits urological consultation. Upon initial contact with the urology service, they may recommend other medical management in the interim before their examination of the patient. Such consultation should be obtained within 4–6 h to prevent long-term sequelae, such as erectile dysfunction. Prognosis is directly influenced by promptness of treatment to induce detumescence; early presentation and initiation of treatment may be the most important factors in a successful outcome (Pantaleo-Gandais et al. 1984; Bertram et al. 1985). The psychiatrist should also provide a focused history (including prior priapisms, other medication, substance misuse) and the results of a thorough physical examination (including genitourinary examination).

Considering the number of case reports in the literature, priapism seems to be associated with most antipsychotic medications, including modern so-called atypicals, thus representing a small but definite risk. In its least desirable trajectory, this side effect may lead to permanent erectile dysfunction. Three clinically relevant points are worth stating: first, a past history of prolonged erection linked to medication use is common before onset of priapism; second, there seems to be no relationship between the treatment and the dosage of antipsy-

Table I.  $\alpha$ 1-Adrenergic blockade affinities of antipsychotics compared with prazosin (according to Richelson 1999).

Drugs	Affinity
Olanzapine	2
Loxapine	4
Haloperidol	6
Thioxanthine	9
Fluphenazine	11
Quetiapine	12
Clozapine	15
Thioridazine	20
Risperidone	37
Ziprasidone	38
Chlorpromazine	38
Prazosin	250

chotic medication in relation to the onset of priapism; and, third, decreased reporting of prolonged erections and delay in reporting priapism lead to increased morbidity (Thompson et al. 1990). Therefore, physicians prescribing these drugs should be aware of this risk, routinely take a psychosexual and urological history, and carefully inform and educate their patients.

## Statement of interest

J. Thome has received lecture fees and support to attend scientific meetings from several pharmaceutical companies including manufacturers of atypical antipsychotics such as Janssen (risperidone) and Lilly (olanzapine).

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## CASE REPORT

# Second run of transcranial magnetic stimulation has no effects on persistent auditory hallucinations

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### Abstract

We report the case of a 30-year-old male schizophrenic patient with persistent auditory hallucinations (AHs) who had received two 1-Hz transcranial magnetic stimulations (TMS) to the left temporoparietal cortex. The patient had suffered from severe persistent AHs for more than 5 years with little improvement in response to various types of combination pharmacotherapy of antipsychotics. Upon commencement of TMS, however, the patient reported substantial improvement by the end of the third week of the treatment. The patient was discharged, but readmitted 6 months later because his AHs returned to their initial severity. A second run of TMS had no therapeutic effect. Further research is needed to establish the therapeutic duration of TMS treatment and to assess whether the second run of TMS after AH reoccurrence has therapeutic benefits.

**Key words:** *Hallucinations, auditory, transcranial magnetic stimulation*

### Introduction

Auditory hallucinations (AHs) are a common symptom of schizophrenia and often persist after other psychotic symptoms subside. The persistent AHs respond poorly to pharmacotherapy. Clozapine or electroconvulsive therapy (ECT) can be tried, but both approaches are associated with serious side effects. Effective treatment alternatives for persistent AHs would benefit those patients with persistent AHs. Since the first report of the positive effects of transcranial magnetic stimulation (TMS) on persistent AHs (Hoffman et al. 1999a), TMS has attracted wide attention as a novel treatment for AHs in schizophrenic patients. Reduction of AHs has been reported when left temporoparietal cortex (Hoffman et al. 2000, 2003; Lee et al. 2005) or left auditory cortex (d'Alfonso et al. 2002) was stimulated with low-frequency TMS. No beneficial effects on AHs were seen with 1-Hz TMS stimulation of Broca's area and the superior temporal gyrus (Schönfeldt-Lecuona et al. 2004) or 10-Hz TMS stimulation of the right prefrontal cortex (Schreiber et al. 2002). To

date, only little information is available on the duration of TMS therapeutic effects and whether second run of TMS stimulation upon recurrence of AHs harbours any therapeutic benefits. Here we report the case of a 30-year-old schizophrenic patient with persistent AHs, who responded well to the first run of TMS sessions, but showed no improvement to the second run of TMS after his AHs had returned to their original level.

### Case report

The patient was a 30-year-old male, unmarried and unemployed with a high school education. He was diagnosed with paranoid schizophrenia at 18 years of age and had been admitted to medical care for treatment of his symptoms on four previous occasions. His chief complaints were persistent AHs, which occurred continuously even during conversations or meals for more than 5 years. The contents of AHs were mostly negative, causing severe distress, but related behavioural disturbances were limited. For the management of the persistent AHs, the

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patient had been put on various types of combination therapy, with clozapine 900 mg as a main drug and risperidone 6 mg, olanzapine 30 mg, haloperidol 18 mg, or amisulpride 1600 mg as add-on drugs with little effect. The patient then agreed to TMS therapy and was admitted after signing his informed consent. Psychotropic medication with clozapine (900 mg) and amisulpride (1600 mg) remained unchanged during the treatment. The patient underwent 3 weeks of consecutive TMS stimulation with one 25-min session per day (5 days/week for a total of 15 days) in an open trial. In each of the 15 sessions, 1000 stimulations were administered in the fashion of 50 trains of 20-s duration, separated by 10 s of rest. Stimulation was carried out with a MagPro magnetic stimulator (Medtronic, Denmark) at a frequency of 1 Hz and an intensity of 90% of the motor threshold. A figure-eight coil, handheld tangentially to the skull, was placed midway between the left temporal ( $T_3$ ) and left parietal ( $P_3$ ) electroencephalogram electrode sites. The stimulation point was determined with an EEG cap on the basis of the international 10–20 electrode placement system. The motor threshold was identified as the minimum magnetic field strength required to produce left thenar muscle activation by single TMS pulses delivered to the motor cortex for at least four out of eight trials. Upon admission, the total Positive and Negative Syndrome Scale (PANSS) score was 92 (25 for the positive subscale; 20 for the negative subscale) and the Psychotic Symptom Rating Scale (PSYRATS; Haddock et al. 1999b) score for AHs was 33. TMS efficacy was assessed using PSYRATS and a 10-point Likert scale, the former evaluated at baseline and 1 day after last session and the latter at baseline and before every other session. The 10-point Likert scale is a 10-point visual analogue scale to which the patient marked the point on the basis of how much the severity of AHs, which is either frequency or loudness of AHs, had improved compared to the one he had experienced at the time of admission. Concerning about the possible psychological effects of admission, TMS was not administered in the first week during which no improvement of AHs was noted. Modest improvement of AHs on the Likert scale was noted after the first session, but AHs returned to baseline levels by the next day (Figure 1). At the beginning of the second week, the patient reported near-complete absence of AHs during the session, which lasted for about 1 thereafter. During the third week, he reported improvement in his ability to concentrate as well as continuing reduction of AHs. By that time, he complained about loneliness, saying ‘the voice no longer talks to me.’ Even though the final Likert scale score was 3, he was still experiencing frequent

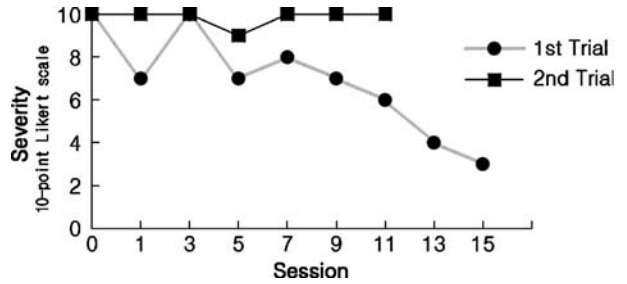


Figure 1. Effect of TMS on the severity of auditory hallucinations.

AHs rated as 3 on the frequency item of the PSYRATS, not fulfilling the remission criteria defined by Larsen et al. (2000). The second PSYRATS total score was 26.

No adverse effects of TMS were identified during the entire treatment period. Satisfied with the results, the patient was discharged 40 days after admission and subsequently followed up as an out-patient. Within 1 month after his discharge the patient's AHs gradually returned to their initial level. Because of the aggravation of AHs and high medical expenses, amisulpride was discontinued and switched to haloperidol again which the patient preferred to be prescribed. Approximately 6 months after discharge, he was readmitted for a second trial of TMS. At the time of his readmission, the dose of haloperidol (15 mg) had been stable for more than a month and compliance was good. However, severity of AHs was even worse than before the first TMS treatment with the PSYRATS score of 38. TMS treatment was repeated in the same fashion as described above without the change of drug doses. It, however, had no effects until the end of the second week and caused him mild to moderate light-headedness. Hence, TMS was terminated. At the time of the submission of this manuscript, the patient was seen as an out-patient, maintaining a combination therapy of clozapine (900 mg) and haloperidol (18 mg) and additional cognitive-behavioural strategies for AHs.

## Discussion

This case illustrates that a first run of TMS can substantially suppress persistent AHs, yet second run after AHs recurrence may be of little therapeutic benefit. Moderate (2 points) and slight (1 point) improvement of the patient's AHs were noted in the areas of amount of distress and controllability of voices and the area of frequency of the PSYRATS respectively. Hoffman et al. (2003) reported that frequency and attentional salience were the two aspects of hallucinatory experience that showed greatest improvement with TMS. The frequency of the Auditory Hallucination Rating Scale (AHRS:

Hoffman et al. 2003) is rated with a 0–9 scaling system, while the frequency of the PSYRATS with a 0–5 scale. Therefore, it should be noted that 1 point of improvement in hallucination frequency on the PSYRATS in the patient may be comparable to the 2 points of improvement in hallucination frequency on the AHRS reported by Hoffman et al. (2003) and delivered great relief to him. As the patient was maintained on high dose of clozapine, it can be assumed that administration of TMS may increase risk for seizure. However, no significant adverse effects were seen, except some degree of light-headedness on the second run of TMS. Interestingly, the patient also noted a significant improvement in his ability to concentrate, which had occurred after the submission of his AHs. To date, little is known about the cognitive improvements associated with temporoparietal or auditory cortex TMS administration. Although we did not objectively evaluate the patient's cognitive improvements, it may be speculated that the improvements were secondary to the attenuation of his AHs.

In terms of the duration of therapeutic effects, TMS is at this point of little promise as the majority of all treated patients experienced an aggravation of AHs 1 day to 4 months after the course of active TMS (Hoffman et al. 2000, 2003) and duration of effects (mean  $\pm$  SD) was  $13.1 \pm 17.0$  weeks (Hoffman et al. 2005). Likewise, we observed an aggravation of AHs to the baseline levels within 2 months after discharge, and the second run of TMS had no effects at all. This finding is in contrast with the result of patient #1 in the Hoffman et al. study (1999), who experienced a reduction of AHs to a meaningless mumble with a second trial of TMS performed when AHs returned to baseline levels. It may be speculated that no effects of second run of TMS in our case could be related to several factors: (a) the severity of AHs on readmission was higher than that observed at the first admission so that TMS could not exert an effect; (b) the second run of TMS had stopped too early; (c) tolerance could have developed to the TMS effects; and (d) as there was no sham control group, the improvement to the first run of TMS might have been a placebo effect.

Further studies with large sample size are needed to examine whether second run of TMS has therapeutic effects on the returned AHs.

### Statement of interest

The authors have no conflict of interest with any commercial associations in connection with the submitted article.

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## CASE REPORT

# Hyponatremia-induced seizure during carbamazepine treatment

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### Abstract

We report the case of a 54-year-old woman who was admitted for benzodiazepine withdrawal. After 6 weeks of carbamazepine treatment (600, then 200 mg) the patient suddenly suffered from a grand mal seizure. Laboratory findings revealed a clinical significant hyponatremia of Na 125 mmol/l (baseline: 143 mmol/l). CCT and ECG were normal. To our knowledge, this is the first description of a seizure related to hyponatremia in an adult carbamazepine-treated patient.

**Key words:** Carbamazepine, oxcarbazepine RAUS, hyponatremia, seizure, benzodiazepine

### Introduction

Carbamazepine (Cbz) is an antiepileptic drug, which is also frequently used in treatment of psychiatric patients as a 'mood stabilizer' for relapse prevention in bipolar disorder. In addition, it is also used for treatment of alcohol withdrawal syndrome, alcohol withdrawal seizures and benzodiazepine withdrawal (Gandelman et al. 1994; Soyka et al. 2002; Palmer et al. 2003). More recently, the drug was also recommended for treatment of alcohol withdrawal in outpatients (Soyka et al. 2002, 2006).

Cbz causes a number of side effects including nausea, sedation, ataxia, allergic skin reactions and leucopenia, among others. There are also multiple interactions of Cbz with other drugs including benzodiazepines, lithium, neuroleptics, SSRI, tricyclic antidepressants and valproate (Benkert et al. 2003). Most of these side effects are mild, transient and reversible. (Gandelman et al. 1994)

Hyponatremia has repeatedly been associated with Cbz treatment. This electrolyte disturbance is usually without clinical significance (Van Amselvoort et al. 1994), but may sometimes lead to serious complications, which may easily be overlooked. By definition, hyponatremia indicates a serum Na level below the normal range, usually below 130 mEq/l, although the laboratory definition may be slightly higher (< 135 mEq/l). One cause of hyponatremia is an inappropriate secretion of antidiuretic hormone (ADH) which has been associated with Cbz therapy

(Kalff et al. 1984; Van Amselvoort et al. 1994; Palmer et al. 2003).

While the Cbz-associated hyponatremia is usually subclinical, in some cases it may have delirious effects. We report the case of a 54-year-old woman with benzodiazepine (Bzd) dependence who, after a 38-day treatment with Cbz suffered from grand mal seizure, probably related to Cbz treatment.

### Case report

Mrs T. was a 54-year-old housewife with a history of agoraphobia, who had repeatedly been treated for benzodiazepine dependence. There was no history of seizures or other significant neurological disorders, except for a toxic delirium associated with Bzd overdose. At baseline, laboratory testing did not reveal any abnormalities. The serum electrolytes were: Na 143 mmol/l (135–148); K 3.8 mmol/l (3.5–5.1); Ca 2.3 mmol/l (2.1–2.6); Cl 105 mmol/l (96–110). Liver: GOT 22 U/l; GPT 14 U/l; GGT 25 U/l. kidney: creatinine 0.83 mg/dl; urea 18 mg/dl; uric acid 4.5 mg/dl.

She had been dependent on benzodiazepines for 8 years before admission to our hospital. No other drugs including alcohol were abused. Before admission she took up to 90 mg bromazepam a day. Clinical and neurological examination did not reveal any significant abnormality. ECG was normal and the otherwise normal EEG showed predominant

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$\beta$ -waves, most probably linked to Bzd medication. On admission, the patient showed mild symptoms of Bzd intoxication, especially mild psychomotor deceleration and slurring of speech, but otherwise was fully orientated. The patient was gradually tapered of Bzd over 4 weeks using diazepam with an initial dose of 40 mg. In addition Cbz (initial 600 mg) was given for prevention of withdrawal seizures. No significant side effects were observed. The patient showed a significant withdrawal syndrome with symptoms of anxiety and sleep disturbance.

In week 6, 15 days after the last Bzd-dose and after reduction of Cbz to 200 mg, the patient suddenly showed symptoms of a mild disorientation and also reported suicidal ideas. A few hours later she experienced an epileptic seizure (grand mal type). While the initial brain CT was normal, the EEG showed a left temporal focus with slow waves. The following brain MRI showed several unspecific white matter lesions but was otherwise normal. Chemical testing revealed: Na 125 mmol/l; K 2.9 mmol/l; Ck 171 U/l; infusion of hypertonic solution was necessary, the serum Na levels were carefully monitored.

Abdomen sonography showed mild fatty liver and multiple cysts in both kidneys and symptoms of chronic impairment. From the internistic point of view it is not probable that this impairment was caused by hyponatremia.

Clinically, the patient quickly recovered and was finally discharged after 63 days of treatment with a Na level of 137 mmol/l. Unfortunately, there has been no more EEG control after the reversal of hyponatremia. at baseline, urine showed 1662 ng/ml Bzd, two weeks before the seizure >5000 and 4 days before 3683 ng/ml. Three days after the seizure the testing resulted in 232 ng/ml. The serum concentration of Bzd 10 days after the seizure was 111 ng/ml alprazolam.

## Discussion

To our knowledge, this is the first report of a hyponatremia-induced seizure in a psychiatric patient following treatment with Cbz. In recent case reports on Cbz-associated new-onset seizures the possibility of an underlying hyponatremia is not even discussed (Monji et al. 2004). While there were no baseline laboratory abnormalities, after the seizure a clinically significant hyponatremia of 125 mmol/l was observed. Taking the increasing number of non-neurological patients treated with Cbz for various disorders including affective and bipolar disorder and schizophrenia into account, the apparent risk of hyponatremia and especially of seizures is of clinical relevance.

The prevalence of hyponatremia associated with Cbz treatment ranged from 4.8% (Van Amselvoort et al. 1994) to 41.5% (Palmer et al. 2003). The differences may reflect differences between the populations in terms of age, mean daily Cbz dosages or serum levels, rates of mono- or polytherapy (Lahr et al. 1985).

Some limitations and alternative explanations for the seizure should be briefly addressed. First, a possible explanation for the seizure may be the BZD withdrawal itself, which often leads to seizures, especially in cases of nearly lifetime abuse of Bzd and high doses of Bzd. In this case, there were no serum levels of Bzd before or directly after the seizure and the semi-quantitative urine testing showed that the Bzd accumulated in the body. The Cbz dose in this case was reduced the day before the seizure from 400 to 200 mg, which may be an insufficient dose. On the other hand, the half-life of Cbz is up to 10 h (Benkert et al. 2003). Second, it is also clinically known that hyponatremia may occur after a seizure. This point cannot finally be cleared, because the only Na level before the seizure is the baseline at admission.

A number of pathophysiological mechanisms have been discussed as reasons for Cbz-associated hyponatremia, including syndrome of inadequate ADH secretion, an antidiuretic effect of Cbz and sensitivity to serum osmolarity. Another pathological mechanism is that Cbz potentiates the action of ADH or acts directly on renal tubular cells (Van Amselvoort et al. 1994; Gandelman et al. 1994), raising the renal sensitivity to normal plasma vasopressin concentrations, resetting the osmoreceptors, so they become 'lazy' (Yassa et al. 1988).

In psychiatric patients, occasionally some form of 'water intoxication' may also be of relevance. The possibility of hyponatremia should not be overlooked in cases of cbz treatment including Cbz overdosage. (Lahr et al. 1985)

Hyponatremic effects correlate with Cbz dose, high therapeutic or toxic serum Cbz concentration, and lower initial sodium levels (Kalff et al. 1984; Yassa et al. 1988; Gandelman et al. 1994; Palmer et al. 2003). In some studies, the risk factors are not so clear; for example, no general consensus can be found for polypharmacy (Kalff et al. 1984; Gandelman et al. 1994). Nicotine may stimulate ADH release, and cigarette smoking has been associated with ADH hypersecretion in psychiatric patients. Higher age has also been associated with a higher risk of developing a hyponatremia (Kalff et al. 1984; Lahr et al. 1985; Van Amselvoort et al. 1994).

Hyponatremia in Cbz-treated patients with affective disorder, schizophrenia, or mental retardation has already been described. In most cases the

hyponatremia was clinically insignificant. In other cases, patients suffered from fatigue, dizziness, lethargy, confusion, decreased ability to concentrate, and irritability. The early symptoms of hyponatremia and Cbz toxicity are very similar; this suggests that some patients who might appear to have non-specific side effects to Cbz actually may be experiencing symptoms of hyponatremia (Ude et al. 1983; Mucklow et al. 1991). Other symptoms can be somnolence, syncope and coma (Gandelman et al. 1994).

For treatment, dosage reduction or drug discontinuation of Cbz usually results in normalization of Na levels. Depending on the Na level, fluid restriction or, in severe cases, perfusion of hypertonic solution, may be necessary, as in the case presented. In this case, risk of central pontine myelinolysis must be considered. If it compromises the management of epilepsy an alternative anticonvulsant, such as sodium valproate or phenytoin or, as in this case, valproate should be substituted (Mucklow et al. 1991).

#### Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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## CASE REPORT

# Butterfly glioma of corpus callosum presenting as catatonia

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### Abstract

Catatonia can occur with functional psychiatric disorders as well as organic conditions. In this case report, catatonia occurred with a butterfly glioma originating from the corpus callosum with bilateral medial frontal extensions. Medial frontal lobe structures have been implicated in the pathophysiology of catatonia.

**Key words:** *Glioma of corpus callosum, medial frontal lobe, catatonia*

### Introduction

Catatonia is a psychomotor syndrome characterized by abnormalities in three domains, motor (akinesia, posturing, catalepsy, waxy flexibility), behavioural (mutism, stupor, stereotypies, perseveration, automatic obedience, echophenomena), and emotional (anxiety, compulsive emotions, aggression, emotional lability) (Gelenberg 1976; Taylor 1990). Although it is commonly associated with functional psychiatric disorders as schizophrenia and mood disorder, various organic causes have been described (Gelenberg 1976). Focal pathology leading to catatonic syndrome lends support to the possible areas involved in generation of such symptoms. We describe a patient with glioma of the corpus callosum with medial frontal extensions, who developed catatonic syndrome.

### Case report

A 45-year-old male without any past or family history of psychiatric or neurological disorder, developed increased frequency of micturition 3 months previously, followed by forgetfulness, crying spells, decreased socialization and hallucinatory behaviour for 1 month, and food refusal, maintaining odd postures, urinary incontinence for 1 week. Examination revealed bilateral rigidity and brisk reflexes, and blurring of the nasal margin of the optic discs was suggestive of early papilloedema. On mental status he was uncooperative, mute, had posturing with

psychological pillow, *mitmachen* (cooperation; the body can be put into any position without any resistance on the part of the patient, although instructed to resist all movements) and negativism. Laboratory investigations including haemogram, liver and renal function tests and serum electrolytes were within normal limits. He was diagnosed as having organic catatonic syndrome. His catatonic symptoms did not improve with intramuscular lorazepam 2 mg t.i.d. Plain CT scan of the brain revealed a large butterfly glioma involving genu and the body of the corpus callosum with extension into adjacent medial frontal cortex and lateral ventricles. He was referred to the neurosurgery department where he eventually succumbed.

### Discussion

Organic causes are responsible for 20–25% of all catatonic syndromes; the common ones being CNS structural damage, encephalitis, seizures and metabolic disturbances (Gelenberg 1976; Carroll and Goforth 2004). Various CNS neoplasms involving the frontal lobe have been associated with the development of catatonia (Galasko et al. 1988), but there has been no previous report of butterfly glioma of the corpus callosum presenting as catatonia.

In a factor analytic study (Abrams et al. 1979) of 55 catatonic patients, two factors emerged suggestive of different catatonic syndromes: (1) akinetic catatonia or akinetic mutism characterized

by mutism, negativism and stupor; (2) one characterized by stereotypy, catalepsy and automatic cooperation. In our case features from both subtypes were present.

It has been noted that the presence of catatonic symptoms are evidence for frontal lobe disease or dysfunction, and this has been proposed to be due to dopamine imbalance in the frontal lobe–basal ganglia–brain stem system (Taylor 1990). Medial frontal cortex lesions have been associated with disturbances of will (e.g. apathy) and motoric abnormalities (e.g. gegenhalten, i.e. opposing all passive movements with the same degree of force as applied by the examiner; waxy flexibility) (Penfield and Welch 1951; Hassler 1980; Luria 1980). According to Mega and Cummings (1994), lesions or dysfunction of the anterior cingulate gyrus lead to development of catatonic syndrome; the pathway includes the anterior cingulate cortex to the globus pallidus and then to the dorsomedial thalamus. Catatonia has been suggested to be due to functional disconnection between the orbitofrontal and premotor/motor cortex (Northoff et al. 2000). In our case, the butterfly glioma originated from the corpus callosum and extended to involve the medial frontal lobes bilaterally, which might explain the catatonic symptoms. In a recent fMRI study (Northoff et al. 2004) on 10 akinetic catatonic patients, catatonic motor symptoms have been related to medial prefrontal activation, whereas behavioural and affective symptoms correlated with orbitofrontal deactivation.

Although the pathophysiology of catatonia is far from clear, medial frontal lobe structures have been implicated. Functional neuroimaging studies on catatonic patients having structural lesions may shed further light into its mechanism. It is important to consider medical causes in all catatonic syndromes, especially in patients with focal neurological findings.

### Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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## CASE REPORT

# Depression as a manifestation of latent chronic hypoparathyroidism

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### Abstract

The exact cause of depression in cases of hypoparathyroidism is not known. We report the first case of an elderly patient with a long history of major depression as a complication of an undiagnosed chronic hypoparathyroidism following surgery on a parathyroid adenoma. Her depression was completely eliminated by calcium supplementation therapy to restore the calcium homeostasis in serum. As it is well known that disturbances in the endocrine hypothalamus–pituitary–thyroid system might be consistent findings of depressive disorders concerning neuroendocrinological alterations, this case report and review of literature strongly supports our claim that also parathyroid diseases like chronic hypoparathyroidism, even in its latent form, might be a relevant factor in the development of depressive symptoms.

**Key words:** Depression, latent postoperative hypoparathyroidism, calcium homeostasis

### Introduction

We report the case of a 68-year-old patient with a long history of severe depression after 1990; while suffering from primary hyperparathyroidism (pHPT) a solitary parathyroid adenoma was extirpated. Chronic postoperative hypoparathyroidism might have been a relevant factor in the development of her depressive symptoms that were treated by intensive medication and required repeated hospitalizations. We report, for the first time, on an unusual manifestation of latent chronic hypoparathyroidism and on the importance of calcium homeostasis for the well-being of patients following surgery on endocrine organs, such as the parathyroid gland, in the context of the international literature.

### Case report

In 1990, the 68-year-old patient with a long history of bone pain was found to have parathormone levels of 386 pg/ml ( $[PTH]_{i.S.}$ ), six-fold above normal levels (NR: 20–70 pg/ml; hPTH RIA, DSL Co., Sinsheim, Germany), and parallel elevation of total calcium in serum of 2.9 mmol/l ( $[Ca^{2+}]_{i.S. total}$ ; NR: 2.1–2.6 mmol/l). Upon suspicion of pHPT, parathyroid surgery was performed in July 1990. Intraoperatively, three parathyroid

glands were identified, the left inferior parathyroid was found to be enlarged to about 2 cm in diameter (weight = 1.1 g) and was removed. Intraoperative frozen-section biopsy of this tissue and of a normal parathyroid gland revealed proliferation of adenomatous and suppressed parathyroid tissue, a finding that was confirmed at final histological analysis. On the fourth postoperative day, the  $[Ca^{2+}]_{i.S. total}$  fell to 1.8 mmol/l unaccompanied by the paresthesias typical of hypocalcemia. Supplementation therapy with calcium (calcium effervescent tablet  $3 \times 1000$  mg) and vitamin D ( $1 \times 0.5$  µg daily calcitriol) was initiated. On the sixth postoperative day, the patient's  $[Ca^{2+}]_{i.S. total}$  was still 1.8 mmol/l. The postoperative unilateral recurrent nerve partial paralysis with discrete hoarseness had by this time completely disappeared. The patient was consequently sent home with the recommendation that she undergo regular monitoring of her calcium levels. Due to her continued good general condition, however, her calcium levels were not followed-up despite occasional highly discrete evening tingling paresthesias of the hands and feet after prolonged physical stress, which however did not cause the patient any subjective impairment. Calcium medication was stopped completely several weeks later in December 1990.

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After 8 months, the patient began to suffer from increasing depression, often combined with feelings of agitation, anxiety and sleep disturbances. She had no family history of depression or psychiatric disorders, also no personal history of psychic abnormalities. Then a major depression was diagnosed following a structured clinical interview based on the DSM-IV Diagnosis Criteria for Mental Disorders. Despite psychiatric treatment beginning in August 1991, when she met DSM-IV criteria for MDE, and drug therapy with tricyclic antidepressants (amitriptyline 50 mg once a day; increased to 75 mg three times daily months later), she showed no significant improvement. The patient's suffering, which remained monopolar for one long episode over the years, was so great that she required two stays in a psychiatric institution in summer 1992 and autumn 1994, also with insufficient therapeutic success. An EEG examination in 1994 was without pathological finding, a structural brain imaging was not performed. Psychopharmacological therapy was first combined with a tranquilizer (clobazam 10 mg 3 times daily) and in the second admission 1994 with an irreversible MAO inhibitor (tranylcypromin 5 mg 3 times daily). Hypocalcemia ( $[Ca^{2+}]_{i.s. total} = 1.75$  mmol/l) was found during her first stay, and was 1.65 and 1.76 mmol/l during the course of her second stay. Both times these laboratory results were not further clarified. There were no further hypocalcemic symptoms after stopping the calcium medication.

In 2001, the patient was brought by her family doctor to our clinic for the first time for an outpatient examination. She exhibited clear functional disability, appearing very down beaten, still suffering from DSM-IV depression. Examination revealed a clearly lowered total calcium of  $[Ca^{2+}]_{i.s.} = 1.6$  mmol/l, with lowered ionized calcium of 0.93 mmol/l (NR: 1.15–1.3 mmol/l). Pathognomonic for the diagnosis of the secondary, postoperative hypoparathyroidism was a drop in  $[PTH]_{i.s.}$  to 5.43 pg/ml. Still no hypocalcemic somatic symptoms existed, so that a latent form of chronic hypoparathyroidism existed.

Replacement therapy with calcium gluconate of up to 2000 mg/day led to complete disappearance of the patient's depression within 3 weeks while the pharmacological treatment has not been changed or further modified since the last years. Additionally no special life-events happened at this time that might have contributed to the improvement. The tricyclic antidepressants were then completely discontinued after 8 weeks. The patient remained inconspicuous in her reported moods and at further psychiatric follow-up examination in March and December 2005 without antidepressant medication.

## Discussion

A rare but complex secondary endocrine disease, permanent postoperative hypoparathyroidism, is by far the most common form of hypoparathyroidism (Hasse et al. 1999). This complication occurs most often after thyroidectomies (DeMeester-Mirkin et al. 1992), much less often after surgery on the parathyroid glands, as was the case in our patient. Treatment of permanent postoperative hypoparathyroidism consists of lifelong calcium and, in some patients, vitamin D supplementation. This therapy cannot, however, prevent the possible late sequelae of the disease, such as joint trouble and calcification of the basal ganglia.

Cases of latent hypoparathyroidism unaccompanied by typical tetanic symptoms or paresthesias are known (Ziegler 1987). The literature, however, contains no mention of a latent chronic postoperative hypoparathyroidism manifesting primarily as depression. In our case it is unlikely the depression was secondary to the largely clinically inconspicuous tingling paresthesias evoked by physical stress, since the patient herself attributed no special importance to them nor did she feel impaired by them. It is known from the literature (Arlt et al. 2002), moreover, that supplementation therapy usually cannot completely restore the well-being of the patient. In our patient the psychiatric component of the disease completely disappeared after initiation of calcium supplementation therapy.

A few studies have shown an association between chronic hypoparathyroidism and other neurological-psychiatric syndromes, including a clinical picture much like Parkinson's disease (Berger and Ross 1981), and tetany mistakenly diagnosed as epilepsy (Armellisasso et al. 2004). Cognitive changes in patients with chronic hypoparathyroidism have been described (Kowdley et al. 1999) and may be associated with intracranial calcification. Also described are cerebellar and extrapyramidal symptoms, the first occurring after a latency of 32 years following thyroidectomy (Kartin et al. 1982).

The exact cause of depression in cases of hypoparathyroidism is not known. Fukuyama and Hayashi (1979) were the only authors who have shown, in two cases, that hypoparathyroidism patients exhibit EEG changes such as disturbances in the cyclical changes of sleep and absence of REM sleep. Interestingly, regular sleep EEG patterns resulted after normalization of calcium serum levels.

In the newest literature, however, Barden et al. (2006) and Lucae et al. (2006) described associations between the P2RX7 gene and both major depressive disorder (unipolar) and bipolar affective disorder. P2RX7 is a purinergic ATP-binding

calcium channel expressed in various brain regions. These findings further support the assumption between calcium homeostasis and depression.

A depressive emotional state in cases of symptomatic (paresthesias in particular) permanent hypoparathyroidism is known (Kaplan and Sadock 1991). Our own post-Chernobyl studies on cancer patients in the Ukraine (which has the largest population of patients with secondary hypoparathyroidism following thyroid gland surgery) showed that up to 12% of symptomatic patients suffer from anxiety, 20% from inner unrest, and 24% from severe depression (Bohrer et al. 2003). About one-third of all patients had a latent form of chronic hypoparathyroidism. But two of these patients also exhibited a depression and were under antidepressant medication, whereas further psychiatric examinations were not performed so far (own unpublished data). Interestingly, it is now known that a direct association exists between hyperparathyroidism and depression (Wilhelm et al. 2004): Most patients reported, after surgical procedure on pHPT, that depression no longer impacted their quality of life. The authors concluded that parathyroidectomy reduces major depression and improves patients' quality of life.

Neuroendocrinological alterations in disturbances of the hypothalamus–pituitary–thyroid system and hypercortisolism are known to cause depression in some patients in biological psychiatry (Tiemeier 2003; Brouwer et al. 2005), e.g., only 20–25% of depressed patients apparently have a reduced TSH response to morning administration of TRH (Loosen, 1985). However, based on the present case and the literature, we propose that also the parathyroid should be included in that system to do full justice to the importance of this endocrine organ for the origin of depression. As we would like to emphasize, parathyroid hormone levels and the resulting calcium homeostasis seem to play an important role in the development of depression. Calcium ions significantly influence the activity of synaptic chemical transmitters. Still, the exact pathway for the onset of depression and the relation to its further course is not clear. Still, the P2RX7 gene might be of significant relevance in that field for further studies.

## Conclusion

Depression can be the main symptom of an otherwise latent, chronic hypoparathyroidism. This postoperative secondary disorder after procedures on the thyroid and parathyroid should be included among the differential diagnostic possibilities for psychiatric disorders associated with hypocalcemia.

If pathognomonic tetany symptoms are lacking, latent postoperative chronic hypoparathyroidism can be definitively diagnosed only by paying attention to a lowered serum calcium level and consecutively assessing parathyroid hormone levels in serum.

Since both hyperparathyroidism and hypoparathyroidism in its symptomatic and asymptomatic form can appear with depression, parathyroid diseases should be observed as possible neuroendocrinological cause in depressive disorders and might be treated successfully according to the underlying pathological somatic finding.

## Statement of interest

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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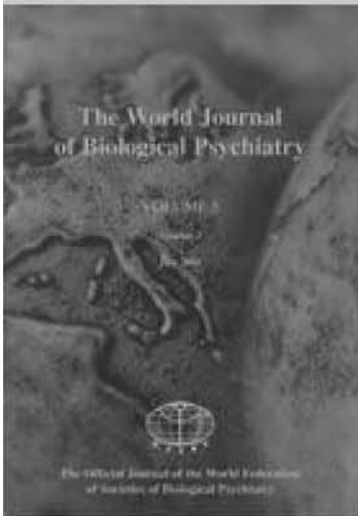
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