International Journal of Neuropsychopharmacology, Page 1 of 10. © CINP 2011
doi:10.1017/S1461145711001507

ARTICLE

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Clozapine protects bone mineral density in female patients with schizophrenia

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Abstract

Decreased bone mineral density (BMD) is common in patients with schizophrenia; however, the pathogenesis is unclear. Different classes of antipsychotic agents may affect BMD. This study systemically examined the effects of clozapine *vs.* other antipsychotics, and several hormonal and metabolic factors that may contribute to BMD in female patients with schizophrenia, who are more vulnerable than males. Forty-eight women with schizophrenia, treated with long-term antipsychotics of the prototype prolactin-sparing (PS) antipsychotic agent clozapine *vs.* prolactin-raising (PR) antipsychotics were enrolled. They were matched for demographic and clinical characteristics. Various factors, including blood levels of prolactin and sex hormones, psychopathological symptoms, global assessment of functioning, physical activity, and menopausal status, were determined to explore their contribution to low BMD (LBMD), defined as a dual-energy X-ray absorptiometer (DEXA) T score < -1. Overall, women receiving clozapine have better bone density than women receiving PR antipsychotics. Compared to PR antipsychotics, PS clozapine therapy is a protective factor (odds ratio 28.2, 95% confidence interval 2.37–336.10, $p = 0.008$) for LBMD. Predictors for higher bone density in the clozapine group included higher clozapine dose ($p < 0.001$), younger age ($p < 0.001$), and higher thyroid-stimulating hormone level ($p < 0.001$); in the PR group, higher body mass index ($p = 0.003$) and lower alkaline phosphatase level ($p = 0.007$) were associated with LBMD. This study suggests that clozapine treatment is beneficial for BMD compared to PR antipsychotic treatment in women with chronic schizophrenia, and clozapine's bone-density protecting effect is dose-related.

Received 22 March 2011; Reviewed 13 July 2011; Revised 26 July 2011; Accepted 23 August 2011

Key words: Bone mineral density, clozapine, osteoporosis, prolactin, schizophrenia.

Introduction

Osteoporosis, associated with high morbidity and mortality, is common in patients with schizophrenia (Renn *et al.* 2009). Patients who receive treatment of potent dopamine D_2 receptor-blocking antipsychotics frequently suffer from hyperprolactinaemia (Halbreich & Palter, 1996; Kane *et al.* 2002, 2009; Lu *et al.* 2008; Meaney *et al.* 2004). Antipsychotic-induced hyperprolactinaemia and/or secondary hypogonadism may be implicated in osteoporosis (Halbreich & Palter, 1996; Meaney *et al.* 2004), but it is inconclusive (Abraham *et al.* 2003; Lean & De Smedt, 2004).

Among prolactin-sparing (PS) antipsychotics (including clozapine, olanzapine, quetiapine, aripiprazole), olanzapine's effects on bone mineral density (BMD) have been studied but the results are

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inconclusive (Abraham *et al.* 2003; Becker *et al.* 2003; Meaney & O'Keane, 2007; O'Keane & Meaney, 2005; Wyszogrodzka-Kucharska & Rabe-Jablonska, 2005*b*). There are a few variables that may have contributed to the discrepant findings. First, the influence of antipsychotic class and dosage remain unclear. Second, osteoporosis has numerous risk factors such as dietary calcium deficiency (Baastrup *et al.* 1980; Wyszogrodzka-Kucharska & Rabe-Jablonska, 2005*a*), polydipsia (de Leon *et al.* 1994), lack of physical activity (Halbreich & Palter, 1996; Kishimoto *et al.* 2008), history of smoking or alcohol drinking (Cantor-Graae *et al.* 2001; Kavanagh *et al.* 2002; Malik *et al.* 2009), and lack of UV light exposure/vitamin D (Halbreich & Palter, 1996). In addition, the role of adjuvant mood stabilizers has been controversial (Stephen *et al.* 1999; Zamani *et al.* 2009). To understand the pathogenesis of BMD in schizophrenia, it is critical to take all these variables into account simultaneously in order to explore the effect of antipsychotics on BMD.

The first PS antipsychotic agent, clozapine, deserves more attention in this issue. Adverse effects of clozapine may be lifelong because being the most efficacious and last-line treatment for schizophrenia, it is difficult to change clozapine for other antipsychotics once it is instituted (Correll *et al.* 2009).

Prevalence and predictors of decreased BMD risk are different between female and male patients (Rozental *et al.* 2010). Female patients usually have more than 2-fold higher prevalence rate of decreased BMD than male patients (Zhu *et al.* 2010), and gender-specific factors such as prolactin and sex hormone levels should be considered for the risk assessment and treatment of osteoporosis (Haney & Bliziotes, 2008; Pietschmann *et al.* 2009). To explore the contributory factors of decreased BMD, our study enrolled two groups of subjects, (1) women receiving prolactin-raising (PR) antipsychotics and (2) women receiving clozapine, and systemically examined demographic effects (such as age and education level), physical effects [such as body mass index (BMI) and hormones], and various previously mentioned factors on BMD in women with schizophrenia, who are more likely to have lower BMD than males.

Methods

Setting

This study was conducted in chronic inpatient units in a major psychiatric centre in Taiwan in order to recruit a study population with supervised nutrition and activity levels. The average duration of hospitalization was 683.7 d. All subjects received regular diet prepared by the hospital which contained 600 mg calcium and 2050 calories per day. The study was approved by the institutional review board and conducted in accordance with the Declaration of Helsinki.

Patients

Women with chronic schizophrenia who had been diagnosed according to DSM-IV criteria (APA, 1994) by experienced psychiatrists and had been hospitalized in chronic wards for at least 6 months were enrolled. All recruited women had been treated with unchanged antipsychotics and doses for at least 6 months (Boling, 2004; Suzuki & Sato, 2010). Women with the following physical or mental conditions which may influence BMD were excluded: eating disorder, substance abuse/dependence (including smoking and alcohol consumption), renal function impairment, electrolyte imbalance, bone metabolism diseases, thyroid or parathyroid diseases, pituitary tumour, pregnancy or lactation, and co-medications known to influence BMD [e.g. glucocorticoids (Suzuki & Sato, 2010), heparin (Wawrzynska *et al.* 2003) and drugs for osteoporosis such as parathyroid hormone (Rosen, 2005), alendronate (Iwamoto *et al.* 2010), selective oestrogen receptor modulators (Kulak *et al.* 2010), bisphosphonates, oestrogens, and calcitonin] except benzodiazepines and antidepressants which were not significantly associated with reduced BMD (Kinjo *et al.* 2005). Written informed consent was obtained after thorough explanation of the study. Two treatment groups of women were enrolled: the PR group (three women treated with chlorpromazine, two with flupentixol, five with haloperidol, eight with risperidone, one with paliperidone, five with sulpiride) and the PS group (24 women treated with clozapine).

Assessment

Previous studies have found that the inverse relationship between BMD and risk of fracture is similar for dual-energy X-ray absorptiometer (DEXA) measurements made at the hip, spine, and appendicular sites (SBU, 1997; Black *et al.* 1992). Therefore we measured BMD of lumbar spine since it is standard (Sturtridge *et al.* 1996). BMD was assayed at lumbar spine L2–L4 by DEXA measurement in patients in a supine position (Placide & Martens, 2003; Theodorou *et al.* 2002), which was reviewed by an experienced radiologist who was blind to the clinical and pharmacological characteristics of the subjects. Osteopenia was defined as the absolute BMD value, T score < -1 and > -2.5 (Czerwinski, 1997), and osteoporosis was defined as

the absolute BMD value T score $\leqslant -2.5$ (Kanis, 2002). A T score < -1 was regarded as low BMD [(LBMD), including osteopenia and osteoporosis] (Czerwinski, 1997). T scores were derived from comparison with the 30-year-old population. DEXA Z score was also measured to help determine whether bone mineral loss resulted from ageing (Swaminathan *et al.* 2009). A Z score < -1 was regarded as bone mineral loss with causes other than age itself (Swaminathan *et al.* 2009).

Blood and urine samples were collected at 08:00 hours for the analysis of bone remodelling-related factors, including complete blood and platelet count, serum oestradiol, testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, cortisol, thyroid hormones, thyroid-stimulating hormone (TSH), GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase), BUN (blood urea nitrogen), creatinine, alkaline phosphatase, and serum and urine calcium. Hyperprolactinaemia is defined as an excess of prolactin above a reference laboratory's upper limit, 25 ng/ml (Iwasa *et al.* 2006; Karasek *et al.* 2006). Women with abnormal GOT, GPT, BUN, or creatinine concentrations were excluded. Pedometers were used to measure activity levels by calculating average steps for 24 h (Kishimoto *et al.* 2008). Physical examination including BMI, and menopausal status were also determined.

Research psychiatrists collected clinical data such as history of psychiatric and physical illness, medication, and substance use by chart review and structured clinical interview. Women with a history of substance use (including alcohol consumption and smoking) were excluded. Positive and Negative Syndrome Scale (PANSS) (Buchanan *et al.* 2005; Kay *et al.* 1987) and Global Assessment of Functioning (GAF) (Axis V of DSM-IV) were used to assess the severity of psychopathological symptoms and overall functioning. An intra-class correlation coefficient (ICC) of 0.95 was obtained between the raters.

All of the above assessments were completed within 3 d for each subject.

Statistical analysis

χ^2 tests or Fisher's exact test were applied for between-group comparisons of categorical data. Continuous data were analysed by t test or the corresponding non-parametric method, Mann–Whitney U test, for variables with non-normal distributions. The Shapiro–Wilk W test was used to examine normality. The logistic regression model was used to calculate the odds ratios for factors related to LBMD. The linear regression model was used to explore the potential prognostic factors related to DEXA T score. All tests were two-tailed, and significance of tests was defined as $\alpha < 0.05$. Data were analysed with SPSS version 17.0 (SPSS Inc., USA) for Windows and SAS version 9.2 (SAS Institute Inc., USA).

Results

Patients

Forty-eight women were enrolled in this study: 24 women receiving PR antipsychotics and 24 women receiving clozapine. They were matched for age, education duration, duration of disease, BMI and menopausal status. Therefore, the two groups had similar demographic and clinical characteristics except that the PR group had higher prolactin levels, a higher *percentage* of hyperprolactinaemia, and less severe psychopathological symptoms (Tables 1 and 2).

Clozapine patients are less prone to low bone density

Since most of the subjects in the present study were premenopausal women who should be less prone to have osteoporosis, we used LBMD as the main outcome measure instead of osteoporosis. Compared to the clozapine group, the women in the PR group were more likely to experience LBMD by both DEXA T score definition (Theodorou *et al.* 2002) [11/24 (45.8%) *vs.* 4/24 (16.7%), $p=0.029$, Table 2] and by DEXA Z score definition (Swaminathan *et al.* 2009) [8/24 (33.3%) *vs.* 0/24 (0%), $p=0.002$, Table 2]. DEXA Z score was used for excluding age effects on BMD (Swaminathan *et al.* 2009).

Predicting variables for low bone density

We utilized multiple logistic regression to identify the risk factors of LBMD (Table 3). Prior to application of multiple logistic regression, a simple logistic regression model separately examined all potentially predicting variables, including age, education duration, duration of schizophrenia, duration of antipsychotic treatment, duration of hospitalization, BMI, waist circumference, menopausal status, hyperprolactinaemia, prolactin level, oestradiol level, testosterone level, PR antipsychotics or clozapine use, antipsychotics dose, duration of antipsychotic exposure, concomitant mood stabilizer use, PANSS total score, GAF score, pedometer steps, cortisol level, T3 level, free T4 level, TSH level, alkaline phosphatase level and calcium level. All the variables (age, testosterone level, and PR *vs.* clozapine) which influenced risk of LBMD in single logistic regression were then

Table 1. Demographic characteristics of schizophrenic women receiving prolactin-raising (PR) antipsychotics or clozapine

Characteristics	PR antipsychotics ($N=24$)		Clozapine ($N=24$)		Test statistic	p
	Mean	S.D.	Mean	S.D.		
Age (yr)	41.88	8.00	41.75	10.23	$t=0.047$	0.963
Education duration (yr)	10.23	2.96	10.06	2.477	$t=0.211$	0.834
Body mass index	25.44	4.18	25.77	4.07	$t=-0.281$	0.780
Duration of disease (month)	245.50	130.02	255.65	108.98	$Z=-0.291$	0.773[b]
Duration of hospitalization (d)	683.38	546.12	944.21	763.95	$t=-1.362$	0.181[b]
Chlorpromazine equivalent dose (mg/d) (Gardner *et al.* 2010)	572.88	431.51	–	–	–	–
Clozapine dose (mg/d)	–	–	224.79	114.64	–	–
PANSS total score	77.88	17.76	87.25	19.34	$t=-1.749$	0.087
PANSS positive symptoms score	18.25	4.58	19.83	5.68	$t=-1.063$	0.293
PANSS negative symptoms score	20.54	8.28	24.46	5.96	$t=-1.880$	0.067
PANSS general symptoms score	39.08	8.87	42.96	10.19	$Z=-1.405$	0.167[b]
Global Assessment of Functioning score	49.33	14.28	41.33	12.53	$t=2.063$	0.045
Pedometer steps	6593.29	6287.29	4944.92	4138.25	$Z=1.073$	0.290[b]
	n	%	n	%		
Menopausal status (post-menopausal)	3	12.5	4	16.7	$\chi^2=0.167$	1.000[c]
Concomitant all mood stabilizers[a]	7	29.2	5	20.8	$\chi^2=0.444$	0.740
Concomitant lithium treatment	2	8.3	1	4.2	$\chi^2=0.356$	1.000[c]
Concomitant valproate treatment	5	20.8	4	16.7	$\chi^2=0.137$	1.000[c]
Concomitant carbamazepine treatment	1	4.2	0	0.0	$\chi^2=1.021$	1.000[c]

PANSS, Positive and Negative Syndrome Scale.
[a] Mood stabilizers include valproate, carbamazepine and lithium.
[b] Mann–Whitney U test, for variables with non-normal distributions.
[c] Fisher's exact test.

selected as predicting variables for the multiple logistic regression model (Table 3). We found that the odds ratio of LBMD in the PR antipsychotic group compared to the PS antipsychotic (clozapine) group was 28.20 [95% confidence interval (CI) 2.37–336.10, $p=0.008$], and the odds ratio of age was 1.27 (95% CI 1.07–1.52, $p=0.007$) (Table 3). However, prolactin level and other variables did not significantly affect the risk of LBMD (Table 3).

Predicting variables for bone density in two treatment groups

Next, we examined predictors for BMD in the two separate treatment groups (Table 4). Because there were only four LBMD women in the clozapine group, we used DEXA T score as the response variable in the multiple linear regression model (Table 4). As mentioned previously, all potentially predicting variables were examined separately in the univariate model; then the variables which showed significant effects in the univariate model were selected for the multiple linear regression model (Table 4). As a result, in the PR group higher BMI ($p=0.003$) and lower alkaline phosphatase level ($p=0.007$) were significantly associated with higher T score (adjusted $R^2=0.438$, Table 3). In the clozapine group, younger age ($p<0.001$), higher clozapine dose ($p<0.001$), and higher TSH level ($p<0.001$) were associated with higher T score (adjusted $R^2=0.627$).

The result of fitted line and scatter plots by fixed-effects model for DEXA T score *vs.* predicted value was better for the clozapine group (adjusted $R^2=0.627$) than for the PR group (adjusted $R^2=0.438$). By the mixed-effects model using the PROC MIXED procedure in SAS, we added β_{0i}s into the model and used the intercept as the random-effect term to describe the potential individual variations of the T score and all those prognostic factors in Table 4 as the fixed-effect terms. Without repeated measurements, the mixed-effects models were over-fitted for the PR and clozapine groups, respectively (Fig. 1*a*, *b*). The fitted model

Table 2. Clinical characteristics of schizophrenic women receiving prolactin-raising (PR) antipsychotics or clozapine

Characteristics	PR antipsychotics ($N=24$)		Clozapine ($N=24$)		Test statistic	p
	n	%	n	%		
Hyperprolactinaemia[a]	23	95.8	5	20.8	$\chi^2=27.771$	<0.001
LBMD using DEXA T score (LBMDT)[b]	11	45.8	4	16.7	$\chi^2=4.752$	0.029
LBMD using DEXA Z score (LBMDZ)[c]	8	33.3	0	0.0	$\chi^2=9.600$	0.002[e]
	Mean	S.D.	Mean	S.D.		
Waist circumference (cm)	85.93	11.22	87.15	8.41	$t=-0.425$	0.673
Duration of antipsychotic treatment (d)	683.67	546.32	959.79	750.55	$t=-1.457$	0.153
Serum calcium (mg/dl)	8.69	0.64	8.73	0.43	$Z=-0.240$	0.812[d]
Urine calcium (mg/dl)	8.91	11.98	9.56	7.19	$Z=-0.227$	0.822[d]
Thyroid-stimulating hormone (mIU/l)	2.33	1.36	2.59	3.87	$Z=-0.311$	0.758[d]
T3 (ng/dl)	95.58	23.33	88.57	23.30	$Z=1.043$	0.303[d]
Free T4 (ng/dl)	0.99	0.15	0.93	0.07	$t=1.640$	0.110
Cortisol (μg/dl)	10.71	4.38	12.57	5.02	$t=-1.374$	0.176
Testosterone (ng/ml)	0.45	0.22	0.54	0.54	$Z=-0.723$	0.475[d]
Follicle-stimulating hormone (IU/l)	18.86	29.76	13.47	18.27	$t=0.756$	0.455
Luteinizing hormone (IU/l)	6.82	6.57	9.52	8.42	$t=-1.240$	0.222
Oestradiol (pg/ml)	46.78	54.39	66.59	56.11	$Z=-1.242$	0.221[d]
Prolactin (ng/ml)	109.01	65.63	19.17	9.96	$Z=6.630$	<0.001[d]
Alkaline phosphatase (U/l)	66.42	19.15	74.75	19.40	$t=-1.497$	0.141

DEXA, Dual-energy X-ray absorptiometer; LBMD, low bone mineral density.

[a] Hyperprolactinaemia is defined as prolactin level >25 ng/ml (Iwasa *et al.* 2006; Karasek *et al.* 2006).

[b] LBMDT was defined as DEXA T score <-1 (Czerwinski, 1997).

[c] LBMDZ was defined as DEXA Z score $\leqslant -1$ (Swaminathan *et al.* 2009).

[d] Mann–Whitney U test, for variables with non-normal distributions.

[e] Fisher's exact test.

for the PR group was significantly improved (adjusted R^2 increased from 0.438 to 0.900). For the clozapine group, the adjusted R^2 increased from 0.627 to 0.859.

Discussion

In the present study, PR antipsychotics use *per se* was associated with LBMD after adjusting other factors related to BMD (Table 3). The current study demonstrated that women receiving clozapine are much less prone to LBMD than women receiving PR antipsychotics (Table 3). This favourable outcome may contribute to less morbidity and mortality in women receiving clozapine compared to other antipsychotics (Meltzer *et al.* 2003; Tiihonen *et al.* 2009), although they tend to be more symptomatic psychiatrically (Table 1).

To our knowledge, this is the first study to find that higher dosage of clozapine is associated with a higher DEXA T score in women with schizophrenia. The relationship between clozapine use and bone metabolism or BMD has not yet been addressed. Clozapine can interact with the glycine site of the *N*-methyl-D-aspartic acid (NMDA) receptor (Schwieler *et al.* 2008) and up-regulate NMDA receptors (Gray *et al.* 2009; Lane *et al.* 2006). NMDA receptors are expressed in human and rat osteoblasts and osteoclasts (Chenu *et al.* 1998; Patton *et al.* 1998), and down-regulation of NMDA receptors may hamper osteogenesis (Ho *et al.* 2005). It is possible that clozapine may protect BMD by activating NMDA receptors. It is unclear whether clozapine can help or protect patients from the effects of other antipsychotics in the present study. If reconfirmed by further studies with longer follow-up duration, clozapine might be a good treatment choice for schizophrenic women with osteopenia or osteoporosis.

Prolactin concentration was higher and oestradiol concentration lower (not significantly) in the PR antipsychotic group; however, neither prolactin level, hyperprolactinaemia, nor oestradiol level was significantly associated with BMD in either treatment

Table 3. Simple and multiple logistic regression analyses of independent predictive factors[a] associated with low bone mineral density (LBMD)[b] in schizophrenic women (stepwise)

	Univariate		Multivariate		
Variable	OR	*p*	OR	95% CI	*p*
Age (yr)	1.21	**0.002**	1.27	1.07–1.52	0.007
Education duration (yr)	0.91	0.439			
Duration of disease (month)	1.006	0.065			
Duration of hospitalization (d)	1.001	0.229			
Duration of antipsychotic treatment (d)	1.001	0.246			
Body mass index (BMI)	0.876	0.118			
Waist circumference (cm)	0.991	0.791			
Menopausal status (post-menopausal)	0.275	0.125			
Hyperprolactinaemia	1.667	0.432			
Prolactin (ng/ml)	1.003	0.594			
Oestradiol (pg/ml)	0.992	0.238			
Chlorpromazine equivalence dose (mg/d)	1.000	0.859			
Concomitant all mood stabilizers	2.826	0.221			
PANSS total score	0.995	0.747			
Global Assessment of Functioning score	1.001	0.964			
Pedometer steps	1.000	0.676			
Cortisol (μg/dl)	0.974	0.697			
Free T4 (ng/dl)	0.709	0.898			
T3 (ng/dl)	0.994	0.668			
TSH (mIU/l)	1.203	0.247			
Alkaline phosphatase (U/l)	1.009	0.582			
Serum calcium (mg/dl)	0.981	0.974			
Urine calcium (mg/dl)	1.033	0.322			
Testosterone level (ng/ml)	0.007	**0.021**			
PR antipsychotic treatment *vs.* clozapine	4.23	**0.035**	28.20	2.37–336.10	0.008

OR, Odds ratio; CI, confidence interval; PANSS, Positive and Negative Syndrome Scale; TSH, thyroid-stimulating hormone; PR, prolactin-raising.

All the potentially predicting variables mentioned previously in Table 2 were examined separately in the univariate model; then the variables (BMI and alkaline phosphatase level in the PR group; and age, drug dose, and TSH level in the clozapine group) which showed significant effects in the univariate model were selected into the multiple linear regression model.

[a] Prior to application of multiple logistic regression, a simple logistic regression model separately examined all potentially predicting variables, including age, education duration, duration of schizophrenia, duration of antipsychotic treatment, duration of hospitalization, BMI, waist circumference, menopausal status, hyperprolactinaemia, prolactin level, oestradiol level, testosterone level, PR antipsychotic or clozapine use, antipsychotic dose, concomitant mood stabilizer use, PANSS total score, Global Assessment of Functioning score, pedometer steps, cortisol level, T3 level, free T4 level, TSH level, alkaline phosphatase level, and calcium level (not shown).

All the variables (age, testosterone level, and PR *vs.* clozapine) which influenced the risk of LBMD in single logistic regressions were then selected to be predicting variables for the multiple logistic regression model.

[b] LBMD was defined as dual-energy X-ray absorptiometer (DEXA) T score < -1 (Czerwinski, 1997).

group or in the studied subjects as a whole. Consistent with previous studies (Abraham *et al.* 2003; Howes *et al.* 2005; Lean & De Smedt, 2004), this finding does not support the role of hyperprolactinaemia/hypogonadism in low bone density in women receiving antipsychotics (Halbreich & Palter, 1996; Meaney *et al.* 2004). However, higher prolactin levels over a longer period, which could not be measured in the present study, may be necessary to lead to BMD loss, since sustained hyperprolactinaemia was found to have an impact on the rate of bone metabolism in a previous study (Abraham *et al.* 2003). Of note, prolactin concentrations might be related to the serum concentrations of antipsychotics and their metabolites (Bai *et al.* 2007) which we did not measure in the present study.

Table 4. Multiple linear regression analysis (stepwise) of independent predictive factors associated with DEXA *T* score in schizophrenic women receiving prolactin-raising antipsychotics or clozapine

Variable	*B* (S.E.)	*t*	*p*
Prolactin-raising (PR) antipsychotics group ($N=24$)			
Body mass index	0.18 (0.05)	3.43	0.003
Alkaline phosphatase level (U/l)	−0.03 (0.01)	−3.02	0.007
(Adjusted $R^2=0.438$)			
Clozapine group ($N=24$)			
Age (yr)	−0.066 (0.02)	−4.27	<0.001
Clozapine dose (mg/d)	0.005 (0.001)	4.91	<0.001
Thyroid-stimulating hormone level (mIU/l)	0.590 (0.14)	4.26	<0.001
(Adjusted $R^2=0.627$)			

DEXA, Dual-energy X-ray absorptiometer.

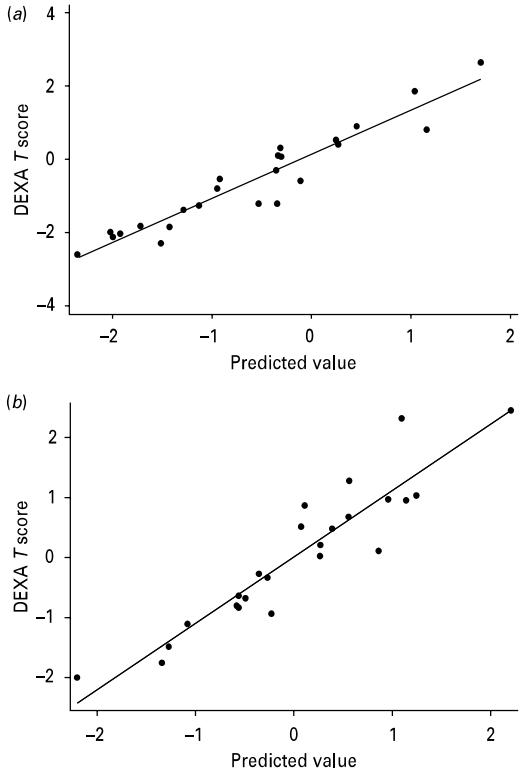

Fig. 1. (*a*) The fitted result for the prolactin-raising antipsychotic group using a mixed-effects model (adjusted $R^2=0.900$). (*b*) The fitted result for the clozapine group using a mixed-effects model (adjusted $R^2=0.859$).

Alkaline phosphatase activity has been found to be negatively correlated with BMD (Hulth *et al.* 1979). In the current study, alkaline phosphatase level, lower in the PR group than the clozapine group, was also negatively correlated with DEXA *T* score in the PR group but not in the clozapine group. In osteoblast-like cells, prolactin exposure can lower expression and activity of alkaline phosphatase (Seriwatanachai *et al.* 2008). In newborn rat pups, prolactin treatment decreases blood alkaline phosphatase by 30% (Coss *et al.* 2000). It seems possible that BMD increases when alkaline phosphatase concentration is reduced by high prolactin levels in women treated with PR antipsychotics.

Higher BMI has been found to be associated with increased BMD (Akdeniz *et al.* 2009; Howes *et al.* 2005; Hummer *et al.* 2005; Kim *et al.* 2008). Accordingly, BMI was associated with BMD in the PR group in the present study. However, we did not have the baseline BMI and BMD values before the initiation of antipsychotic treatment. It is possible that women have had different BMD before treatment due to genetic or other predisposing factors, thereby lowering the R^2 of the multiple linear regression model. Prospective studies are needed to verify the effect of clozapine and PR antipsychotics by comparing BMD after long-term medication use with baseline BMD.

Higher TSH concentration was associated with higher *T* score in the clozapine group in our study. Murphy *et al.* (2010) found that higher TSH levels protected healthy euthyroid post-menopausal women from non-vertebral fracture. Baqi *et al.* (2010) demonstrated a favourable influence of TSH on BMD in adult women. Another study (Kim *et al.* 2010) also suggested a positive association between serum TSH concentration and BMD in men. It is possible that thyroid function is important for BMD in patients receiving clozapine, whose BMD is normal. However, the effect

of TSH may be insufficient to sustain BMD in women treated with PR antipsychotics.

There are several limitations of this study. First, the sample size was small. The second limitation of this cross-sectional study is that we did not collect the baseline BMI and BMD values before treatment. Third, we did not check genetic or other predisposing factors for lower BMD. Moreover, we may not be able to apply the findings to all women with schizophrenia since the population of the present study had relatively longer duration of disease and more severe symptoms than community controls.

In summary, this study suggests that clozapine treatment is beneficial for BMD compared to PR antipsychotic treatment in women with chronic schizophrenia. Factors associated with DEXA *T* scores were different between women receiving PR antipsychotics and those receiving clozapine; clinicians should take note of different factors according to their antipsychotic treatments when assessing the risk for osteoporosis. The study also suggests that clozapine's bone density-protecting effect is dose-related with higher dosage associated with denser BMD. Clozapine may enhance osteogenesis by activating NMDA receptors on the bone cells; further studies are needed to explore this possibility. A prospective study is needed to confirm the favourable effect of clozapine use in BMD and its possible mechanisms in women with schizophrenia.

Acknowledgements

This study was funded by the National Science Council (Taiwan) NSC-97-2314-B-039-006-MY3, NSC-98-2627-B-039-001, NSC 99-3114-B-182A-003, DOH99-TD-I-111-TM001, the National Health Research Institutes (Taiwan) NHRI-EX-100-9904NI, and Taiwan Department of Health Clinical Trial and Research Center of Excellence DOH100-TD-B-111-004.

Statement of Interest

None.

References

Abraham G, Paing WW, Kaminski J, Joseph A, *et al.* (2003). Effects of elevated serum prolactin on bone mineral density and bone metabolism in female patients with schizophrenia: a prospective study. *American Journal of Psychiatry* **160**, 1618–1620.

Akdeniz N, Akpolat V, Kale A, Erdemoglu M, *et al.* (2009). Risk factors for postmenopausal osteoporosis: anthropometric measurements, age, age at menopause and the time elapsed after menopause onset. *Gynecological Endocrinology* **25**, 125–129.

APA (1994). *Structured Clinical Interview for DSM-IV*. Washington, DC: American Psychiatric Press.

Baastrup PC, Christiansen C, Transbol I (1980). Calcium metabolism in schizophrenic patients on long-term neuroleptic therapy. *Neuropsychobiology* **6**, 56–59.

Bai YM, Ting Chen T, Chen JY, Chang WH, *et al.* (2007). Equivalent switching dose from oral risperidone to risperidone long-acting injection: a 48-week randomized, prospective, single-blind pharmacokinetic study. *Journal of Clinical Psychiatry* **68**, 1218–1225.

Baqi L, Payer J, Killinger Z, Susienkova K, *et al.* (2010). The level of TSH appeared favourable in maintaining bone mineral density in postmenopausal women. *Endocrine Regulations* **44**, 9–15.

Becker D, Liver O, Mester R, Rapoport M, *et al.* (2003). Risperidone, but not olanzapine, decreases bone mineral density in female premenopausal schizophrenia patients. *Journal of Clinical Psychiatry* **64**, 761–766.

Black DM, Cummings SR, Genant HK, Nevitt MC, *et al.* (1992). Axial and appendicular bone density predict fractures in older women. *Journal of Bone and Mineral Research* **7**, 633–638.

Boling EP (2004). Secondary osteoporosis: underlying disease and the risk for glucocorticoid-induced osteoporosis. *Clinical Therapeutics* **26**, 1–14.

Buchanan RW, Davis M, Goff D, Green MF, *et al.* (2005). A summary of the FDA-NIMH-MATRICS workshop on clinical trial design for neurocognitive drugs for schizophrenia. *Schizophrenia Bulletin* **31**, 5–19.

Cantor-Graae E, Nordstrom LG, McNeil TF (2001). Substance abuse in schizophrenia: a review of the literature and a study of correlates in Sweden. *Schizophrenia Research* **48**, 69–82.

Chenu C, Serre CM, Raynal C, Burt-Pichat B, *et al.* (1998). Glutamate receptors are expressed by bone cells and are involved in bone resorption. *Bone* **22**, 295–299.

Correll CU, Rummel-Kluge C, Corves C, Kane JM, *et al.* (2009). Antipsychotic combinations *vs.* monotherapy in schizophrenia: a meta-analysis of randomized controlled trials. *Schizophrenia Bulletin* **35**, 443–457.

Coss D, Yang L, Kuo CB, Xu X, *et al.* (2000). Effects of prolactin on osteoblast alkaline phosphatase and bone formation in the developing rat. *American Journal of Physiology. Endocrinology and Metabolism* **279**, E1216–E1225.

Czerwinski E (1997). Radiologic diagnosis and densitometry of osteoporosis. *Przeglad Lekarski* **54**, 220–225.

de Leon J, Verghese C, Tracy JI, Josiassen RC, *et al.* (1994). Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biological Psychiatry* **35**, 408–419.

Gardner DM, Murphy AL, O'Donnell H, Centorrino F, *et al.* (2010). International consensus study of antipsychotic dosing. *American Journal of Psychiatry* **167**, 686–693.

Gray L, van den Buuse M, Scarr E, Dean B, *et al.* (2009). Clozapine reverses schizophrenia-related behaviours in the metabotropic glutamate receptor 5 knockout

mouse: association with N-methyl-D-aspartic acid receptor up-regulation. *International Journal of Neuropsychopharmacology* **12**, 45–60.

Halbreich U, Palter S (1996). Accelerated osteoporosis in psychiatric patients: possible pathophysiological processes. *Schizophrenia Bulletin* **22**, 447–454.

Haney EM, Bliziotes MM (2008). Male osteoporosis: new insights in an understudied disease. *Current Opinion in Rheumatology* **20**, 423–428.

Ho ML, Tsai TN, Chang JK, Shao TS, *et al.* (2005). Down-regulation of N-methyl D-aspartate receptor in rat-modeled disuse osteopenia. *Osteoporosis International* **16**, 1780–1788.

Howes OD, Wheeler MJ, Meaney AM, O'Keane V, *et al.* (2005). Bone mineral density and its relationship to prolactin levels in patients taking antipsychotic treatment. *Journal of Clinical Psychopharmacology* **25**, 259–261.

Hulth AG, Nilsson BE, Westlin NE, Wiklund PE (1979). Alkaline phosphatase in women with osteoporosis. *Acta Medica Scandinavica* **206**, 201–203.

Hummer M, Malik P, Gasser RW, Hofer A, *et al.* (2005). Osteoporosis in patients with schizophrenia. *American Journal of Psychiatry* **162**, 162–167.

Iwamoto J, Sato Y, Uzawa M, Takeda T, *et al.* (2010). Seven years' experience with Alendronate in postmenopausal Japanese women with osteoporosis. *Therapeutics and Clinical Risk Management* **6**, 201–206.

Iwasa T, Matsuzaki T, Tanaka N, Minakuchi M, *et al.* (2006). Comparison and problems of measured values of LH, FSH, and PRL among measurement systems. *Endocrine Journal* **53**, 101–109.

Kane JM, Carson WH, Saha AR, McQuade RD, *et al.* (2002). Efficacy and safety of aripiprazole and haloperidol *vs.* placebo in patients with schizophrenia and schizoaffective disorder. *Journal of Clinical Psychiatry* **63**, 763–771.

Kane JM, Correll CU, Goff DC, Kirkpatrick B, *et al.* (2009). A multicenter, randomized, double-blind, placebo-controlled, 16-week study of adjunctive aripiprazole for schizophrenia or schizoaffective disorder inadequately treated with quetiapine or risperidone monotherapy. *Journal of Clinical Psychiatry* **70**, 1348–1357.

Kanis JA (2002). Diagnosis of osteoporosis and assessment of fracture risk. *Lancet* **359**, 1929–1936.

Karasek M, Pawlikowski M, Lewinski A (2006). Hyperprolactinemia: causes, diagnosis, and treatment. *Endokrynologia Polska* **57**, 656–662.

Kavanagh DJ, McGrath J, Saunders JB, Dore G, *et al.* (2002). Substance misuse in patients with schizophrenia: epidemiology and management. *Drugs* **62**, 743–755.

Kay SR, Fiszbein A, Opler LA (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin* **13**, 261–276.

Kim BJ, Lee SH, Bae SJ, Kim HK, *et al.* (2010). The association between serum thyrotropin (TSH) levels and bone mineral density in healthy euthyroid men. *Clinical Endocrinology* **73**, 396–403.

Kim YM, Hyun NR, Shon HS, Kim HS, *et al.* (2008). Assessment of clinical risk factors to validate the probability of osteoporosis and subsequent fractures in Korean women. *Calcified Tissue International* **83**, 380–387.

Kinjo M, Setoguchi S, Schneeweiss S, Solomon DH (2005). Bone mineral density in subjects using central nervous system-active medications. *American Journal of Medicine* **118**, 1414.

Kishimoto T, Watanabe K, Shimada N, Makita K, *et al.* (2008). Antipsychotic-induced hyperprolactinemia inhibits the hypothalamo-pituitary-gonadal axis and reduces bone mineral density in male patients with schizophrenia. *Journal of Clinical Psychiatry* **69**, 385–391.

Kulak Jr. J, Kulak CA, Taylor HS (2010). SERMs in the prevention and treatment of postmenopausal osteoporosis: an update. *Arquivos Brasileiros de Endocrinologia e Metabologia* **54**, 200–205.

Lane HY, Huang CL, Wu PL, Liu YC, *et al.* (2006). Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to clozapine for the treatment of schizophrenia. *Biological Psychiatry* **60**, 645–649.

Lean M, De Smedt G (2004). Schizophrenia and osteoporosis. *International Clinical Psychopharmacology* **19**, 31–35.

Lu ML, Shen WW, Chen CH (2008). Time course of the changes in antipsychotic-induced hyperprolactinemia following the switch to aripiprazole. *Progress in Neuro-psychopharmacology & Biological psychiatry* **32**, 1978–1981.

Malik P, Gasser RW, Kemmler G, Moncayo R, *et al.* (2009). Low bone mineral density and impaired bone metabolism in young alcoholic patients without liver cirrhosis: a cross-sectional study. *Alcoholism, Clinical and Experimental Research* **33**, 375–381.

Meaney AM, O'Keane V (2007). Bone mineral density changes over a year in young females with schizophrenia: relationship to medication and endocrine variables. *Schizophrenia Research* **93**, 136–143.

Meaney AM, Smith S, Howes OD, O'Brien M, *et al.* (2004). Effects of long-term prolactin-raising antipsychotic medication on bone mineral density in patients with schizophrenia. *British Journal of Psychiatry* **184**, 503–508.

Meltzer HY, Alphs L, Green AI, Altamura AC, *et al.* (2003). Clozapine treatment for suicidality in schizophrenia: International Suicide Prevention Trial (InterSePT). *Archives of General Psychiatry* **60**, 82–91.

Murphy E, Gluer CC, Reid DM, Felsenberg D, *et al.* (2010). Thyroid function within the upper normal range is Associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **95**, 3173–3181.

O'Keane V, Meaney AM (2005). Antipsychotic drugs: a new risk factor for osteoporosis in young women with schizophrenia? *Journal of Clinical Psychopharmacology* **25**, 26–31.

Patton AJ, Genever PG, Birch MA, Suva LJ, *et al.* (1998). Expression of an N-methyl-D-aspartate-type receptor by human and rat osteoblasts and osteoclasts suggests a novel glutamate signaling pathway in bone. *Bone* **22**, 645–649.

Pietschmann P, Rauner M, Sipos W, Kerschan-Schindl K (2009). Osteoporosis: an age-related and gender-specific disease – a mini-review. *Gerontology* **55**, 3–12.

Placide J, Martens MG (2003). Comparing screening methods for osteoporosis. *Current Women's Health Reports* **3**, 207–210.

Renn JH, Yang NP, Chueh CM, Lin CY, *et al.* (2009). Bone mass in schizophrenia and normal populations across different decades of life. *BMC Musculoskeletal Disorders* **10**, 1.

Rosen CJ (2005). The role of parathyroid hormone in the management of osteoporosis. *Hormone Research* **64** (Suppl. 2), 81–85.

Rozental TD, Shah J, Chacko AT, Zurakowski D (2010). Prevalence and predictors of osteoporosis risk in orthopaedic patients. *Clinical Orthopaedics and Related Research* **468**, 1765–1772.

SBU (1997). Bone density measurement – a systematic review. a report from SBU, the Swedish Council on Technology Assessment in Health Care. *Journal of Internal Medicine* **739** (Suppl.), 1–60.

Schwieler L, Linderholm KR, Nilsson-Todd LK, Erhardt S, *et al.* (2008). Clozapine interacts with the glycine site of the NMDA receptor: electrophysiological studies of dopamine neurons in the rat ventral tegmental area. *Life Sciences* **83**, 170–175.

Seriwatanachai D, Thongchote K, Charoenphandhu N, Pandaranandaka J, *et al.* (2008). Prolactin directly enhances bone turnover by raising osteoblast-expressed receptor activator of nuclear factor kappaB ligand/osteoprotegerin ratio. *Bone* **42**, 535–546.

Stephen LJ, McLellan AR, Harrison JH, Shapiro D, *et al.* (1999). Bone density and antiepileptic drugs: a case-controlled study. *Seizure* **8**, 339–342.

Sturtridge W, Lentle B, Hanley DA (1996). Prevention and management of osteoporosis: consensus statements from the Scientific Advisory Board of the Osteoporosis Society of Canada. 2. The use of bone density measurement in the diagnosis and management of osteoporosis. *Canadian Medical Association Journal* **155**, 924–929.

Suzuki Y, Sato S (2010). Secondary osteoporosis UPDATE. clinical significance of glucocorticoid-induced osteoporosis. *Clinical Calcium* **20**, 645–653.

Swaminathan K, Flynn R, Garton M, Paterson C, *et al.* (2009). Search for secondary osteoporosis: are z scores useful predictors? *Postgraduate Medical Journal* **85**, 38–39.

Theodorou DJ, Theodorou SJ, Sartoris DJ (2002). Dual-energy X-ray absorptiometry in diagnosis of osteoporosis: basic principles, indications, and scan interpretation. *Comprehensive Therapy* **28**, 190–200.

Tiihonen J, Lonnqvist J, Wahlbeck K, Klaukka T, *et al.* (2009). 11-year follow-up of mortality in patients with schizophrenia: a population-based cohort study (FIN11 study). *Lancet* **374**, 620–627.

Wawrzynska L, Tomkowski WZ, Przedlacki J, Hajduk B, *et al.* (2003). Changes in bone density during long-term administration of low-molecular-weight heparins or acenocoumarol for secondary prophylaxis of venous thromboembolism. *Pathophysiology of Haemostasis and Thrombosis* **33**, 64–67.

Wyszogrodzka-Kucharska A, Rabe-Jablonska J (2005*a*). Calcium balance and regulation in schizophrenic patients treated with second generation antipsychotics. *Psychiatria Polska* **39**, 1157–1171.

Wyszogrodzka-Kucharska A, Rabe-Jablonska J (2005*b*). Decrease in mineral bone density in schizophrenic patients treated with 2nd generation antipsychotics. *Psychiatria Polska* **39**, 1173–1184.

Zamani A, Omrani GR, Nasab MM (2009). Lithium's effect on bone mineral density. *Bone* **44**, 331–334.

Zhu H, Fang J, Luo X, Yu W, *et al.* (2010). A survey of bone mineral density of healthy Han adults in China. *Osteoporosis International* **21**, 765–772.