Abstract

Context: Patients with primary adrenal insufficiency (Addison’s disease) receive more glucocorticoids than the normal endogenous production, raising concern about adverse effects on bone.

Objective: To determine i) the effects of glucocorticoid replacement therapy on bone, and ii) the impact of glucocorticoid pharmacogenetics.

Design, setting and participants: A cross-sectional study of two large Addison’s cohorts from Norway (n = 187) and from UK and New Zealand (n = 105).

Main outcome measures: Bone mineral density (BMD) was measured; the Z-scores represent comparison with a reference population. Blood samples from 187 Norwegian patients were analysed for bone markers and common polymorphisms in genes that have been associated with glucocorticoid sensitivity.

Results: Femoral neck BMD Z-scores were significantly reduced in the patients (Norway: mean −0.28 (95% confidence intervals (CI) −0.42, −0.16); UK and New Zealand: −0.21 (95% CI −0.36, −0.06)). Lumbar spine Z-scores were reduced (Norway: −0.17 (−0.36, +0.01); UK and New Zealand: −0.57 (−0.78, −0.37)), and significantly lower in males compared with females (P = 0.02). The common P-glycoprotein (ABCB1) polymorphism C3435T was significantly associated with total BMD (CC and CT > TT P = 0.015), with a similar trend at the hip and spine.

Conclusions: BMD at the femoral neck and lumbar spine is reduced in Addison’s disease, indicating undesirable effects of the replacement therapy. The findings lend support to the recommendations that 15–25mg hydrocortisone daily is more appropriate than the higher conventional doses. A common polymorphism in the efflux transporter P-glycoprotein is associated with reduced bone mass and might confer susceptibility to glucocorticoid induced osteoporosis.

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Introduction

Primary adrenal insufficiency (Addison’s disease) is a rare disease mainly caused by autoimmune destruction of the adrenal cortex. The treatment is a replacement of mineralocorticoids and glucocorticoids (1), but conventional replacement doses replenish more glucocorticoids than are normally produced (2, 3). The serum cortisol profile obtained with present medication features peaks, troughs and abolished circadian pattern (4–6). Studies have reproducibly shown impairment of health-related quality of life in these patients, indicating that the replacement therapy is not optimal (7–9).

Glucocorticoids accelerate reduction in bone mass by inhibition of osteoblast activity, stimulation of osteoclast activity and inhibition of intestinal vitamin D dependent calcium absorption. Furthermore, patients with adrenal insufficiency lack adrenal androgens, possibly conferring additional risk of osteoporosis. It is not known whether osteoporosis is more common in patients with Addison’s than in the general population. A series of small studies in Addison’s disease have shown
inconsistent correlation between bone mineral density (BMD) and disease duration, glucocorticoid type and dose; the main findings are summarized in Table 1 (10–16). Most of these studies found reduced mean BMD that was not statistically significant, but none of the studies were sufficiently powered to reliably determine these effects. Gurnell et al. published the largest trial so far of adrenal androgens in Addison’s disease suggesting that 12 months DHEA replacement slightly but significantly increased BMD at the femoral neck, but not at other skeletal sites (9).

Little is known about the individual susceptibility to glucocorticoid-induced osteoporosis (GIOP), neither in patients with Addison’s disease nor in general. Single nucleotide polymorphisms (SNPs) in genes that govern glucocorticoid metabolism and sensitivity (Fig. 1) are likely to confer some of this susceptibility. Multiple SNPs with possible functional effects exist in these genes (17–20), but most of the variants are rare and would require large patient populations to investigate. Therefore, we chose to include common SNPs with likely functional effects.

Glucocorticoids are substrates for the efflux transporter P-glycoprotein (encoded by the ATP-binding cassette B1 (ABCB1) gene) (21–24), and common polymorphic variation in this gene, particularly three common SNPs in linkage disequilibrium, associates with altered pharmacokinetics and pharmacodynamics of numerous drugs (17, 18, 25, 26). Only a few studies have addressed the association between ABCB1 polymorphisms and clinical outcome of glucocorticoid therapy (27–29). Among the common SNPs, rs1045642 has been most consistently associated with reduced function of P-glycoprotein: we hypothesized that this SNP could lead to increment in intracellular cortisol levels, since less is pumped out of the cell, and thus increased risk of GIOP.

Variable levels of the co-chaperone FK506 binding protein (FKBP51; encoded by FKBP5) determines glucocorticoid sensitivity (30, 31). The common SNP rs1360780 may be associated with increased sensitivity to glucocorticoids (19, 32, 33); hence we postulated increased risk of GIOP in carriers of this SNP. Variable activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is associated with the risk of osteoporosis, possibly due to its role in peripheral activation of cortisone to cortisol (34). Reduced activity of this enzyme is associated with the common SNP rs12086634 in HSD11B1 (20), which could possibly protect against GIOP. A few SNPs in the glucocorticoid (GR) gene confer variation in sensitivity to glucocorticoids; rs6195 is associated with enhanced sensitivity and rs6189/rs6190 with reduced sensitivity. These SNPs are likely to influence the risk for GIOP, but they are relatively rare variants with allele frequencies below 5% (35).

The aims of this study were i) to evaluate whether the conventional replacement of glucocorticoids in two large cohorts of patients with Addison’s disease is associated with reduced bone mass, and ii) to test the hypothesized effects on BMD and bone markers of selected common gene polymorphisms.

### Table 1 Previous studies of bone mineral density in Addison’s disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Men</th>
<th>Women</th>
<th>n</th>
<th>BMD</th>
<th>n</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Devolagear et al. (10)</td>
<td>1987</td>
<td>12</td>
<td>Ns</td>
<td>21</td>
<td>↓</td>
<td>60</td>
<td>Ns</td>
</tr>
<tr>
<td>Florkowski et al. (11)</td>
<td>1994</td>
<td>5</td>
<td>Ns</td>
<td>9</td>
<td>↓</td>
<td>22</td>
<td>Ns</td>
</tr>
<tr>
<td>Zeilissen et al. (12)</td>
<td>1994</td>
<td>31</td>
<td>Ns</td>
<td>60</td>
<td>Ns</td>
<td>19</td>
<td>Ns</td>
</tr>
<tr>
<td>Valero et al. (13)</td>
<td>1999</td>
<td>19</td>
<td>Ns</td>
<td>22</td>
<td>Ns</td>
<td>10</td>
<td>Ns</td>
</tr>
<tr>
<td>Braatvedt et al. (14)</td>
<td>2003</td>
<td>7</td>
<td>Ns</td>
<td>19</td>
<td>Ns</td>
<td>8</td>
<td>Ns</td>
</tr>
<tr>
<td>Jodar et al. (15)</td>
<td>2006</td>
<td>6</td>
<td>Ns</td>
<td>19</td>
<td>Ns</td>
<td>8</td>
<td>Ns</td>
</tr>
</tbody>
</table>

↓, significantly reduced and Ns, not significantly different from normal.

Confined to men with low testosterone.

Confined to post-menopausal women.
Materials and methods

Subjects and design

A Norwegian cohort ($n=187$) was recruited from the Norwegian Registry of Addison’s disease. Inclusion criteria were aged 30 years or more and had verified Addison’s disease, as documented by low serum cortisol and simultaneously elevated ACTH. Adrenal insufficiency after adrenalectomy and congenital adrenal hyperplasia were exclusion criteria. The patients were assessed at five Norwegian university clinics in the period November 2004–November 2006, according to a standardized protocol. Adjusted glucocorticoid dosage was calculated as hydrocortisone equivalents (0.8, 5 and 20 times the doses of cortisone acetate, prednisolone and dexamethasone respectively) per kg body weight. BMD was measured in all patients, and spinal X-rays performed in patients aged 50 years or more. After overnight medication fast of glucocorticoids a serum sample was obtained. DNA was available from a bio-bank for most patients. The patients gave written informed consent before entering the study, which was approved by the western Norway ethics committee for medical research.

A cohort of Addison’s disease patients from UK and New Zealand (UK/NZ, $N=105$), aged 18–65 years with disease duration of at least 1 year was previously studied in a 12-month trial of DHEA replacement in primary adrenal insufficiency (9); baseline clinical data and BMD measurements (obtained before 2003) were made available for this study. The diagnosis was substantiated by documented hypocortisolemia, and raised serum ACTH (or hyperpigmentation). Exclusion criteria were pregnancy, past personal history of hormone-dependent malignancy and any intercurrent significant medical or psychiatric condition.

Control populations The Lunar database was used as reference for BMD measurements, as a previous study demonstrated that BMD in the femur and total body of the subjects in this database complied well with the general Norwegian population (36). The results of dual X-ray absorptiometry (DXA) measurements and vertebral morphometry (spinal X-rays) were compared with the results of the European vertebral osteoporosis study in which normative data from Norwegian and UK populations were available (37, 38). Eighty-nine blood donors (47 males and 42 females) aged 20–69 were used as controls for the bone marker analyses.

Measurements

Bone mineral density BMD at the femoral neck, total hip, lumbar spine and total body was measured by DXA, according to a standardized protocol. In the Norwegian cohort four study centres applied Lunar scanners and one centre used a Hologic scanner. The UK and New Zealand cohort was assessed with Hologic scanners. To standardize the measurements between the centres the hip and spine scores of all the patients assessed with Hologic scanners were transformed to Lunar scores by standard equations (39). Z-scores were calculated for all the patients using regression equations from the Lunar database (36), as this database was found to be representative for the Norwegian population (36). Total body BMD Z-scores were analyzed as given by the scanners without any adjustment. The within-day and between-day coefficients of variation (CV) were below 2% for all the scanners tested. In vivo cross-calibration of the Lunar DXA scanners at three of the study sites was undertaken in 15 volunteers, showing only minor variation (40); and no significant difference compared with the Hologic scanner with phantom measurements. All patients who had DXA measurements performed more than 3 years previously were assessed for longitudinal Z-score changes.

Spinal X-rays Standardized spinal X-rays were taken for morphometric analysis of all patients 50 years or older in the Norwegian cohort, and interpreted according to the algorithm developed by McCloskey et al. (41).

Biochemical analyses Serum samples were stored under light-protected conditions at $-80^\circ$C. All bone markers were analyzed in the same laboratory in duplicates and in one batch. The bone marker assays were analyzed with ELISAs. For the analysis of serum osteocalcin, we used an assay from Nordic Bioscience Diagnostics (Herlev, Denmark), which measures both intact osteocalcin and the N-terminal mid-region fragment of osteocalcin. The mean variation between pairs of samples measured was 4.1%. Inter-assay CV was 6.9% in the low range and 2.3% in the high range. CrossLaps (Nordic Bioscience Diagnostics) measures the degradation product of C-terminal telopeptides of type-I collagen (CTX). Mean variation between sample pairs was 12.8%. Inter-assay CV was 24.2% in the low range and 7.8% in the high range. Bone-specific alkaline phosphatase (ALP) was determined with an assay from Quidel Corporation (San Diego, CA, USA). The mean variation between the pairs of samples was 5.0%, inter-assay CV was 7.0% in the low range and 2.6% in the high range.

Genetic analyses We elected to study genes that are anticipated to influence glucocorticoid sensitivity, i.e. $ABCB1$, $FKBP5$, $HSD11B1$ and $GR$ (Fig. 1). Multiple SNPs with possible functional effects exist in these genes, but most of the variants are rare and would require large patient populations to investigate; therefore, in this study, we included SNPs with allele frequencies $>0.1$, except $GR$ for which well validated SNPs are found at lower frequencies. Genotyping was...
performed by allelic discrimination analysis using the 7900HT fast real-time PCR system and Taqman allelic
discrimination assays (Applied Biosystems, Foster City,
CA, USA) for the SNPs: rs1045642 (ABCB1),
rs1360780 (FKBP5), rs12086634 (HSD11B1) and
rs6195 (GR). The assays were performed following the
recommendations from the supplier although less
volume of the reagents was used (only 2 μl dried DNA
with the addition of 2 μl mastermix containing enzyme,
primers and probes). Twenty of the DNAs were run
twice and the genotypes reproduced for all. The coupled
SNPs rs6189/rs6190 (GR) were analyzed by PCR and
DNA sequencing of exon 2 by using the forward primer
5’-GCTGCCCTTCTACTGAGATCA-3’ and the reverse
primer 5’-GCTGCATTTGCTTACTGAG-3’.

Statistical analysis

BMD is expressed by Z-scores, which represent age and
gender-adjusted SDS, which are presented with 95% confidence intervals (CI). CI different from zero indicates
Z-scores statistically different from the reference popu-
lation. The influence of age, gender, disease duration,
cumulative glucocorticoid use, concomitant endocrine
diseases (diabetes mellitus type 1 and/or autoimmune
thyroid disease), current smoking, family history and
genotypes on the Z-scores was analysed by linear
regression analysis in a backward-stepwise fashion.
For women premature hypogonadism was also entered
as a variable in the regression analysis. Power
calculations indicated that ~250 patients would be
required to exclude mean effects on Z-scores of 0.25
with significance level 0.05 and power 0.80.

Results

Patients

Characteristics of the 187 Norwegian patients and the
105 patients from UK and New Zealand are shown in
Table 2. The frequency of self-reported concomitant endocrine diseases was as expected. The Norwegian
patients were older and had longer duration of Addison’s disease than the patients from UK and New Zealand. The Norwegian patients mostly used cortisone acetate, whereas almost all the patients from UK and New Zealand were on hydrocortisone replacement. For seven out of the 15 patients on bisphosphonate therapy pre-treatment DXA scans were available, which were
used in the further analysis.

Table 2 Characteristics and replacement therapy mean (s.d.) of patients with Addison’s disease in Norway and UK and New Zealand.

<table>
<thead>
<tr>
<th></th>
<th>Norwegian</th>
<th>UK and New Zealand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>n</td>
<td>124</td>
<td>63</td>
</tr>
<tr>
<td>Age (year)</td>
<td>53 (14)</td>
<td>52 (16)</td>
</tr>
<tr>
<td>Disease duration (year)</td>
<td>17 (14)</td>
<td>19 (13)</td>
</tr>
<tr>
<td>Replacement therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucocorticoid dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC equivalents (mg)</td>
<td>31.5 (11)</td>
<td>33.2 (11)</td>
</tr>
<tr>
<td>HC equivalent/body weight (mg/kg)</td>
<td>0.47 (0.1)</td>
<td>0.41 (0.1)</td>
</tr>
<tr>
<td>Glucocorticoid type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone (%)</td>
<td>1.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Cortisone acetate (%)</td>
<td>98.4</td>
<td>98.4</td>
</tr>
<tr>
<td>Prednisolone (%)</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Dexamethasone (%)</td>
<td>0.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Concomitant endocrine diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid disease (%)</td>
<td>51</td>
<td>27</td>
</tr>
<tr>
<td>Type 1 diabetes (%)</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>APS1 (%)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Antiresorptive therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisphosphonates (n)</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Oestrogen replacement (n)</td>
<td>18</td>
<td>--</td>
</tr>
</tbody>
</table>

AP51, autoimmune polyglandular syndrome type 1. NR, not reported.
*HC equivalents, cortisone acetate estimated at 80% of hydrocortisone (HC) potency.
**Bone mineral density**

Femoral neck BMD was significantly lower in the patients than in the reference database: as expressed by mean Z-scores as illustrated in Fig. 2A (Norway: −0.28 (95% CI −0.42, −0.16); UK and New Zealand: −0.21 (95% CI −0.36, −0.06)). Total hip Z-scores were significantly reduced in the Norwegian cohort (−0.29 (−0.46, −0.15)), but not in the UK and New Zealand cohort (0.09 (−0.26, +0.27)). Lumbar spine Z-scores in the patients were lower than in the Lunar reference population (Fig. 2B: UK and New Zealand: −0.57 (−0.78, −0.37); Norway: −0.17 (−0.36, +0.01)), and significantly lower in males compared with females (P=0.02). Total body BMD was only available for the Norwegian patients (Table 3), which was not significantly different from normal.

In Norwegian patients, the Z-scores at the femoral neck (Fig. 3, P=0.02), total hip (P<0.001) and total body (P=0.02), but not those at lumbar spine (P=0.27), were significantly associated with weight-adjusted glucocorticoid dose. These associations were not explained by variation in weight, and persisted when patients taking synthetic glucocorticoids (prednisolone or dexamethasone, n=10) were omitted.

![Figure 2](image-url)

**Figure 2** BMD Z-scores in Addison’s disease patients from Norway (n=187) and UK and New Zealand (n=105). (A) Femoral neck. (B) Lumbar spine.

<table>
<thead>
<tr>
<th>Genes</th>
<th>n</th>
<th>Femoral neck</th>
<th>P value*</th>
<th>Total hip</th>
<th>P value*</th>
<th>Lumbar spine</th>
<th>P value*</th>
<th>Total body</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>58</td>
<td>−0.22 (1.1)</td>
<td>0.09†</td>
<td>−0.32 (0.89)</td>
<td>0.12†</td>
<td>−0.36 (0.94)</td>
<td>0.28†</td>
<td>−0.28 (0.94)</td>
<td>0.021†</td>
</tr>
<tr>
<td>FKBP5</td>
<td>67</td>
<td>−0.15 (0.97)</td>
<td>0.081†</td>
<td>−0.28 (0.85)</td>
<td>0.12†</td>
<td>−0.36 (0.88)</td>
<td>0.28†</td>
<td>−0.32 (0.86)</td>
<td>0.021†</td>
</tr>
<tr>
<td>HSD11B1</td>
<td>61</td>
<td>−0.29 (0.71)</td>
<td>0.15</td>
<td>−0.32 (0.78)</td>
<td>0.12†</td>
<td>−0.32 (0.78)</td>
<td>0.28†</td>
<td>−0.32 (0.78)</td>
<td>0.021†</td>
</tr>
<tr>
<td>GR</td>
<td>161</td>
<td>−0.37 (0.94)</td>
<td>0.15</td>
<td>−0.27 (0.81)</td>
<td>0.12†</td>
<td>−0.36 (0.97)</td>
<td>0.28†</td>
<td>−0.32 (0.89)</td>
<td>0.021†</td>
</tr>
<tr>
<td>Overall</td>
<td>187</td>
<td>−0.36 (1.2)</td>
<td>0.021†</td>
<td>−0.28 (1.1)</td>
<td>0.12†</td>
<td>−0.36 (1.1)</td>
<td>0.28†</td>
<td>−0.28 (1.1)</td>
<td>0.021†</td>
</tr>
</tbody>
</table>

*P* values for †analysis of the genotypes in a co-dominant model (linear regression analysis), and ‡comparison of genotypes in recessive model for the variant allele (Student’s *t*-test).

Table 3 BMD Z-scores (mean (s.d.)) according to genotypes in Norwegian patients with Addison’s disease.

**Table 3** BMD Z-scores (mean (s.d.)) according to genotypes in Norwegian patients with Addison’s disease.
any location. Longitudinal data obtained from 47 patients did not differ from the women with a history of premature gonadal insufficiency (7% of the female population). In the cross-sectional analysis, the BMD of the women having had none of these differences were statistically significant (mean Z-score: femoral neck −0.67 vs −0.24 (P = 0.13), total hip −0.59 vs −0.13 (P = 0.14), lumbar spine −0.93 vs −0.30 (P = 0.10) and total body −0.47 vs + 0.17 (P = 0.19)). Disease duration had no impact on the Z-scores at any location. Longitudinal data obtained from 47 patients (mean observation interval 8 years (s.d. 2.7)) showed no change in femoral neck Z-scores in those without antiresorptive therapy (n = 40, mean annual change + 0.006 (−0.004, + 0.017)). In those receiving bisphosphonates or hormone replacement therapy (HRT), femoral neck Z-score tended to increase (n = 7, mean annual change + 0.035, CI (−0.014, + 0.084)).

Concomitant endocrine diseases such as diabetes mellitus type 1 or autoimmune thyroid disease did not correlate with Z-scores at any site in either patient population. In the cross-sectional analysis, the Z-scores were marginally lower in smokers (22% of patients) than in non-smokers, and marginally higher in women on HRT than in those without; at all locations, however, none of these differences were statistically significant (data not shown). The BMD of the women having had premature gonadal insufficiency (7% of the female patients) did not differ from the women with a history of normal gonadal function (data not shown).

Spinal X-rays
X-rays of the thoracolumbar spine were obtained from 84 patients of 50 years and older in the Norwegian cohort (mean age 63.0 years). Fourteen patients (16.8%, CI (8.7–24.7), men: 4/29 (13.8%), mean age 62.4 years; women 10/55 (18.1%), mean age 66.3 years) had one or more vertebral fractures. The frequency of patients with vertebral fractures was not different from that reported in a Norwegian reference population (men: 15.7%, mean age 65.3 years; women: 19.2%, mean age 65.4 years) (38).

Genotypes and BMD
DNA was available from 171 of the Norwegian patients. All the SNPs were in Hardy–Weinberg equilibrium. The BMD Z-scores for the ABCB1, FKBP5, HSD11B1 and GR genotypes are shown in Table 3. There was a significant correlation between the ABCB1 rs1045642 genotype and total body Z-score, and this correlation was similar in the patients that were assessed with the Hologic scanner as those assessed with Lunar scanners. The correlation was significant both in a recessive model (CC (wildtype) and CT versus TT (variant) genotypes, (Student’s t-test, P = 0.015)) as well as in a co-dominant model (CC versus CT versus TT, linear regression analysis, P = 0.021), but not in a dominant model (CC versus CT and TT, Student’s t-test, P = 0.15). The same pattern between ABCB1 genotypes and Z-scores was observed at the other skeletal regions, although statistically non-significant. Similar results were obtained if weight-adjusted glucocorticoid dose was entered into the model and when patients taking synthetic glucocorticoids were excluded from the analyses (data not shown).

Biochemical analyses
Sera were available from 168 of the Norwegian patients. The PTH concentration was normal (mean 4.2 nmol/l, s.d. 2.7; five patients with APS1 and hypoparathyroidism were excluded). The levels of osteocalcin, bone-specific ALP and CTX in patients and controls are shown in Table 4. Osteocalcin and CTX levels were higher in patients than in controls; whereas bone-specific ALP was similar in the two groups. None of these parameters correlated with weight-adjusted glucocorticoid dose. Compared with homozygous FKBP5 rs1360780 CC (wildtype) carriers, there was a trend towards lower levels of bone formation and resorption markers in individuals carrying the FKBP5 TT (variant) genotype. No statistical differences were demonstrated between ABCB1 or HSD11B1 genotypes.

Discussion
We here demonstrate that BMD at the femoral neck and lumbar spine is slightly but significantly lower than normal in patients with Addison’s disease. The patients
in this study received mean replacement doses 26.5 mg hydrocortisone or 40.1 mg of cortisone acetate divided into two or three daily doses, which is higher than the recent suggestions that 15–20 mg hydrocortisone daily may be more appropriate (1, 4–6, 42). The inverse association between glucocorticoid dosage and BMD in the Norwegian cohort, which was also reported in the recent suggestions that 15–20 mg hydrocortisone daily may be more appropriate (1, 4–6, 42). The inverse association between glucocorticoid dosage and BMD in the Norwegian cohort, which was also reported in the two largest previous studies (12, 14), indicates that the Norwegian cohort, which was also reported in the two largest previous studies (12, 14), indicates that the clinical relevance of adrenal androgen depletion in Addison’s disease has been much debated. The recent 12 months DHEA replacement study by Gurnell et al. found minor, but significant improvement of BMD at the femoral neck, but no effect on any other location. All the other DHEA replacement trials have been small and short-term, which have shown inconsistent results on bone markers (49–51). Thus, DHEA replacement may improve BMD and ultimately reduce fracture risk, but more studies are needed.

Although the adverse effects of glucocorticoid replacement doses may be limited, genetic variation is likely to influence the individual risk of reduced bone mass. The number of patients in this study allowed only for analysis of common SNPs in selected genes, but rare variants in these and other genes are likely to have greater impact on glucocorticoid sensitivity. We found that the common polymorphism rs1045642 in the efflux transporter P-glycoprotein (ABCB1) is significantly associated with total body BMD and with a similar pattern at all the skeletal sites studied. The observed associations between BMD and glucocorticoid pharmacogenetics are remarkable, given the large normal variability in BMD. Rs1045642 is associated with impaired function of the P-glycoprotein efflux pump (25, 26), which could lead to increment in intracellular cortisol levels. This is the first population-based study of association between ABCB1 genotype and glucocorticoid effects on bone. We speculate that
this association could be even more pronounced in pharmacological treatment with synthetic glucocorticoids, as the P-glycoprotein shows higher affinity for both prednisolone and dexamethasone than for cortisol (23). Addison's disease might be perceived as a model for glucocorticoid effects on bone since these patients lead normal lives and have less confounding factors than other patients treated with glucocorticoids.

Only a few studies have addressed the association between ABCB1 polymorphisms and clinical outcome of glucocorticoid therapy. Paediatric heart transplant patients homozygous for the rs1045642 wildtype allele were significantly more steroid dependent 1 year after transplantation than those carrying one or two variant alleles (27). Both this and the results of our investigation are in keeping with the experimental studies (21, 22, 52), and support the hypothesis that reduced activity of the P-glycoprotein efflux pump expose the target cells to higher concentrations of glucocorticoids. Conversely, a study of steroid-responsive nephrotic syndrome in children showed longer time to remission in patients homozygous for ABCB1 haplotypes containing the variant allele (29). Another study found an association between the variant allele or two variant alleles (27). Both this and the results of our investigation are in keeping with the experimental studies (21, 22, 52), and support the hypothesis that reduced activity of the P-glycoprotein efflux pump expose the target cells to higher concentrations of glucocorticoids.

The glucocorticoid receptor co-chaperone FKBP51 (encoded by FKBP5) plays a pivotal role for glucocorticoid sensitivity (30). A common SNP in FKBP5 associates with frequency of depressive episodes and response to antidepressant therapy, and with risk of post-traumatic stress disorder in adults who had experienced childhood abuse (19, 32, 33); both conditions might relate to drug interactions, as ABCB1 expression is induced by a number of compounds by activation of the nuclear receptor steroid and xenobiotic receptor (53).

The glucocorticoid receptor co-chaperone FKBP51 (encoded by FKBP5) plays a pivotal role for glucocorticoid sensitivity (30). A common SNP in FKBP5 associates with frequency of depressive episodes and response to antidepressant therapy. This is particularly true for the analysis of genotype effects, which must be viewed as observations rather than evidence. Different glucocorticoids as used in these cohorts might also have different impact on bone. We found no elevation of PTH levels in the Norwegian patients, and a previous analysis of 30 patients showed normal vitamin D levels (Falch, unpublished data, 1994). Still, vitamin D status is a possible confounding factor, as other factors such as thyroid status and sex hormone levels.

We conclude that BMD at the femoral neck and lumbar spine was slightly but significantly lower than normal in patients with Addison's disease. Such undesirable metabolic effects indicate that the recent recommendations of lower glucocorticoid replacement doses are appropriate. The common rs1045642 polymorphism in the efflux transporter P-glycoprotein is associated with BMD in patients with Addison's disease, and might be important for susceptibility to glucocorticoid induced osteoporosis. Glucocorticoid pharmacogenomics is likely to explain some of the variation in the effects of glucocorticoids on bone, and genotyping of the ABCB1, FKBP5, HSD11B1 and GR genes might become part of future pharmacogenomic individual tailoring of both replacement and pharmacological therapy with glucocorticoids.

Declaration of interest
There are no conflicts of interest.

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