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Reduced bone density in cystic fibrosis: ΔF508 mutation is an independent risk factor

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ABSTRACT: The aim of this cross-sectional study was to determine the prevalence and identify determinants of reduced bone mineral density (BMD) in adults with cystic fibrosis (CF).

Adults (88) with CF (mean±SD age 29.9±7.7 yrs; forced expiratory volume in one second (FEV_1) 58.2±21.5% of the predicted value) were studied. BMD at the lumbar spine (LS) and femoral neck (FN) and body composition were measured using dual-energy X-ray absorptiometry. Blood and urine were analysed for hormones, bone turnover markers, and the cytokines tumour necrosis factor-α, and interleukin-6 and -1β. FEV_1 (% pred); CF genotype; malnutrition; history of growth, development or weight gain delays; and corticosteroid use were analysed.

BMD Z-scores were -0.58±1.30 (mean±SD) at the LS and -0.24±1.19 at the FN. Z-scores of <-2.0 were found in 17% of subjects. Subjects who were homozygous or heterozygous for the ΔF508 mutation exhibited significantly lower Z-scores than those with no ΔF508 allele. Multiple linear regression showed that the ΔF508 genotype and male sex were independently associated with lower BMD at both sites. Other factors also independently associated with lower BMD included malnutrition, lower 25-hydroxyvitamin D level, lower fat-free mass and lower FEV_1 (% pred).

In conclusion, reduced bone mineral density in cystic fibrosis is associated with a number of factors, including ΔF508 genotype, male sex, greater lung disease severity and malnutrition.

KEYWORDS: Bone mineral density, cystic fibrosis, genotype, nutrition, osteoporosis

Cystic fibrosis (CF) is associated with mutation of the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR). Approximately 1,000 different CFTR mutations have been reported, the commonest being the ΔF508 mutation [1]. An increased lifespan and the availability of lung transplantation have exposed additional complications associated with longevity. These include reduced bone mineral density (BMD) and increased fracture rates [2]. Fractures have the potential to increase morbidity in CF patients as a result of pain and deterioration in respiratory status [2, 3].

Reduced BMD has been reported in studies of CF populations [2, 4–7]. A longitudinal study of BMD in CF patients concluded that both reduced bone accretion and accelerated bone loss contribute to the reduced BMD [8]. Elevated levels of bone turnover markers, including serum osteocalcin, urinary N-telopeptide and bone-specific alkaline phosphatase, have been reported in CF patients [5, 6, 9, 10], and it has been suggested that, in CF, there is an imbalance between bone formation and bone resorption in favour of resorption [11, 12]. An association between reduced 25-hydroxyvitamin D levels and reduced BMD has been reported in CF patients [7]. Poor nutritional status has also been reported to be associated with reduced BMD [4, 5, 7, 10, 13, 14]. Delayed puberty has been associated with reduced BMD in some studies [2, 15], but, in other studies, age of menarche in females did not correlate with BMD [4, 6, 7]. An association between reduced BMD and more severe lung disease has been suggested [4–6, 15]. There is evidence that elevated levels of circulating cytokines in CF may be associated with reduced BMD [10, 16, 17].

The aims of the present cross-sectional study were to determine the prevalence of reduced BMD in a large population of adults with CF; and

For editorial comments see page 9.

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to identify determinants of reduced BMD in a multivariate model, in order to identify risk factors which might potentially be targeted in the prevention of osteoporosis.

MATERIAL AND METHODS

Study subjects and design

The present study was a prospective cross-sectional study of adults with CF, which comprised a randomly selected sample of ~50% of the population of 174 adults who had not undergone transplantation attending the regional Adult Cystic Fibrosis Service at the Alfred Hospital, Melbourne, Australia. The aim was to obtain a large study sample that was representative of an adult CF population. The sex, age, forced expiratory volume in one second (FEV_1) as a percentage of the predicted value, CF genotype, height and pancreatic status of all 174 patients were recorded. The study population of 88 patients showed similar genotypic and phenotypic characteristics to the non-studied population of 86 patients, confirming that the study population was representative of the total clinic population.

The diagnosis of CF had been made previously, using a sweat chloride test and on the basis of an appropriate CF phenotype. CF patients were excluded from the study if they were already receiving treatment for reduced BMD, pregnant or unable to complete the study requirements. Patients (88, all Caucasian, 53 males) aged 19–59 yrs were enrolled in the study between May 2000 and April 2001. Twenty healthy volunteers of similar age to the CF subjects were also recruited, to provide a blood sample. The study was approved by the Alfred Hospital Institutional Ethics Committee and written informed consent was obtained prior to participation.

Clinical characteristics: cystic fibrosis subjects

Clinical characteristic information was collected from the subject's medical record and at interview (table 1). In the present study, pancreatic exocrine status was defined as pancreatic insufficient or pancreatic sufficient according to whether or not pancreatic enzyme replacement therapy was required to control maldigestion, malabsorption or otherwise unexplained nutritional failure, and was reviewed on a periodic basis at routine outpatient clinic consultations.

TABLE 1 Clinical characteristics of cystic fibrosis (CF) subjects

Characteristic	Frequency %
CF genotype[#]	
Homozygous for ΔF508 mutation	46
Heterozygous for ΔF508 mutation	42
No allele for ΔF508 mutation	12
Pancreatic insufficient	88
CF-related diabetes mellitus	18
Liver disease[¶]	10
Use of inhaled corticosteroids[+]	39
Use of intravenous or oral corticosteroids[§]	47
History of malnutrition[§,ƒ]	14
History of GDW delay/difficulty in adolescence	30

GDW: growth, development or weight gain. [#]: n=85; three patients did not undergo genotype testing; [¶]: features of liver disease identified by ultrasound or clinical examination, and/or two or more liver enzymes elevated to more than twice the upper limit of the normal range; [+]: current usage; [§]: current or previous usage; [ƒ]: indicated by use of supplementary enteral nutrition.

The median estimated cumulative oral/intravenous corticosteroid dose in prednisolone equivalents was 6,007 mg over a mean of 31.2 patient-yrs. The median age of menarche in females was 14 yrs (range 10–17 yrs). Ten (11%) subjects received ⩾400 IU vitamin D daily from supplements. Thirty-five (40%) subjects reported that they had previously sustained one or more bone fractures. The total number of fractures reported was 74. All fractures were associated with significant trauma.

Lung function was assessed by measuring FEV_1 using a Lilly pneumotachometer attached to a Jaeger spirometer (Master screen pneumo, version 4.0; Jaeger, Würzburg, Germany). The best lung function in the 3 months prior to the measurement of BMD was recorded. Current height and weight were used to calculate body mass index (BMI) (in kilograms per metre squared) and FEV_1 (% pred) using the prediction equations of KNUDSON *et al.* [18].

Physical activity was assessed using the Baecke Questionnaire of Habitual Physical Activity [19]. Each type of activity is scored 1–5, and the total score for the questionnaire ranges 3–15. Dietary intake was assessed by a trained dietitian using a 4-day household measures food record. Mean daily intakes from the diet and supplements of energy and calcium were estimated using a computerised dietary analysis program (Foodworks, version 2.10; Xyris Software Australia, Brisbane, Australia).

Radiographic assessment and bone mineral density and body composition measurements: cystic fibrosis subjects

Subjects underwent BMD and body composition measurement using dual-energy X-ray absorptiometry scanning (DPX-IQ, version 4.7e; Lunar Radiation Corporation, Madison, WI, USA) of the: 1) whole body, to determine fat mass, fat-free mass and whole-body BMD; and 2) lumbar spine (LS) and femoral neck (FN), to determine their BMDs.

BMDs were expressed as T- and Z-scores. These were calculated using the population reference data provided by the manufacturer (Lunar Radiation Corporation), which were derived from BMD measurements in >4,000 Caucasian adults aged 20–79 yrs from a range of countries. T-scores were calculated by subtracting the sex-specific population mean BMD for young adults (aged 20–40 yrs) from the CF subject's BMD, and this value was divided by the SD of the sex-specific young adult mean. Z-scores were calculated by subtracting the sex- and age-specific population mean BMD from the CF subject's BMD, and this value was divided by the SD of the sex- and age-specific mean. Lateral spinal films of subjects were taken and assessed for osteoporotic fractures using standard radiological criteria [20].

Biochemical measurements

Cystic fibrosis subjects

A venous blood sample was taken, when the subjects were clinically stable, and analysed for serum levels of calcium,

phosphate, alkaline phosphatase and albumin (Hitachi 747 and 917 analysers; Roche Diagnostics, Mannheim, Germany); 25-hydroxyvitamin D (Cobra II Auto-gamma counter; Packard Instrument Co., Downers Grove, IL, USA); testosterone (in males), sex-hormone-binding globulin, parathyroid hormone, tumour necrosis factor-α (TNF-α), and interleukin (IL)-1β and IL-6 (immunometric immunoasssays using chemiluminescent substrates) (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA); oestradiol (in females), thyroid-stimulating hormone, follicle-stimulating hormone and luteinising hormone (microparticle enzyme immunoassay) (Abbott AxSYM Immunoassay Analyser; Abbott Diagnostics Division, Chicago, IL, USA); and osteocalcin (two-site immunoradiometric assay) (kit DSL-7600; Diagnostic Systems Laboratories, Inc., Webster, TX, USA). A urine sample was collected for determination of the bone resorption marker N-telopeptide (Osteomark ELISA kit; Ostex International, Inc., Seattle, WA, USA). N-telopeptide levels were corrected for variations in urine concentration and expressed as nanomoles of bone collagen equivalents per millimole of urinary creatinine. The intra- and interassay coefficients of variation for the bone turnover and cytokine assays, respectively, were: osteocalcin 2.7 and 4.0%; N-telopeptide 7.6 and 4.0%; TNF-α 3.2 and 5.2%; IL-1β 4.2 and 6.2%; and IL-6 4.7 and 6.4%.

Healthy volunteers

A venous blood sample was collected and analysed for serum levels of TNF-α and IL-1β and IL-6 using the same methods as for the CF subjects.

Statistical analysis

Data were analysed for normality, and, where appropriate, underwent logarithmic transformation. Data were summarised as mean±SD or median (interquartile range) as appropriate. Chi-squared or Fisher's exact tests were performed in order to identify differences in categorical variables between subgroups. Unpaired t-tests or Mann–Whitney U-tests were performed to compare means or medians. Univariate analyses using Pearson's correlations, Spearman's correlations and one-way ANOVA were undertaken to assess correlation between variables. The influence of the CF genotype was analysed using three categories: homozygous for ΔF508, heterozygous for ΔF508, and no ΔF508 allele. Oral/intravenous corticosteroid use was analysed as a binomial variable due to the potential for recall and information bias in the estimation of cumulative prednisolone equivalent dose. Multiple linear regression was performed in order to identify which variables were significantly and independently associated with LS and FN BMD Z-scores. This analysis was performed using data from the 85 subjects who had undergone genotype testing. It was undertaken using a backward elimination technique incorporating all variables except those excluded below, in order to incorporate significant correlates in this and other studies, and to reduce the impact of occult confounding in the univariate analyses. Sex-specific variables (age of menarche in females and testosterone level in males) were excluded, as was weight, whose effect was incorporated within other variables (fat-free mass and fat mass), physical activity and dietary intake, for which the data were incomplete and for which there were no correlations with outcome variables on univariate analyses. The largest possible patient group was modelled at each step and the least significant variables eliminated. Regression models were further validated using a stepwise selection approach. The significance level was taken as a p-value of <0.05.

RESULTS

Clinical measurements: cystic fibrosis subjects

Table 2 shows CF subject demographics, dietary intake and physical activity results.

Lateral LS films were taken for 75 subjects. Of these, four (5%) met the radiological criteria for osteoporotic fractures [20].

Biochemical results

The biochemical results for CF subjects are shown in table 3. There was a trend towards higher 25-hydroxyvitamin D levels in subjects studied in the summer months compared to those studied in the winter months (October–March 71 nM (median) *versus* April–September 52 nM; $p<0.05$ (Mann–Whitney test)). A low 25-hydroxyvitamin D level did not correlate with pancreatic status ($p=0.25$).

IL-1β was not detected in any CF subject or healthy volunteer (<5 pg·ml^{-1}). IL-6 was not detected in 15 (75%) of the volunteers (<5 pg·ml^{-1}). TNF-α was not detected in 10 (50%) of the volunteers (<4 pg·ml^{-1}). The lower 95% ranges for IL-6 and TNF-α in volunteers were taken as the reference ranges, the upper limits of which were 10.4 and 11.7 pg·ml^{-1} respectively. TNF-α was not detected in 29 (36%) of the CF subjects, whereas IL-6 was not detected in 39 (48%) of the CF subjects. IL-6 levels were above the reference range in 19 (23%) of the CF subjects, whereas TNF-α levels were elevated in five (6%) of the CF subjects. IL-6 and TNF-α levels did not correlate in the 28 CF subjects in whom both were detected.

Bone mineral density measurements

Results of the BMD and body composition measurements are shown in table 4. Thirty-seven (42%) subjects had a BMD Z-score of <-1 at one or more of the three sites tested, with 15 (17%) having a BMD Z-score of <-2. The prevalence of osteoporotic

TABLE 2 Details of cystic fibrosis subjects

	Total	Males	Females
Subjects n	88	53	35
Age yrs	29.9±7.7	29.0±7.3	31.2±8.2
FEV$_1$ % pred	58.2±21.5	54.8±23.5	63.3±17.1
Height cm	168.6±8.9	173.1±7.0	161.7±6.7^{++}
Weight kg	61.0±10.0	64.4±10.2	55.8±7.0^{++}
Body mass index kg·m^{-2}	21.4±2.6	21.4±2.7	21.3±2.4
Dietary EI$^{\#}$			
MJ·day^{-1}	11.1±2.6	12.5±2.4	9.3±1.7^{++}
%RDI	99.8±18.5	100.8±18.3	99.0±18.8
Dietary Ca$^{\#}$ mg·day^{-1}	1220±515	1403±509	977±421^{+}
Physical activity score¶	7.8±1.4	8.0±1.6	7.5±1.2

Data are presented as mean±SD. FEV$_1$: forced expiratory volume in one second; EI: energy intake; RDI: recommended daily intake for adult Australians [21]. $^{\#}$: n=56, ¶: n=55. $^{+}$: $p<0.005$; $^{++}$: $p<0.0001$ *versus* males (unpaired t-test).

TABLE 3 Biochemical parameters in cystic fibrosis patients

	Subjects n	Concentration	RR	>RR %	<RR %
Bone metabolism markers					
25-hydroxyvitamin D nM	84	60.0 (35.0–83.5)	38–108	10.7	27.4
Osteocalcin $\mu g \cdot L^{-1}$					
Males	46	16 (12–25)	12.1–18.9	47.8	28.3
Females	34	10.5 (7.3–16.0)	6.2–19.0	14.7	14.7
Urinary NTx nmol BCE·mmol UCr^{-1}					
Males	47	48.3 (40.6–85.4)	19.6–62.6	36.2	4.3
Females	34	39.1 (28.4–64.6)	11.5–62.3	29.4	0
Alkaline phosphatase $U \cdot L^{-1}$	85	112 (93–137)	<110	52.9	0
Calcium mM	85	2.33±0.12	2.1–2.6	0	2.4
Phosphate mM	85	1.24±0.24	0.7–1.3	34.1	3.5
Parathyroid hormone pM	85	4.5±3.6	1.3–7.6	14.6	11.0
Hormones					
Testosterone (males) nM	49	14.8 (12.5–20.0)	6.9–28.1	6.1	0
Oestradiol (females) pM	32	212 (154–392)	143–1864	0	21.9
LH $U \cdot L^{-1}$					
Males	48	5.15 (3.65–7.00)	2–12	6.3	4.2
Females	32	6.90 (3.55–9.55)	0.4–105	0	0
FSH $U \cdot L^{-1}$					
Males	48	5.25 (4.65–7.05)	1–8	18.9	0
Females	32	5.55 (4.10–7.10)	2–22	0	9.4
SHBG nM					
Males	49	29 (24–33)	13–70	0	8.2
Females	33	53 (29–124)	11–114	15.2	0
TSH $mU \cdot L^{-1}$	84	1.62±0.92	0.4–4.7	1.2	1.2
Inflammatory markers					
Albumin $g \cdot L^{-1}$	85	35.3±3.2	35–52	0	38.9
TNF-α $pg \cdot mL^{-1}$	80	5.45 (ND–7.30)	<11.7	6.3	0
IL-6 $pg \cdot mL^{-1}$	81	6.3 (ND–10.1)	<10.4	23.4	0

Data are presented as mean±SD or median (interquartile range). Reference ranges (RRs) are those reported by the testing laboratory, except for interleukin (IL)-6 and tumour necrosis factor-α (TNF-α), for which the reference ranges are the lower 95% range of the healthy volunteers tested. NTx: N-telopeptide; LH: luteinising hormone; FSH: follicle-stimulating hormone; SHBG: sex-hormone-binding globulin; TSH: thyroid-stimulating hormone; ND: not detected.

T-scores (<-2.5) was 15%, with a further 42% of subjects having a T-score ranging -1.0– -2.5 at one or more sites. Compared to females, males had significantly lower BMD Z-scores at all sites, indicating that males exhibited greater deficits in bone mineral relative to the age-matched normal population.

Correlates of bone mineral density

Mean BMD Z-scores at the LS and FN were significantly lower in subjects who were homozygous (-0.67±1.33 and -0.37±1.24, respectively) or heterozygous (-0.73±1.16 and -0.44±1.03, respectively) for the ΔF508 mutation, compared to those with no ΔF508 allele (0.43±1.26 and 0.80±1.26, respectively; $p<0.05$ (one-way ANOVA)) (fig. 1).

Correlation of all variables significantly associated with LS and/or FN BMD Z-score are shown in tables 5 and 6, as is the correlation of age with BMD. There was no significant difference in mean BMD Z-scores in subjects who were pancreatic insufficient compared with those who were pancreatic sufficient, at either the LS (-0.58±1.33 and -0.61±1.14, respectively; $p=0.94$) or the FN (-0.23±1.24 and -0.29±0.89, respectively; $p=0.88$). Age, serum levels of calcium, phosphate, parathyroid hormone, 25-hydroxyvitamin D, osteocalcin, TNF-α, IL-6, testosterone in males and oestradiol in females, physical activity score, and dietary intakes of energy and calcium were not associated with either LS or FN BMD Z-score. The physical activity score did not correlate significantly with disease severity, as measured by FEV_1 (% pred).

Multiple linear regression analysis of associations with lumbar spine and femoral neck bone mineral density Z-scores

Multiple linear regression analysis showed that the most significant independent predictors of lower LS BMD Z-scores were presence of the ΔF508 CF genotype (homozygote or heterozygote), malnutrition as indicated by the requirement for supplementary enteral nutrition, male sex, lower serum phosphate level, lower serum 25-hydroxyvitamin D level and higher fat mass (table 7). For FN BMD Z-score, the best predictors were male sex, lower fat-free mass, ΔF508 genotype and greater disease severity as measured by FEV_1 (% pred) (table 7). The significant variables identified accounted for 44%

TABLE 4 Bone mineral density (BMD) and body composition in adults with cystic fibrosis

	Total	Males	Females
Subjects n	88	53	35
Lumbar spine			
BMD g·cm^{-2}	1.10±0.17	1.08±0.17	1.14±0.16
T-score	-0.94±1.38	-1.30±1.39	-0.41±1.20#
Z-score	-0.58±1.30	-0.87±1.35	0.15±1.11*
Femoral neck			
BMD g·cm^{-2}	0.97±0.16	0.98±0.16	0.96±0.16
T-score	-0.50±1.28	-0.71±1.22	-0.18±1.33
Z-score	-0.24±1.19	-0.45±1.14	0.08±1.22*
Whole body¶			
BMD g·cm^{-2}	1.15±0.09	1.16±0.09	1.14±0.09
T-score	-0.38±1.20	-0.73±1.16	0.15±1.08#
Z-score	0.13±1.05	-0.16±1.02	0.58±0.93#
Fat-free mass¶ kg	49.6±9.1	55.0±7.4	41.3±3.7#
Fat mass¶ kg	10.2±5.7	8.3±5.3	13.2±4.9#

Data are presented as mean±SD. ¶: n=86. *: p<0.05; #: p<0.005 *versus* males (unpaired t-test).

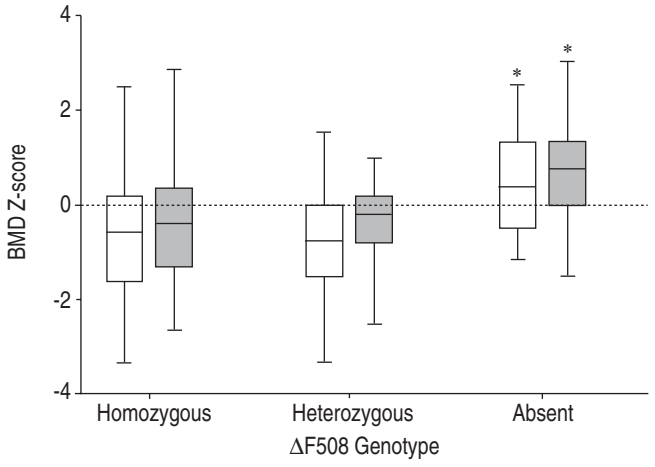

FIGURE 1. Bone mineral density (BMD) Z-scores at the lumbar spine (□) and femoral neck (■) in adult cystic fibrosis (CF) patients for each CF genotype category (homozygous: n=39; heterozygous: n=36; absent: n=10). Boxes represent the median and interquartile range, and whiskers the 5th and 95th percentiles. *: p<0.05 (one-way ANOVA).

TABLE 5 Univariate correlates of lumbar spine and femoral neck bone mineral density Z-score in adults with cystic fibrosis

Variable	Comparison	Subjects n	Lumbar spine Z-score		Femoral neck Z-score	
			Δmeans	p-value	Δmeans	p-value
ΔF508 genotype	Absent *versus* present	85	1.12	<0.01	1.20	<0.005
Sex	Male *versus* female	88	-0.73	<0.05	-0.53	<0.05
GDW delay	Ever *versus* never	88	-0.66	<0.05	-0.37	NS
Supplementary enteral nutrition	Ever *versus* never	88	-1.50	<0.0005	-1.27	<0.0005
Oral/intravenous corticosteroids	Ever *versus* never	88	0.08	NS	-0.62	<0.05
Inhaled corticosteroids	Ever *versus* never	88	-0.24	NS	-0.54	<0.05

Δmeans: difference in means; GDW: growth, development or weight gain; NS: nonsignificant.

TABLE 6 Univariate correlates of lumbar spine and femoral neck bone mineral density Z-score in adults with cystic fibrosis

Variable	Subjects n	Lumbar spine Z-score		Femoral neck Z-score	
		Correlation coefficient	p-value	Correlation coefficient	p-value
Age of menarche (females)	35	-0.50	<0.005	-0.41	<0.05
Log serum alkaline phosphatase	85	-0.30	<0.01	-0.14	NS
Log N-telopeptide	81	-0.28	<0.05	-0.26	<0.05
FEV$_1$ (% pred)	88	0.27	<0.05	0.38	<0.005
BMI	88	0.10	NS	0.21	<0.05
Age	88	0.15	NS	0.05	NS

FEV$_1$: Forced expiratory volume in one second; BMI: body mass index; % pred: percentage of the predicted value; NS: nonsignificant.

TABLE 7 Significant variables associated with bone mineral density Z-score in cystic fibrosis subjects on multiple linear regression analysis

Variable	Lumbar spine		Femoral neck	
	Coefficient b	p-value	Coefficient b	p-value
ΔF508 absent	1.46	<0.001	0.95	<0.01
Supplementary enteral nutrition	-1.32	<0.001	NS	NS
Male sex	-0.86	<0.005	-1.19	<0.005
Serum phosphate level	1.61	<0.005	NS	NS
Serum 25-hydroxyvitamin D level	0.01	<0.01	NS	NS
Fat-free mass	NS	NS	0.06	<0.005
Fat mass	-0.07	<0.01	NS	NS
Log serum alkaline phosphatase	-0.97	<0.05	NS	NS
Liver disease	1.37	<0.05	NS	NS
GDW delay	-0.62	<0.05	NS	NS
FEV_1 (% pred)	NS	NS	0.01	<0.05
Age	–	0.12	–	0.33
R^2	0.44		0.34	

GDW: growth, development or weight gain; FEV_1: forced expiratory volume in one second; % pred: percentage of the predicted value; NS: nonsignificant.

of the variability in LS BMD Z-score and 34% of the that in FN BMD Z-score.

Further analysis showed that CF genotype was not associated with sex (Chi-squared test), and that there were no significant differences in age, BMI, or serum 25-hydroxyvitamin D, osteocalcin, parathyroid hormone or urinary N-telopeptide level across the three genotype categories (one-way ANOVA). There was no association between CF genotype and FEV_1 (% pred), even when the effect of age was accounted for. However, pancreatic status and genotype were associated, with 100% of the subjects who were homozygous for ΔF508 being pancreatic insufficient, compared to 78% for those who were heterozygous for ΔF508 and 90% for those who had no ΔF508 allele ($p<0.01$; Chi-squared test).

DISCUSSION

In the present large cross-sectional study, reduced BMD was found to be common in adults with CF, with 17% having a BMD Z-score of <-2.0 at one or more sites. This confirms other reports from Europe, the UK and Australia [4–6, 22, 23]. A recently published Australian study reported similar mean BMD T- and Z-scores in adults with CF to those in the present study, and a similar proportion of adults exhibited a BMD Z-score of <-2 [22]. In the present study, a strong association between reduced BMD and the factors ΔF508 CF genotype and male sex was shown in a large sample representative of an adult CF population, independent of lung disease, pancreatic insufficiency, vitamin D deficiency and nutritional deficiencies. The independent variables identified in the multivariate models accounted for 44% of the variability in LS BMD Z-score and 34% of the variability in FN BMD Z-score in adults with CF.

The present study extends previous work characterising the aetiology of reduced BMD in CF. The results suggest that reduced BMD in CF appears to have a genetic component, independent of the influence of lung disease severity and nutritional deficits. Although genetic factors have been reported to contribute a significant proportion of the variability in bone mass in the general population [24], there are no previous reports in CF patients of genotypic dependency of BMD.

In the present study, mean BMD Z-scores at both the LS and FN were significantly lower in subjects who were either homozygous or heterozygous for the ΔF508 CF mutation, compared to those who had no ΔF508 allele. The present findings may have significant clinical implications, as they suggest that the risk of reduced BMD can be stratified according to whether or not the ΔF508 mutation is present. Although the present authors accept that the majority of CF patients carry the ΔF508 mutation, the proportion without ΔF508 remains significant. The proportion (12%) of subjects with no ΔF508 allele was greater than that found in other studies (4–6%), providing greater power for undertaking the analysis. In addition, the ΔF508 mutation was independently associated with reduced BMD when important confounders such as pancreatic status and vitamin D level were accounted for. In the present study, 100% of subjects who were homozygous for ΔF508 showed pancreatic insufficiency. Pancreatic insufficiency was not associated with reduced BMD or low serum levels of 25-hydroxyvitamin D, and there was no association between genotype and serum levels of 25-hydroxyvitamin D. The contribution of ΔF508 to reduced BMD would therefore seem not to be explained by pancreatic or vitamin D insufficiency. The present findings not linking BMD and pancreatic status are consistent with other reports in CF patients [6, 13].

To the present authors' knowledge, no previous studies have reported that genotype and BMD are correlated in CF. The present finding that there was no difference in mean BMD between homozygotes and heterozygotes is consistent with the findings reported for other studies [5, 15]. Although the CFTR

has been shown to be expressed in a number of body tissues, there are no reports regarding whether or not it is expressed in bone [25]. The mechanism whereby the ΔF508 mutation is associated with reduced BMD remains unclear. It may be related to increased bone turnover, as it has been reported that levels of bone turnover markers are higher in CF patients who are homozygous for ΔF508 than in those who are not homozygous [5]. Longitudinal studies are required in order to examine the influence of CF genotype on the rates of accretion and loss of bone mineral in CF patients.

In the present study, male sex was independently associated with lower BMD at both the LS and FN on multivariate analysis. These findings are consistent with other studies [4, 5], and suggest that adult males with CF show a greater deficit in bone mineral relative to their healthy peers than do females. There was no evidence in the present study of current male hypogonadism, and testosterone levels did not correlate with BMD. There was no correlation between sex and genotype, which rules out sex as a possible confounder of the association found between CF genotype and BMD. Males tended to exhibit increased bone turnover, having higher median levels of osteocalcin and N-telopeptide.

The present finding may reflect the sex bias in survival in CF, since fewer females survive into adulthood [26]. Longitudinal studies of BMD from childhood to adulthood may help to identify whether the sex bias in survival, or other factors, such as greater nutritional imbalances in males, are responsible for the observed sex differences in BMD in adults with CF. There is evidence suggesting that males with CF experience greater nutritional deficits than do females [27–29].

Poor nutritional status and lower serum 25-hydroxyvitamin D levels were also associated with reduced BMD. Lower fat-free mass was associated with lower LS BMD Z-score, whereas higher fat mass was associated with lower FN BMD Z-score. These findings are consistent with those of IONESCU *et al.* [10], who also postulated that reduced BMD and reduced fat-free mass may be linked *via* other features of CF, such as physical inactivity, corticosteroid use and loss of body protein as a result of catabolism associated with chronic lung disease. These findings, together with the present results, suggest that the relationships between pulmonary infection, nutritional status and bone metabolism merit further study, and confirm the emphasis given to optimising nutritional status as an integral part of CF management [30].

Lung disease severity, as measured by FEV1 (% pred), was found, in the present study, to be independently associated with FN BMD Z-score. This supports the findings of other studies [4–6, 14]. A link between reduced BMD and chronic and acute infection and inflammation has been suggested [5, 10], and alterations in bone metabolism markers have been reported to accompany exacerbation of lung infection in CF [31]. In the present study, there was no correlation between genotype and FEV1 (% pred), even when the effect of age was taken into account, which is consistent with other reports of a poor correlation between genotype and severity of lung disease [1].

Serum levels of TNF-α and IL-6 levels did not correlate with either LS or FN BMD Z-score. This finding is in contrast to associations reported by others [10]. In the present study, cytokine levels were measured once, when subjects were clinically stable. The authors accept this as being a limitation to the cross-sectional study design. Serial measurements of both BMD and cytokines may assist with characterising the role of cytokines in bone metabolism in CF. Nevertheless the present results provide important information about cytokine levels in a heterogeneous group of stable CF patients.

It is possible that physical activity level has an influence on the association between lung disease severity and reduced BMD found in the present study. However, no correlation was found between BMD and physical activity, nor were physical activity and FEV1 (% pred) correlated. This suggests that physical activity level is not confounding the association between disease severity and reduced BMD. The present finding is consistent with that of IONESCU *et al.* [10], but not with those of others who have reported associations between physical activity and BMD, in univariate [4, 5] or multivariate [22] analyses.

The present authors acknowledge the limitations associated with the cross-sectional design of the current study, which include the seasonal variation in 25-hydroxyvitamin D levels, the lack of data on the frequency and severity of exacerbations of lung disease, the difficulties in quantifying physical activity levels over the long time period during which bone mineral is accreted and the lack of physical activity tools specific for CF. Nevertheless, the authors feel that the present data, derived from a large and representative CF population sample and analysed using multivariate techniques, offer novel insights into the potential mechanisms contributing to reduced BMD in CF patients.

In conclusion, reduced bone mineral density is common in adults with cystic fibrosis. The ΔF508 cystic fibrosis mutation, male sex, poorer nutritional status and more severe lung disease are independently associated with reduced bone mineral density. The mechanism of action of ΔF508 in reducing bone density in cystic fibrosis remains to be elucidated. The present authors recommend that all adult cystic fibrosis patients undergo assessment of bone mineral density, and timely intervention to prevent and treat problems such as acute pulmonary deterioration, growth and pubertal delay, and nutritional deficits. Further studies are required to determine the natural history of reduced bone mineral density in cystic fibrosis and to optimise therapeutic interventions.

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