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ORIGINAL ARTICLE

Bone demineralisation in a large cohort of Wilson disease patients

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Abstract
Aims and background We compared the bone mineral density (BMD) of adult Wilson disease (WD) patients (n=148), with an age- and gender-matched healthy control population (n=148). Within the WD cohort, correlations of BMD with WD disease parameters, lab results, type of treatment and known osteoporosis risk factors were analysed.
Methods Hip and lumbar spine absolute BMD and T-score were measured by dual-energy X-ray absorptiometry. Osteoporosis and osteopenia were defined as a T-score≤−2.5, and between −1 and −2.5, respectively.
Results There were significantly more subjects with abnormal T-scores in the WD population (58.8 %) than in the control population (45.3 %) (χ^2=6.65, df=2, p=0.036), as there were 50.0 % osteopenic and 8.8 % osteoporotic WD patients, vs. 41.2 % and 4.1 %, respectively, in the controls. Especially L2-L4 spine BMD measurements (BMD and T-scores) differed significantly between the WD population and matched controls. L2-L4 spine BMD for WD patients was on average 0.054 g/cm^2 (5.1 %) lower than in matched normal controls (0.995±0.156 vs 1.050±0.135; p=0.002). We found no significant correlation between BMD values and any of the WD disease parameters (e.g. the severity of liver disease), lab results, type of treatment or known osteoporosis risk factors. Duration of D-penicillamine treatment was negatively correlated with femoral BMD value, but in a clinically irrelevant manner, compared to age and gender. Importantly, BMD remained significantly lower in WD patients (n=89) vs. controls after excluding WD patients with cirrhosis (p=0.009).
Conclusions Our study suggests that WD is intrinsically associated with bone demineralisation.

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Prof Steven Boonen unfortunately succumbed during the study

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Abbreviations

ALT	Alanine transaminase
AP	Alkaline Phosphatase
AST	Aspartate transaminase
BMD	Bone mMineral density
DXA	Dual X-ray Absorptiometry
GGT	Gamma glutamyl transpeptidase
INR	International normalised ratio
MELD	Model for end-stage liver disease
PTH	Parathyroid hormone
SD	Standard deviation
WD	Wilson disease

Introduction

Wilson disease (WD, OMIM#277900) is an autosomal recessive disorder of copper metabolism with an estimated

prevalence of 1 in 30,000. Copper accumulation is caused by mutations in the gene encoding a copper transporting P-type ATPase (ATP7b), on chromosome 13. In WD, the copper transport from hepatocyte to bile and the incorporation of copper into coeruloplasmin are impaired. As a result, excess copper accumulates in many organs and tissues, particularly the liver and brain. The disease is characterised by the presence of liver disease, neurological and psychiatric symptoms (Ferenci 2006; Weiss 1999).

Osteoporosis is a metabolic bone disease characterised by an imbalance between bone formation and resorption, that leads to a net decrease in bone mass with reduced bone strength and increased susceptibility to fracture (Gielen et al 2011). Osteoporotic fractures are associated with significant morbidity, loss of quality of life, economic cost and even mortality.

While skeletal changes have been reported in WD, (Golding and Walshe 1977; Xie et al 1985; Rodríguez Nieva et al 2004; Mindelzun et al 1970) until very recently only two studies had quantified bone mineral density (BMD) in these patients, one in an adult population (n=21) (Hegedus et al 2002) and one in children with WD (n=31) (Selimoglu et al 2008). Both studies reported a high prevalence of osteoporosis in WD. Last year, Quemeneur et al reported on 85 WD patients, and showed increased fractures related to high-energy trauma, associated with neurological involvement and male sex. In addition, they showed an association between low BMD and vertebral fractures within the WD group. They did not compare BMD results of WD patients with controls (Quemeneur et al 2014).

The goal of this paper is twofold: firstly, to assess the prevalence of osteoporosis and osteopenia in a large cohort of WD patients, compared to age- and gender-matched normal controls. Secondly, to gain insight in the correlation of bone disease in WD, with disease specific parameters (e.g. the presence or absence of cirrhosis), with lab results, with type and duration of WD treatment, and with known osteoporosis risk factors, in order to acquire pathophysiological insight into the possible causes of osteoporosis in WD.

Materials and methods

Patients A total of 148 adult WD patients were included; 19 patients from Leuven University Hospitals and 129 patients from Heidelberg University Hospital. Diagnoses were established as reported in previous WD publications from Leuven and Heidelberg separately and jointly (Weiss et al 2011; Lowette et al 2010; Pfeiffenberger et al 2014). All patients signed a written informed consent, approved by the centers' ethical committees. Only the first BMD data at the time of diagnosis or during follow-up were included in the analyses. The mean age of WD patients at the first time of BMD measurement was 36.3 years (SD=10.6) with 60.8 % of the patients being females.

Control population The age- and gender-matched control subjects were extracted from a database with lumbar spine and femoral neck measurements from 345 healthy subjects (204 men and 141 women), collected at the Metabolic Bone Disease Unit at the University Hospitals Leuven. These healthy subjects were selected, because they do not carry any risk factor or risk behaviour known to be associated with bone demineralisation besides age and gender, and therefore represent 'normal BMD' individuals.

Endpoints

Primary endpoint Bone mineral density (BMD, g/cm2) values were measured by dual-energy X-ray absorptiometry (DXA) at the lumbar spine (L2-L4 or L1-L4) and the hip (femoral neck region). All measurements were made on the Hologic Discovery A scanner. For each BMD, T scores were calculated using the Hologic reference values from the National Health and Nutrition Examination Survey (NHANES) III (1988–1994) (Looker et al 1997). A T-score is the difference in standard deviation (SD) between the BMD of the patient and the average value of a young-adult, healthy sex-matched population (T-score=(BMD patient - BMD peak)/SD).

Osteoporosis and osteopenia were defined as a T-score≤ -2.5, and between -1 and -2.5, respectively, according to the World Health Organisation criteria (World Health Organization Study Group on Assessment of Fracture Risk and Its Application to Screening and Postmenopausal Osteoporosis 1994).

Secondary endpoints WD associated problems or disease manifestations (presence or absence of cirrhosis at diagnosis or at time of BMD measurement), WD medication, laboratory results (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), gamma glutamyltransferase (GGT), albumin, bilirubin, quick test, INR (international normalised ratio), creatinine, copper (Cu), coeruloplasmin, model for end-stage liver diseases (MELD) score, calcium, phosphate, vitamin D3 and parathyroid hormone (PTH)) were collected for all patients. Risk factors for osteoporosis including smoking, alcohol use (≥3 units per day or not), diabetes, hyperthyroidism, known hypogonadism, chronic malnutrition (body mass index (BMI) <18) and malabsorption and rheumatoid arthritis were registered for each patient.

Statistical analysis

Descriptive statistics of all demographic, baseline variables and study parameters were provided overall. Continuous data were summarised by their mean, standard deviation, median, minimum and maximum. Categorical and ordinal data were summarised by frequency and percentages.

WD patients versus normal controls

The effect of age, gender and disease status (WD versus normal controls) on L2-L4 spine and femoral neck BMD measurements (absolute BMD values and T-scores) was explored using multiple linear regression.

A Chi-square test of independence was used to verify if the prevalence of osteopenia/osteoporosis differed between WD patients and age/gender matched controls. In the case of a significant Chi-square test, a post hoc test was performed using +/−1.96 as the critical value for the standardised residual.

Relation between BMD measurements and risk factors for osteoporosis in the WD population

An exploratory study of the measured variables was performed. In a first step, the nature and strength of the bivariate relationship between the continuous variables was investigated by a scatter plot matrix and the Pearson correlation coefficient. The relation between the BMD measurements and categorical parameters was explored using boxplots. Next, the data were analysed using multiple linear regression analysis. Forward stepwise regression was used for this purpose. Once the main effects were identified, it was checked whether there were any significant interactions that should be added to the model. Once the final model was elucidated, we investigated whether the assumption for linear regression held. Finally, the quality of the model fit and the assumption underlying the linear regression model were assessed.

All statistical tests were performed in R version 2.15.3 using a two-sided significance level of 5 %.

Results

Prevalence of osteoporosis in WD patients, compared to matched controls

WD population characteristics

Summary statistics for lumbar and femoral neck bone density measurements (absolute and T-scores) in the WD patients cohort are presented in Table 1.

Table 1 Bone mineral density (BMD) of WD patients

Variable	All	Male	Female
n (%)	148 (100 %)	58 (39.2 %)	90 (60.8 %)
Age (years)			
Mean (SD)	36.3 (10.7)	36.5 (11.2)	36.2 (10.2)
Minimum–median–maximum	18–36–67	18–38.5–60	18–35 - 67
Lumbar			
Mean (SD) (g/cm^2)	0.995 (0.156)	0.988 (0.172)	1.000 (0.145)
Minimum–median–maximum	0.617–0.970–1.398	0.618–0.966–1.398	0.617–0.974–1.385
Lumbar T-score			
Mean (SD)	−0.85 (1.42)	−1.12 (1.54)	−0.67 (1.32)
Minimum–median–maximum	−4.3–−1.05–2.78	−4.3–− 1.36–2.57	−4.2–−0.95–2.78
Lumbar Z-score			
Mean (SD)	−0.56 (1.44)	−1.01 (1.54)	−0.27 (1.30)
Minimum–median–maximum	−4.02–−0.76–3.06	−4.02–−1.18–3.06	−3.66–−0.42–3.03
Femur neck			
Mean (SD) (g/cm^2)	0.813 (0.129)	0.839 (0.142)	0.790 (0.117)
Minimum–median–maximum	0.486–0.814–1.178	0.522–0.820–1.178	0.486–0.808–1.066
Femur neck T-score			
Mean (SD)	−0.55 (1.05)	−0.67 (1.05)	−0.48 (1.05)
Minimum–median–maximum	−3.27–−0.64–1.95	−3.00–−0.80–1.82	−3.27–−0.38–1.95
Femur neck Z-score			
Mean (SD)	−0.26 (1.01)	−0.30 (0.99)	−0.23 (1.02)
Minimum–median–maximum	−2.51–−0.34–2.39	−2.33–−0.48–2.09	−2.51–−0.15–2.39
Diagnosis[a]			
Normal, *n* (%)	61 (41.2 %)	22 (47.9 %)	39 (43.3 %)
Osteopenia, *n* (%)	74 (50.0 %)	28 (48.3 %)	46 (51.1 %)
Osteoporosis, *n* (%)	13 (8.8 %)	8 (13.8 %)	5 (5.6 %)

[a] Osteopenia, BMD value between 1–2.5 SD below mean of young adult reference group; Osteoporosis, BMD value below 2.5 SD below mean of young adult reference group

Control population characteristics

The controls were matched with the WD patients by gender and age. The matching by age was an approximation, since the subjects from the control database (age range: 25 to 81 years, median: 50.7 years) were generally older than the WD patients (age range: 18 to 67 years, median: 36 years).

Comparison between WD and control population

L2-L4 spine BMD The effect of age, gender and disease status (WD versus normal controls) on L2-L4 spine BMD measurements (absolute BMD values and T-scores) was explored using multiple linear regression. The disease status (WD or normal control) was the only predictor that was significantly related to the BMD (absolute values and T-scores). L2-L4 spine BMD for WD patients was on average 0.054 g/cm^2 (95 % CI: −0.087; −0.021, p=0.002) lower in comparison to matched normal controls. The L2-L4 spine T-score for WD patients was on average 0.453 SD's (95 % CI: −0.756; −0.150, p=0.004) lower in comparison to matched normal controls (Fig. 1).

Femoral neck BMD The effect of age, gender and disease status (WD versus normal controls) on femoral neck BMD measurements (absolute BMD values and T-scores) was explored using multiple linear regression. Age and gender were significantly related to BMD values whereas the disease status was not (results not shown).

Bone demineralisation is more prevalent in WD than in controls and seems to predominantly affect the lumbar spine There were significantly more subjects with abnormal T-scores in the WD population (58.8 %) than in the control population (45.3 %) (χ^2=6.65, df=2, p=0.036), as there were 50.0 % osteopenic and 8.8 % osteoporotic WD patients, vs. 41.2 % and 4.1 %, respectively, in the controls (Table 2).

In the control population, 50 % (3/6) of the subjects diagnosed with osteoporosis, demonstrated an osteoporotic L2-L4 spine, while 83 % (5/6) had an osteoporotic femoral neck. In the WD population however, 84.6 % (11/13) of the osteoporotic subjects had an osteoporotic L2-L4 spine, while only 38.5 % (5/13) had an osteoporotic femoral neck. This suggests that the region of demineralisation differs between WD patients and the matched controls.

However, as the age matching between WD patients and controls was not exact, and as it is known that–in general - spine demineralisation starts at younger age than femoral neck demineralisation, the possibility remains that the regional difference of demineralisation suggested by our data, is related to age rather than to disease status (WD vs. normal). In addition, the numbers of patients with osteoporosis in the WD cohort and the control cohort are too small to draw final conclusions on the predominance of the region of demineralisation.

Correlations between BMD and WD manifestations, type of treatment, duration of D-penicillamine treatment, lab results and known osteoporosis risk factors

Summary statistics of the number (proportion) of patients, classified according to the presentation at diagnosis and the manifestations of the disease, are presented in Table 3. The type of WD medication the patients were on, is shown in Table 4.

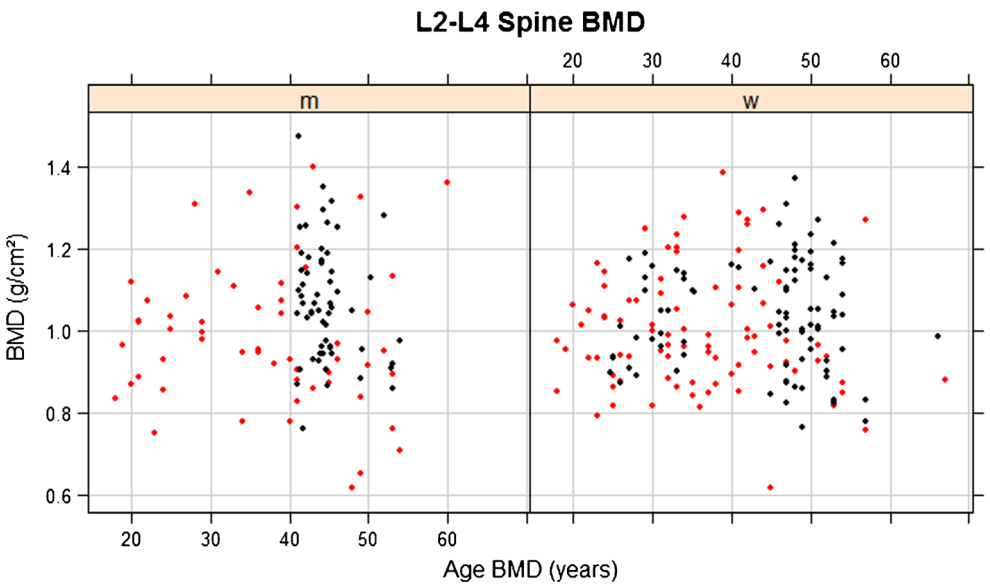

Fig. 1 **a**–**b** L2-L4 spine BMD by age and gender (m: men, left; w: women, right): of the Wilson patients (red dots) and the controls (*black dots*)

Table 2 Prevalence of osteopenia and osteoporosis in WD patients and matched controls

Diagnosis	Control	WD
Normal (T-score >−1), *n* (%)	81 (54.7)	61 (41.2) [A]
Osteopenia (T-score >−2.5 and≤−1), *n* (%)	61 (41.2)	74 (50.0)
Osteoporosis (T-score ≤−2.5), *n* (%)	6 (4.1)	13 (8.8)

Significant difference in prevalence between the control and WD population (χ^2 =6.65, df=2, p =0.036), [A] WD patients with normal condition were under represented (less then expected, standardise residual=−2.33<−1.96)

Table 4 WD medication and other medication (dpen=D-penicillamine)

Variable	All	First line
WD medication		97 (65.5)
Dpen, *n* (%)	86 (58.1)	81 (83.5)
Zinc, *n* (%)	27 (18.2)	8 (8.3)
Dpen+zinc, *n* (%)	8 (5.4)	1 (1.0)
Trientine, *n* (%)	27 (18.2)	7 (7.2)

NA: not applicable; *Dpen*: D-penicillamine

There was no difference in the range of blood parameters (AST, ALT, AP, GGT, albumin, bilirubin, quick, INR, creatinin, copper (Cu), coeruloplasmin, MELD score, calcium, phosphate, vitamin D3 and PTH (results not shown)) for WD patients classified as 'normal BMD', osteopenic or osteoporotic. Phosphate is the only parameter with slightly reduced values for the group with osteoporosis. This trend was observed at both sites (Heidelberg and Leuven) but one must take into account the small number of measurements.

The relation between BMD values and known risk factors for osteoporosis, the severity of liver disease, WD manifestations (Table 3), lab results or the type of treatment (Table 4) was investigated. Highly correlated (Pearson r>0.80) predictors are not desired in regression analyses, therefore the following parameters were also excluded from the model: AST (correlated with ALT), bilirubin, Quick and INR (correlated with MELD), coeruloplasmin (correlated with Cu).

The following parameters were also excluded from the analyses because they were not reported for the included WD patients: anorexia, hypogonadism, rheumatoid arthritis, corticosteroid treatment, diabetes and chronic malnutrition or malabsorption. As expected, based on the ages of the WD women (Table 1), menopause was only present in 8/90 WD women at the time of BMD measurement. Of the five women with osteoporosis in the entire WD cohort (23, 45, 47, 53 and 57 years old), only the 57 year old woman was menopausal. Inversely, six out of eight menopausal women had osteopenia and a single 51 year old menopausal woman had a normal BMD.

L2-L4 spine BMD Risk factors for osteoporosis including smoking, alcohol use and hyperthyroidism were checked for correlation with normal/osteopenia/osteoporosis classes as well as with the BMD values for each patient. Only one parameter (albumin) was significantly correlated with the L2-L4 spine BMD measurements but the correlations were very low (r=−0.17). According to the stepwise forward regression however, none of the known risk factors for osteoporosis or the type of treatment were significantly correlated with the L2-L4 spine BMD measurements.

Table 3 Distribution of WD associated problems

Variable	All	Male	Female
Initial presentation			
Hepatic, *n* (%)	75 (50.7)	29 (50.0)	46 (51.1)
Neurologic, *n* (%)	33 (22.3)	10 (17.2)	23 (25.6)
Hepatic & neurologic, *n* (%)	21 (14.2)	10 (17.2)	11 (12.2)
Asymptomatic, *n* (%)	19 (12.8)	9 (15.5)	10 (11.1)
Initial KFR, *n* (%)	83 (56.1)	27 (46.6)	56 (62.2)
Unknown, *n* (%)	18 (12.2)	11 (19.0)	7 (7.8)
Initial ALF, *n* (%)	8 (5.4)	0 (0)	8 (8.9)
Cirrhosis initial, *n* (%)	56 (37.8)	18 (31.0)	38 (42.2)
Confirmed at BMD measurement, *n* (%)–missing *n* (%)	59 (39.9)–4 (2.7)	19 (32.8)–1 (1.7)	40 (44.4)–3 (3.3)
Mobility			
Wheelchair	1 (0.7)	1 (1.7)	0 (0)
Severe deficiency but can walk	13 (8.8)	4 (6.9)	9 (10.0)
Slight deficiency able to walk	35 (23.6)	11 (19.0)	24 (26.7)
Normal	99 (66.9)	42 (72.4)	57 (63.3)

KFR Kayser-Fleischer ring; *ALF* acute liver failure

Femoral neck BMD There was a significant negative correlation between age and femoral neck BMD ($r=-0.27$, $p<0.001$) and a significant positive correlation between BMI and femoral neck BMD ($r=0.18$, $p<0.05$). WD patients with hyperthyroidism were found to have lower femoral neck BMD values than the WD patients without hyperthyroidism. Forward stepwise regression showed that none of the known risk factors for osteoporosis, the severity of liver disease or the type of treatment were significantly related with the femur neck BMD measurements (results not shown).

Liver disease Of note, neither the presence or absence of cirrhosis at the time of diagnosis or at the time of BMD measurement, nor the MELD score at the time of diagnosis or at BMD measurement, were correlating with lumbar or femur neck BMD values. In addition, BMD remained significantly lower in WD patients ($n=89$) vs. controls after excluding WD patients with cirrhosis ($n=59$, $p=0.009$).

Correlation between duration of D-penicillamine treatment and BMD

The majority of the WD patients received D-penicillamine as first line therapy whereas only a few patients received zinc or trientine as first line therapy (Table 4). The BMD for both L2-L4 spine and femoral neck appeared to decrease in function of D-penicillamine treatment duration. From the previous analyses we know that age significantly affects femoral neck BMD. We can also assume that the older the patients, the longer they have WD and the longer they received D-penicillamine as first line therapy. Indeed, the duration of WD disease and D-penicillamine treatment duration were highly correlated (Pearson's $r=0.94$, df=63, $p<0.001$). Therefore the influence of D-penicillamine treatment duration on BMD was investigated in function of age category.

The effect of age category, gender and D-penicillamine treatment duration on L2-L4 spine and femoral neck BMD was explored using multiple linear regression. L2-L4 spine BMD was only affected by age category, WD patients older than 45 years had on average significantly lower BMD in comparison with the younger age categories ($p=0.015$).

Femoral neck BMD was significantly affected by age category ($p<0.001$), medication duration ($p=0.049$) and gender ($p=0.017$).

Within the same age group and for the same gender, the mean femoral neck BMD is expected to decrease with 0.003 g/cm^2 (95 % CI: −0.006; −0.0001, $p=0.049$) when the D-penicillamine therapy duration increases with one year. In comparison, the effect of age and gender on bone mineralisation changes, are clearly more pronounced. We see that, when medication duration is kept constant, the average femoral neck BMD is significantly lower for the older patients (age 45+) in comparison with younger patients (mean difference age 45+ versus (1)≤25 years: 0.109 g/cm^2, $p=0.023$ and (2) 26–35 year: 0.137 g/cm^2, $p=0.002$). In addition, we see that within the same age category and when medication duration is kept constant, women have on average a 0.066 g/cm^2 lower femoral neck BMD than men ($p=0.019$). We can therefore conclude that the duration of D-penicillamine treatment has a statistically significant effect on femoral neck BMD. This effect is however clinically irrelevant when compared to the effect of age and gender.

Discussion

L2-L4 spine BMD measurements (absolute BMD values and T-scores) were significantly lower in the WD patients than in matched controls. In contrast, femoral neck BMD measurements did not differ between the WD and matched controls. There were significantly more subjects with abnormal T-scores in the WD population (58.8 %) than in the control population (45.3 %) ($\chi^2=6.65$, df=2, $p=0.036$), as there were 50.0 % osteopenic and 8.8 % osteoporotic WD patients, vs. 41.2 % and 4.1 %, respectively, in the controls. Further analyses showed that the higher prevalence of osteoporosis appeared to be mainly caused by lower L2-L4 BMD measurements within the WD population.

The WD patients were matched by age and gender with the controls. One must keep in mind that exact matching by age was not feasible for our cohorts, therefore the subjects from the control population (age range: 25 to 66 years, median: 45 years) were generally older than the WD patients (age range: 18 to 67 years, median: 36 years). As age is clearly correlated with BMD, there might be an underestimation of the burden of bone demineralisation in WD, in our study.

Within the WD population, none of the known risk factors for osteoporosis, the severity of liver disease, the lab results or the type of treatment were significantly associated with changes in BMD measurements. The correlation of age, gender and BMI with BMD values, was also observed in the controls and is therefore not specific for WD disease.

The effect of therapy duration for the WD patients that received D-penicillamine as first line treatment was also investigated. From these analyses we can conclude that L2-L4 spine BMD was not affected by D-penicillamine therapy duration, but femoral neck BMD was. Compared to the effect of age and gender on BMD, duration of D-penicillamine treatment affected femoral neck BMD only in a minimal way. In addition, as the type of WD treatment did not correlate with BMD values, we can exclude the possibility that D-penicillamine treatment can explain the demineralisation we see in the WD population on the whole.

Copper is an essential but also toxic metal. It is an important component of many proteins and enzymes, and is

essential for life. As copper is available in an oxidised state (Cu^{2+}) and in a reduced state (Cu^{+}), it is a useful component in various metabolic pathways.

WD is typically associated with accumulation of copper in various tissues, including bone tissue, with skeletal copper content reported to be increased about four times (Xie et al 1985). In a study by Hegedus et al, beta-cross-laps, a marker of bone remodeling, were found to be significantly increased in WD patients, suggesting increased bone resorption as a key mechanism contributing to bone loss and osteoporosis in these patients (Hegedus et al 2002). WD in our study, in line with previous reports, is indeed associated with significantly reduced BMD.

Indirect causes of bone disease in WD patients were excluded in our study. Osteoporosis in the context of chronic liver disease is well known and different mechanisms have been implied (Nakchbandi and van der Merwe 2009). However, in WD patients, previous reports did not find a relation between the degree of liver dysfunction and reduced BMD (Hegedus et al 2002; Selimoglu et al 2008). In our study, BMD did not correlate with presence or absence of cirrhosis at the time of diagnosis or at the time of BMD measurement, or with MELD score at diagnosis or at BMD measurement. Excluding WD patients with cirrhosis from the analyses showed that BMD remained significantly lower in WD vs. controls.

Other causal but indirect mechanisms linking WD to osteoporosis include tubular dysfunction, caused by copper accumulation in tubular cells, with high renal tubular phosphate excretion and hypercalciuria, or D-penicillamine treatment (Sözeri et al 1997; Walshe 1969). Renal function, calcium, phosphate, PTH and 25-OH-vitamin D levels were normal in our group of patients, in line with previous findings (Hegedus et al 2002) but this does not fully exclude phosphate loss or hypercalciuria due to tubulopathy. The duration of D-penicillamine treatment in our cohort had no meaningful impact on BMD, as discussed above.

Because the severity of liver disease is unrelated to the degree of bone loss as assessed by DXA, and D-penicillamine treatment or other indirect causes of bone demineralisation in WD patients do not appear to be paramount, we hypothesize here that copper accumulation may directly affect bone metabolism. Accumulation of copper causes production of reactive oxygen species (ROS), (Sokol et al 1994; Strand et al 1998; von Herbay et al 1994; Gu et al 2000) which is a risk factor for osteoporosis and may accelerate the effect of aging on bone by stimulating osteoclast differentiation and bone resorption and inhibiting osteoblast differentiation (Huh et al 2006; Maggio et al 2003; Bax et al 1992; Kikuyama et al 2002; Li et al 2014). Excess copper on the other hand causes disease by causing mitochondrial dysfunction. The known mechanisms by which excess copper directly causes mitochondrial dysfunction and damage, are via (1) a deficiency in the mitochondrial respiratory chain, at the level of the copper-dependent complex IV (cytochrome C oxidase); (2) a cross-linking of mitochondrial membranous proteins, resulting in contraction of the mitochondrial membrane; and (3) an increase in sphingomyelinase activity, thereby changing the ceramide content of the membranes (mitochondrial membrane and cell membrane), leading to a pro-apoptotic phenotype of the cell (Roberts et al 2008; Seth et al 2004; Gaetke and Chow 2003; Arnal et al 2013; Lang et al 2007; Zischka et al 2011).

Mitochondrial dysfunction and mitochondrial toxicity are causally related to bone demineralisation. Treatment with antiretroviral therapy in HIV patients causes mitochondrial dysfunction with lactic acidosis and bone demineralisation (Herman and Easterbrook 2001). Inborn errors of the mitochondrial respiratory chain function are associated with osteoporosis (Trifunovic et al 2004). An association study of common variants in mitochondrial DNA (mtDNA) found two mtDNA variants correlating with the risk for developing osteoporosis (Guo et al 2011). All these facts suggest that the mitochondrial toxicity that is caused by copper accumulation could indeed contribute to the bone demineralisation we describe here.

In conclusion, our study suggests that WD is intrinsically associated with bone demineralisation, predominantly in the lumbar spine.

Compliance with ethics guidelines

Conflict of interest None.

Human and animal rights and informed consent All procedures followed were in accordance with the ethical standards of the responsiblecommittee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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