Variation In The PTH Gene, Hip Fracture And Femoral Neck Geometry In Elderly Women

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ABSTRACT

Purpose: Parathyroid hormone (PTH) is a principal regulator of calcium homeostasis. Previously, we studied single nucleotide polymorphisms (SNPs) present in the major genes in the PTH pathway (PTH, PTHrP, PTHR1, PTHR2) in relation to bone mineral density (BMD) and fracture incidence. We found that haplotypes of the PTH gene were associated with fracture risk independent of BMD. In this present study, we evaluated the relationship between PTH haplotypes and femoral neck bone size.

Methods: Hip structure analysis (HSA) and BMD of the femoral neck was assessed by DXA in elderly women from the Malmö Osteoporosis Prospective Risk Assessment (OPRA) study. Data on hip fracture, sustained as the result of low trauma after the age of 45 years, was also analysed. Haplotypes derived from six polymorphisms in the PTH locus were analysed in 750 women.

Results: Carriers of Haplotype 9 had lower values for hip geometry parameters: Cross Sectional Moment of Inertia ($p=0.029$), Femoral Neck Width ($p=0.049$) and Section Modulous ($p=0.06$) suggestive of increased fracture risk at the hip. However, this did not translate into an increased incidence of hip fracture in the studied population. Women who suffered a hip fracture compared to those who had not, had longer hip axis length (HAL) ($p <0.001$). HAL was not significantly different among haplotypes.

Conclusions**:** Polymorphisms in the PTH gene associate to differences in aspects of femoral neck geometry in elderly women; however, the major predictor of hip fracture in our population is HAL to which PTH gene variation does not contribute significantly**.**

INTRODUCTION

Osteoporosis is a common skeletal disease characterised by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue. The ultimate outcome of osteoporosis is fracture, the most serious of that is hip fracture in terms of morbidity, mortality and economic costs [1-2].

Bone strength is influenced by bone mass and bone quality, which encompasses both structural and biomechanical aspects. Bone strength is determined by a combination of genetic and environmental factors. Family and twin studies have shown a significant heritable component, accountable for up to 80% with both BMD and femoral structure [3-4], between 51% and 79% for hip axis length [5] and from 60%-80% for the other aspects of bone geometry as well as bone quality [3, 6-7]. Data modelled in twins indicates that both specific and shared genetic factors act on individual bone phenotypes [8-9] and may explain the partially BMD-independent associations often observed with fracture [10-11].

Parathyroid hormone (PTH) is a key regulator of calcium metabolism, maintaining the levels of calcium ion in serum by modulating osteoclastic bone resorption and calcium reabsorption in the kidneys [12]. PTH secretion is induced by low serum calcium levels, resulting in the activation of osteoclasts. In the case of hyperparathyroidism, a continuously elevated PTH level may lead to osteoporosis and fracture. However PTH has a dual action and when elevation is intermittent, PTH promotes bone formation. Consequently, when administrated intermittently through stimulation of osteoblast proliferation and differentiation. PTH has been proven to be a useful bone anabolic agent [12-14]. Associated with PTH treatment are beneficial micro-architectural changes affecting trabecular width, connectivity and a denser trabecular structure leading to increased bone strength, particularly in the vertebrae [15-16]. However, a differential effect on cortical bone has also been noted, with initially decreasing

BMD values at the hip and distal forearm [13, 17-18]. Another protein in the PTH pathway, the parathyroid hormone related peptide (PTHrP), has been shown to be important for skeletal development during early bone growth and as a para-malignant phenomenon [19-20].

Previously we studied several genes within the PTH complex (PTH, PTHrP, PTHR1 and PTHR2) in a cohort of elderly Swedish women and reported an association between haplotypes of *PTH* and fracture, while no such association was found for the other genes in the pathway [21]. The relationship with fracture was independent of an effect on bone mass.

Given the evidence for the effects of *PTH* on bone size and the fact that bone size itself is an independent risk factor for fracture, the aim of this study was to investigate whether the SNPs within *PTH* play a role in the regulation of femoral neck geometry and bone quality in elderly women. Additionally, within this cohort whose age confers a high risk of fracture, we also evaluated the contribution to hip fracture risk.

MATERIALS AND METHODS

Subjects

The study cohort comprises the Malmö Osteoporosis Prospective Risk Assessment study (OPRA) of 1604 women aged 75 years at invitation which has been previously described in detail [22]. Briefly, these women were randomly selected from the city files in Malmö and included between December 1995 and May 1999. 1044 (65%) subjects accepted baseline investigation and no exclusion criteria were used. The vast majority were self ambulatory [23], and of Caucasian origin [22]. At baseline, bone mineral density (BMD) was assessed and blood samples were collected for DNA analysis and determination of serum PTH concentration. The participants also answered a questionnaire regarding general health, medication and previous falls and fractures. The Ethics Committee of Lund University approved the study.

Fracture

At the baseline visit, data on self-reported fractures sustained between the age of 20 and 75 years were collected by questionnaire and verified from radiological files as previously reported [24]. Prospective fracture data was also collected and similarly verified [25]. The mean prospective follow-up time was 7.0 years (range 5.4 – 9.0 years). In this study, we report only on hip fractures.

Bone mineral density and hip structure analysis

All image files were reanalysed by a single operator, who was blinded for fracture status, using hip strength analysis software provided by Lunar Instruments Corporation (Madison, WI, USA). The x-ray absorption data of the proximal femur are, with this software, extracted

from the output image data file and the amount of bone mineral and its distribution within the femoral neck is calculated. The reproducibility of the hip strength analysis was determined by five repeated scans in six young healthy subjects after repositioning of the subject. The standard error of the measurement (SEM) and the coefficient of variation (CV) of BMD and the hip strength indices were calculated. On average, the SEM of hip strength measurements was between 0.02 to 0.06 of their mean and the CV was between 0.6 to 3.7 %.

CSMI is an estimate of the ability of the femoral neck to withstand bending forces and was calculated using the mass distribution of the absorption curve[26]. The CSMI estimated with DXA has been found to be highly correlated with the CSMI measured directly on cadaver specimens (r^2=0.96)[26]. The automatic identification of the weakest cross-section of the femoral neck is the central part of the hip strength analysis software and this cross-section level is then used for the subsequent calculations of section modulus (SM, cm^3) and the femoral neck width (FN width, cm). The section modulus is also an estimate of the ability of the femoral neck to withstand bending forces, and is calculated as CSMI divided by the distance from the center of the mass to the superior neck margin. The hip axis length (HAL, cm) is defined as the linear distance from the pelvic rim to the lateral aspect of the femur along the femoral neck axis defined in the hip strength analysis and measured using the DXA ruler option.

Quantitative Ultrasound

Ultrasound measurements, speed of sound (SOS), broadband ultrasound attenuation (BUA), and stiffness index (SI), a derivative of BUA and SOS, were performed using a Lunar Achilles® system. The right calcaneus was measured unless there was a history of previous

injury or fracture on the right side. Precision of ultrasound in our hands was 1.5%[27]. The long-term stability of the apparatus was checked by daily calibrations.

DNA genotyping

Genotyping of the PTH gene as described in detail previously was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA, USA) [21]. All polymorphisms were amplified according to standard conditions (50°C for 2 min, 10 min at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C). After PCR amplification, genotypes were determined using an ABI Prism 7900HT sequence detector (Applied Biosystems). In this report we present data on 750 women for whom complete genotype data for all six PTH polymorphisms was available.

PTH Polymorphisms and haplotypes

We studied 6 single nucleotide polymorphisms (SNPs) in and around the region of the PTH gene (11p15.3-p15.1). These SNPs were selected from Ensemble representing the most commonly occurring haplotypes according to Haploview. The polymorphisms selected were: rs307253 located ~4kb downstream; rs307247 located ~100bp downstream; rs6254 in intron 2 (also known by its restriction enzyme recognition site *Bst*BI); rs1459015 which lies ~5kb upstream, rs10500783 located ~56kb upstream and rs10500784 located 56.2kb upstream.

As previously reported, 5 common haplotypes defined by the 6 SNPs in LD were identified, reported in the order rs307253 (C/T) –rs307247 (C/T) – rs6254 (A/G) – rs1459015 (G/A) – rs10500783 (C/T) – rs10500784 (A/C)): haplotype 5, CCAACC (36.7%), haplotype 9, TTGGCA (19.3%), haplotype 2, CCGATA (15.3%), haplotype 8, TTGACA(14.8%), haplotype 1,CCGACA(13.1%) (Table 1).

Statistics

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc.). Hardy-Weinberg equilibrium (HWE) was calculated by the Chi^2 test. PTH Haplotypes were inferred from the population genotype data using the program PHASE version 2.02 (http://stephenslab.uchicago.edu/software.html) [28]. Haplotype analyses were performed, according to whether individuals possessed 0, 1 or 2 copies of the haplotype allele. Association between genotypes and bone variables were made using GLM ANOVA correcting, as appropriate, for confounding factors. Spearman's correlation was used to identify relationships between clinical measurements, bone geometry as well as quality parameters. Significance was set at $p<0.05$ and the p-values reported are nominal without correction for multiple testing.

RESULTS

Subject characteristics

The clinical characteristics of the OPRA study participants including BMD, ultrasound and femoral neck geometry measurements are shown in Table 2. In brief, all women were 75 years old (range 75.01 and 75.98 yrs) and only 14% were current smokers. Serum PTH levels were normally distributed for age and all no exclusions were made. The PTH genotype distribution was in Hardy-Weinberg equilibrium and the five common haplotypes, which accounted for >98% of alleles at the PTH locus are reported in Table 1.

Relationship between femoral neck geometry and anthropometric variables

Correlations between femoral neck geometry, BMD and height and weight were evaluated. Significant positive correlations were observed between all the variables ($p<0.01$). Current weight correlated more strongly with femoral neck width ($R=0.43$; $p<0.001$) CSMI ($R=0.51$; $p<0.001$) and SM ($R=0.50$; $p<0.001$) than did current height (Table 3).

Femoral neck geometry, bone quality and hip fracture

We compared women who had suffered a hip fracture during their lifetime (n=117/750) (includes retrospective and prospective fractures) against those who had not (n=633/750) (Table 4). Weight and current height were not significantly different between the two groups, although women who suffered a hip fracture were significantly taller at age 20 ($p<0.001$). HAL was greater while SM was lower, as was BMD in the women with hip fractures ($p<0.01$) but there were no significant differences in either FN width or CSMI. Of those women who had sustained a hip fracture, three women suffered the fracture between the ages of 45-54 yrs

and excluding them from the analysis did not appreciably alter the results. All ultrasound measures at the calcaneus were lower in the hip fracture women.

Haplotype associations with femoral neck geometry and ultrasound

Measures of femoral neck geometry in relation to PTH haplotype are reported in Table 5. We observed modest associations between Haplotype 9 and femoral neck width (p=0.029), CSMI (p=0.049) and trends towards association for SM (p=0.063) after correction for current height and weight. No association was observed with HAL (p=0.78). Individuals with 2 copies of haplotype 9 had the lowest values and the relationship was dose dependent. Comparison of those with $\geq$1 copies against 0 copies of the haplotype increased the significance of these associations (p=0.008, 0.018 and 0.029 for femoral neck width, CSMI and SM respectively).

Although the haplotype 5 was associated with current height (p=0.038), no significant association between femoral neck geometry and the other PTH haplotypes was observed,. Individually, PTH polymorphisms were not associated with femoral neck geometry, with the exception of rs1459015 which was associated with femoral neck width (34.4 vs 33.9 vs 32.8; p=0.046).

We found no association between PTH haplotypes or individual genotypes and measures of BUA, SoS or stiffness index (Table 5). However, for all the haplotypes studied, all values were lowest in those carrying two copies of the haplotype.

Haplotype associations with hip fracture

A hip fracture was sustained by 117 women and all of these were the result of low energy trauma and occurred after the age of 45. Individuals carrying one or more copies of haplotype 9 were found to be under-represented among those who had sustained a hip fracture compared

to those who had not fractured (Chi2 analysis, χ^2=4.1, p=0.043). No differences in distribution were observed for the other haplotypes analysed.

Compared to the population as a whole, among the women who had sustained a hip fracture, measures of femoral neck geometry were not significantly different between individuals who carried or did not carry haplotype 9 (Table 6).

DISCUSSION

In addition to BMD, other bone phenotypes also have a strong genetic component and several genes like *RANKL, eNOS, TNFα, WNT10b, FZD1, IGF-1, ESR 1 and ESR2* have been associated with aspects of femoral neck geometry or indices of bone strength [29-35].

PTH is an important modulator of skeletal regulation and we have shown recently that genetic variation within the PTH locus is associated with fracture through mechanisms/pathways independent of BMD [21]. Furthermore, another study suggests a possible effect of PTH polymorphisms on bone dimensions in middle aged women [36]. In light of these observations, we evaluated variation in the PTH locus in relation to measures of bone quality and femoral neck bone geometry in elderly Swedish women at risk of fracture.

Age and BMD are the major contributing factors for fracture risk, but a number of other risk factors also need to be taken into account. Bone strength is a complex trait that is not captured by BMD alone and both macro- and micro-structural components play an important role. One facet of this structural component is hip geometry that has been implicated as an independent risk factor for fracture [37-38].

Hip structure analysis allows the extraction of geometric bone strength information from hip DXA scans [39]. The analysis relies on three main variables; hip axis length, femoral neck diameter and distribution of bone mass, from which a number of other indices are derived. We analysed variables shown to be most relevant to fracture in other studies. In a large Australian study, smaller femoral neck diameter and CSMI were found to be independent risk factors for hip fracture [37]. Another study in Caucasian and South American women found that the hip axis length and femur strength index were, alongside BMD, independent predictors of hip fracture.[38].

In our study, we report that PTH haplotype 9 displays lower values for all measures of femoral neck geometry, although only significantly associated with femoral neck width, CSMI and SM. Despite these findings that might suggest a higher susceptibility to hip fracture, through reduced mechanical strength individuals carrying this haplotype were not over-represented among those who had suffered a hip fracture. This is in keeping with our previous observation of a slight protective effect of this haplotype on fracture overall. We also reported previously that PTH haplotypes 1 and 5 were at slightly increased risk of fracture overall, however this does not appear to extend to fractures sustained at the hip. Furthermore, these haplotypes do not appear to make a significant contribution to bone dimensions at this site.

The literature relating to femoral neck width and its relationship with hip fracture is conflicting, with both increased and decreased widths reported among fracture patients, mirroring the fact that mechanical strength is dependant on both size and distribution of bone mass [40-41]. In our cohort of elderly women, hip axis length was significantly longer in women with a hip fracture, and the bending strength index (section modulus) was lower yet femoral neck width and CSMI were similar in both fracture and non-fracture groups. This may be explained by counter-actions between femoral neck diameter and thinning of the cortices in hip fracture patients [42], alternatively femoral neck diameter may be dichotomously distributed in hip fracture patients.

Only a limited number of studies have examined *PTH* gene variations and bone phenotypes, and only two other studies have examined bone size. Gong et al reported a significant association between *PTH* genotype and radiogrammetric bone dimensions, however analyses were restricted to the hand i.e. cross-sectional cortical area of the metacarpal and annual rate of change in radial cortical area [36]. In contrast, Lei et al found no association with bone size

at the lumbar spine and hip [43]. These results are suggestive of skeletal site-specific genetic effects of PTH on bone size.

The anabolic effect of PTH is exploited as a treatment for osteoporosis contributing both to aspects of bone strength and BMD. Animal studies indicate that changes in iliac crest bone biopsies after PTH treatment correlate with microstructure in both femoral neck and vertebrae [15]. In a prospective randomized multi-centre study, teriparatide has been shown to positively influence HSA parameters such as section modulus and cross-sectional area [44]. There is no effect however on hip axis length, since this is determined by the end of adolescence and is unchanged with age whereas, expansion of the circumference of the femoral neck and cortical thickness increases with age and varies in response to the magnitude of biomechanical loading. This is in keeping with our observed association that haplotypes of PTH affect only those aspects of femoral neck geometry (femoral neck diameter, CSMI and SM) which can be altered, suggesting that variation in the PTH gene may affect bone geometry throughout life. However, since not all patients respond to PTH treatment, it is likely that there is a complex mechanism involving other genetic and environmental factors in the response to PTH.

Our study is of interest since it is the only one to evaluate PTH genotype in relation to femoral neck geometry and hip fracture in an age-relevant cohort of elderly women whose age confers a high risk of fracture. The proportion with hip fracture is relatively high at 16% in the cohort, although it may still be considered low in the context of genotype distribution. However, the study population is large and ethnically homogenous and the narrow age band eliminates age associated confounding factors. Other strengths include the comprehensive selection of SNPs encompassing the *PTH* locus, capturing genetic variation in both the 5′ and 3′ regions, thus ensuring capture of as much as possible of the genetic variation across the region of the gene.

Since none of the SNPs included in our study has a known function, we must assume linkage disequilibrium with other functional polymorphisms within or close to the gene. A mutation at an intron-exon donor splice site, resulting in the loss of the signal peptide encoding exon 2, has been described in familial hypoparathyroidism [45] while mutations in the 3'UTR may affect mRNA stability [46]. The genes in the immediate vicinity of *PTH* have not been implicated in bone metabolism, but interactions between polymorphisms in other calcium regulatory pathway genes e.g. Calcium sensing receptor (*CaSR*), *Klotho* and fibroblast growth factor-23 (*FGF23*) should be considered and further explored.

There are a few potential limitations to our study. Although HSA of femoral neck geometry has been shown to be prognostic of hip fracture [47], QCT measurements would have enhanced the information obtained relating to microstructure and biomechanical parameters since QCT gives 3-dimensional information [39]. The number of hip fractures in the population is not sufficiently high to be able to further sub-divide them by type i.e trans-cervical or inter-trochanteric, which may have provided useful insight into the relationship between specific HSA parameters and fracture. Finally, our findings in elderly, Caucasian women may not be applicable to women of other ethnicities or to men.

Conclusions

This study was performed on the basis of our previous finding that variation in the PTH gene contributed to fracture risk in elderly women, independent of an effect on bone mass, thus we studied femoral neck bone geometry, a phenotype contributing to bone strength. We conclude that polymorphisms in the PTH gene contribute to differences in femoral neck geometry in elderly women; however, this does not translate into a discriminatory ability between those with and without hip fracture.

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Statement of disclosure

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Table 1. PTH haplotypes observed in the OPRA population.

PTH Haplotype	*Frequency in the Population*	Number of women with Copies of the Haplotype			
		0	**1**	**2**	**Total**
Hap 5	36.7%	309	332	109	750
Hap 9	19.3%	490	230	30	750
Hap 2	15.3%	536	199	15	750
Hap 8	14.8%	539	200	11	750
Hap 1	13.1%	560	183	7	750

Table 2. Clinical characteristics of the OPRA cohort

Variable	**Mean ± SD**
Age (years)	75.2 ± 0.1
Height at baseline (cm)	160.7 ± 5.9
Height at age 20 years (cm)	164.2 ± 5.6
Weight (kg)	67.8 ± 11.6
BMI (kg/m^2)	26.2 ± 4.1
Serum PTH (pmol/L)	4.69 ± 1.99
No of current smokers	101 (13.5%)*
Spine BMD (g/cm^2)	0.989 ± 0.195
Femoral neck BMD (g/cm^2)	0.747 ± 0.129
HAL (mm)	105.4 ± 6.0
Femoral neck width (mm)	34.1 ± 3.5
CSMI (cm^4)	11035 ± 4660
Section modulus (cm^3)	630 ± 203
SoS (m/s)	1512 ± 25
BUA (db/mHz)	101 ± 10
Stiffness Index	71 ± 13

* Number (Percentage)

Table 3. Spearman's Correlations between anthropometric variables and femoral neck geometry

	Height	**Weight**	**FN BMD**	**FN width**	**CSMI**	**SM**
Height		0.37	0.21	0.31	0.34	0.33
Weight			0.49	0.43	0.51	0.50
FN BMD				0.23	0.52	0.58
FN width					0.82	0.74
CSMI						0.98
SM						

Correlations are all significant at the level $p<0.01$

Table 4. Measures of femoral neck geometry and ultra sound parameters in women with hip and women with no hip fracture

Variable	**Hip Fracture** (n=117) Mean (SD)	**No Hip Fracture** (n=633) Mean (SD)	*p-value*
Weight (kg)	66.27 (11.7)	68.13 (11.5)	0.11
Height (cm)	161.2 (6.18)	160.6 (5.80)	0.31
Height at age 20 yrs (cm)	165.8 (5.80)	163.9 (5.5)	0.001
FN BMD (g/cm2)	0.673 (0.124)	0.761 (0.126)	<0.001
HAL (mm)	108.3 (6.7)	104.9 (5.8)	<0.001
Fem Neck width (mm)	34.23 (3.95)	34.13 (3.47)	0.681
CSMI (cm^4)	10415 (5128.4)	11151 (4561.4)	0.13
Section modulus (cm^3)	586.2 (217.7)	638.4 (198.8)	0.014
SoS (m/s)	1499.7 (27.18)	1513.9 (24.4)	<0.001
BUA (db/mHz)	96.9 (11.9)	102.1 (9.6)	<0.001
Stiffness Index	64.01 (14.34)	71.94 (12.70)	<0.001

Values are mean (Standard Deviation).

Table 5. PTH haplotypes and measures of femoral neck geometry and ultrasound measures in the OPRA cohort

	HAL	FN width	CSMI	SM	BUA	SoS	Stiffness Index
Haplotype 9							
0 copies	105.4 (6.1)	34.4 (3.7)	11291 (4748)	640 (206)	102.2 (10.2)	1511 (26)	70.3 (13.5)
1 copy	105.7 (5.7)	33.9 (3.0)	10660 (4501)	615 (195)	102.1 (10.2)	1515 (24)	72.3 (12.6)
2 copies	104.1 (7.3)	32.9 (3.4)	10021 (4060)	595 (189)	98.7 (8.1)	1509 (21)	69.2 (9.9)
	p=0.78	p=0.029	p=0.049	p=0.063	p=0.3	p=0.1	p=0.11
Haplotype 5							
0 copies	105.0 (6.0)	34.0 (3.5)	10924 (4443)	628 (196)	101.6 (9.9)	1513 (26)	71.2 (13.2)
1 copy	105.6 (6.2)	34.3 (3.5)	11112 (4688)	632 (201)	101.6 (10.1)	1512 (24)	71.0 (13.1)
2 copies	106.1 (5.6)	34.3 (3.9)	11177 (5140)	633 (223)	100.2 (11.0)	1510 (26)	69.4 (13.4)
	p=0.90	p=0.80	p=0.99	p=0.90	p=0.4	p=0.5	p=0.5
Haplotype 1							
0 copies	105.6 (5.9)	34.2 (3.6)	11118 (4857)	633 (209)	101.1 (10.2)	1512 (25)	70.7 (13.1)
1 copy	104.7 (6.3)	33.9 (3.5)	10808 (3985)	622 (180)	102.1 (10.0)	1512 (26)	71.3 (13.5)
2 copies	108.1 (7.1)	34.0 (2.7)	11290 (4417)	653 (209)	101.2 (84)	1508 (22)	69.9 (12.0)
	p=0.67	p=0.89	p=0.94	p=0.93	p=0.4	p=0.3	p=0.8

Values are means (SD) adjusted for height and body weight. (femoral neck geometry) or BMI, smoking and femoral neck BMD (ultrasound)

Table 6. PTH haplotype 9 and measures of femoral neck geometry in women with hip fracture

Women With Hip Fracture	**HAL**	**FN width**	**CSMI**	**SM**
Haplotype 9				
0 copies	108.4 (6.6)	34.5 (3.9)	10628 (4859)	597 (209)
1 copy	107.6 (7.4)	33.9 (4.3)	10076 (6043)	566 (246)
2 copies	111.4 (5.0)	31.5 (3.4)	7666 (4068)	474 (2)
	p=0.65	p=0.39	p=0.58	p=0.55

Values are means (SD) adjusted for BMI, smoking and femoral neck BMD