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Genetic architecture of hand quantitative ultrasound measures: A population-based study in a Sardinian genetic isolate

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ABSTRACT

It is now recognized that quantitative ultrasound (QUS) measures may predict osteoporotic fracture risk independently of bone mineral density. Although many studies have examined genetic and environmental components of bone mineral density and calcaneal QUS measures, few of them were addressed to phalangeal QUS phenotypes, and none to graphic trace parameters. This study aims to evaluate the relative contribution of genetics in the expression of phalangeal QUS traits in the adult healthy population of a Sardinian genetic isolate. Our sample includes 6056 men and women aged 30–103 years, from 43 extended pedigrees recruited in 10 villages of Ogliastra region in occasion of a large epidemiologic survey. Amplitude-dependent speed of sound (AD-SoS), fast wave amplitude (FWA), signal dynamic (SDy), bone transmission time (BTT) and ultrasound bone profile index (UBPI) were obtained from the non-dominant hand using the IGEA DBM Sonic Bone Profiler™. These phenotypes were first regressed on age, anthropometric and bioimpedance measures, serum calcium, phosphorus and alkaline phosphatase, alcohol and caffeine consumption, smoking status, exercise and also months since menopause and estrogens use in women. Adjusted QUS parameters were then analyzed by univariate and bivariate variance component models to obtain heritability estimates and genetic and environmental correlations. QUS parameters were correlated to age, anthropometric and bioimpedance measures, serum phosphorus, alkaline phosphatase and to reproductive history and menopause in women. All phenotypes demonstrated substantial heritabilities ranging from 0.29 ± 0.03 for SDy to 0.55 ± 0.03 for FWA. Proportion of variance due to all covariates ranged from 36% for SDy to 59% for BTT. Many significant genetic and environmental correlations were found between the different QUS measures. In this study, genetic factors appear to play a relevant role in determining hand QUS measures even when taking into account various important environmental factors. Furthermore, the modest genetic correlations may imply the existence of partially unique sets of genes affecting different QUS traits, thus suggesting that QUS parameters measure different properties of bone tissue.

Introduction

Phalangeal quantitative ultrasound (QUS) has been proposed as a technique that provides additional information on bone mineral status and osteoporotic fracture risk beyond that obtained from bone density, as it has been demonstrated that quantitative ultrasonography assesses qualitative characteristics of the bone, such as microarchitecture, geometry and elasticity [1–3]. Osteoporotic fractures are an important cause of excessive morbidity and mortality among elderly individuals [4]. In Italy almost 4,000,000 women are affected by osteoporosis and thus at risk of fracture, with a prevalence higher than 40% in women older than 60 years of age [5]. With the progressive ageing of the population, an exponential increase in the incidence of osteoporotic fractures, in particular hip fractures, is expected. For this reason, the social costs related to hip fractures are relevant [6].

In recent years, the genetic contribution to osteoporosis susceptibility has been well documented [7]. Twin studies have shown that variation in calcaneal and phalangeal QUS parameters among individuals are largely determined by genetic factors and the estimated heritability has been found to range between 74% and 82% [8]. Relatively few studies have examined the genetics of QUS measures in extended pedigrees, in males or in individuals spanning a wide range of ages; even

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fewer of them were done on phalangeal QUS parameters, including those related to QUS attenuation, or taking into account the effects of many potential environmental risk factors and determinants of osteoporosis. Since osteoporosis is determined by environmental and genetic factors, we decided to study this complex phenotype in Ogliastra, a peculiar isolated region within Sardinia (Italy). Genetic isolates like Ogliastra represent an important and powerful tool in investigating inheritance of multifactorial disorders [9,10]. The existence of multigenerational pedigrees descended from a small number of founders several generations ago, environmental and phenotypic homogeneity, restricted geographical distribution and the presence of exhaustive and detailed records correlating individuals in very well ascertained pedigrees are all features that make such populations ideal for identifying the genetic and non-genetic risk factors of complex diseases because of reduced background variability [11].

The aim of this study is to investigate the environmental determinants and the genetic architecture of different phalangeal QUS parameters in the adult population of 10 isolated villages in the central-eastern part of Sardinia.

Materials and methods

Population features

Ogliastra is one of the most isolated and anciently populated area in Sardinia. It is a mountainous region flanking the eastern coastal areas. Its small communities lie on the mountainside and entertained very few exchanges among each other and with large towns due to geographic barriers and lack of roads.

The Ogliastra population has grown slowly and uniformly through time with no relevant immigration: from 12,000 inhabitants in the 17th century, it numbers now 65,000 people. They are dispersed into 23 villages, many of whom with less than 1500 residents.

Our study was carried out in 10 villages: Baunei, Escalaplano, Loceri, Perdasdefogu, Seui, Seulo, Talana, Triei, Urzulei and Ussassai, all characterized by a great deal of homogeneity in life style and eating habits, a high rate of endogamy and consanguinity.

Study design and sample

Study sample comes from a large epidemiologic survey on complex diseases carried out on about 11,000 subjects living in the Ogliastra region. Briefly, the study design is cross-sectional and population based. People living in the villages were invited to take part in the study by means of information campaigns and letters sent to every family. Furthermore, pass the word, especially among family members, made a high level of recruitment possible. Participants gave a blood sample, underwent anthropometric measurements, bioelectrical impedance, quantitative ultrasonography and a standardized interview collecting socio-demographic, lifestyle, medical and pharmacological history data and family history of many pathologies, including osteoporosis. During the interviews, particular care was used to identify and record the best known risk factors for osteoporosis. The research adheres to the tenets of the Declaration of Helsinki and written informed consent was obtained from all enrolled people.

The sample cohort analyzed in the present study is aged 30–103 years and consists of 6056 individuals (4166 women and 1890 men) for whom at least one set of hand QUS measurement was available. In Baunei, Seulo and Ussassai, quantitative ultrasonography was performed only on women.

Quantitative ultrasound and covariates measurements

The IGEA DBM Sonic Bone Profiler™ was used for phalangeal QUS measurements. The device is based on the transmission of ultrasounds through the proximal phalanges (digits II–V), probes are applied to the lateral surfaces of the fingers. The coupling of the probes with the skin is mediated by standard ultrasound gel. Five QUS parameters, measured on the non-dominant hand, were included in the present analysis: amplitude-dependent speed of sound (AD-SoS, m/s), three parameters describing the graphic trace of the QUS signal – fast wave amplitude (FWA, mV), signal dynamic (SDy, $mV/\mu s^2$), bone transmission time (BTT, μs) – and finally ultrasound bone profile index (UBPI), which describes the probability that the subject belongs to the non-fractured group. Nine operators, all trained to the proper use of the QUS device by the same high experienced technician, performed the measurements. Reproducibility was evaluated for each of the 9 operators; each operator performed 5 repeated measurements, on different days, on at least 2 adult subjects. The three DBM Sonic Bone Profiler devices we used were cross-calibrated at the start up of the study using a special phantom provided by IGEA. Additionally, quality controls were done every time QUS devices were used, according to the manufacturer's guideline to verify the stability and calibration of all the devices.

Among living habits, we gathered information on physical (never/occasional/moderate/intense) and sport activity (never/occasional/1–2 per week/>2 per week), current and past diet (diet on medical grounds for more than 1 year), current smoking (number of cigarettes smoked per day) and alcohol consumption (number of glasses per day of wine, beer and spirits). We recorded age at onset and duration of menopause, age at menarche and number of pregnancies in women, previous diagnosis of osteoporosis, rheumatoid arthritis, chronic endocrinopathy and gastropathy. Finally, we collected data on chronic use of steroids, chemotherapy and treatments affecting bone metabolism. Biochemical analyses including serum calcium (SCa), phosphorus (S-phosphorus) and alkaline phosphatase (ALP) were done in our central laboratory in Perdasdefogu (Targa 3000 Biotecnica Instruments). For anthropometric measurements (weight, height, waist, wrist and hip circumferences), subjects were examined wearing only their underwear. Body mass index (BMI) in kg/m^2 and waist to hip ratio (WHR) were calculated. Bioelectrical impedance (BIA 101, RJL/Akern Systems), measuring the resistance and reactance, was used to determine phase angle, which is an indicator of cellular health and integrity, total body water, extracellular water, fat, fat free, lean, muscular and body cell masses.

Pedigree structure

Thanks to the availability of complete municipal and parish archives going back to the seventeenth century, it was possible to recreate deep rooted genealogical trees (up to 20 generations) to connect all people living in the villages into large families with common ancestors. Using the software PedNavigator [12], the 6056 individuals in this study sample were included in 43 extended pedigrees (mean size, 675.6). We computed pair statistics running Pedstats [13]. Overall, the pedigrees count 29050 members, 2454 parent-offspring pairs, 3229 siblings, 307 grandparent–grandchild pairs, 5296 avunculars, 71 half-siblings and 10650 first cousins. Although our quantitative genetic analysis only include fully phenotyped individuals, it is best to use the largest possible pedigree for the computation of kinship and IBD matrices because genetic information is lost when pedigrees are broken up; it is typical that ancestors and founders are not phenotyped, but there is still great benefit from including them in the pedigree.

Statistical analysis

Statistical analyses of non-genetic factors were performed by using SPSS 13.0 (SPSS Inc. Chicago). The root mean square coefficient of

variation (RMS CV%) for intra-operator and inter-operator precision were determined as:

$$\text{RMS CV\%} = \left(\frac{\text{SD}}{\sum_{i=1}^{m} x_j / m} \right) \cdot 100\%$$

where x_j is the mean value obtained for each subject j and m the number of subjects. The standard deviation (SD) was calculated according to the equation:

$$\text{SD} = \sqrt{\sum_{i=1}^{m} \text{SD}_j^2 / m}$$

First, correlation analysis was used to assess the linear relationship between QUS parameters and all the quantitative variables we collected data on. Subsequently, generalized linear regression was employed to find best models explaining QUS data putting all variables we collected data on as covariates. The genetic analysis of QUS parameters was performed using a standard quantitative genetic variance-components model implemented in the software Sequential Oligogenic Linkage Analysis Routines (SOLAR version 4.1.0) [14]. SOLAR is designed for incorporating all available information of extended pedigrees in parameters estimation. Univariate heritability, the proportion of phenotypic variance attributable to additive genetic effects was estimated for each QUS measure after accounting for all covariates whose effect was significant at the $p \leq 0.05$ level. The significance of heritability estimates was tested by comparing the likelihoods of nested models using the likelihood ratio test. A power analysis was performed and indicated that we have over 90% power to detect a heritability estimate of at least 0.20. Bivariate quantitative genetic analyses were conducted to determine the extent of genetic and environmental covariation among QUS measures and to investigate how much of the genetic component is shared between traits and how much is trait specific. The phenotypic correlation between two traits is partitioned into additive genetic and random environmental components as given in the equation:

$$\rho_P = \rho_G \sqrt{h_1^2} \sqrt{h_2^2} + \rho_E \sqrt{1 - h_1^2} \sqrt{1 - h_2^2}$$

where ρ_G is the genetic correlation, ρ_E is the environmental correlation between trait pairs and h_1^2 and h_2^2 are the heritabilities of trait 1 and trait 2, respectively [15]. Additive genetic correlations can range from −1 to 1, where a value of 1 indicates complete positive pleiotropy (the same genes are affecting the two traits in the same manner), a value of zero between the traits indicates that different genes influence them and a value of −1 indicates complete negative pleiotropy (genes acting to increase the value of one trait decrease the value of the other one). A genetic correlation significantly different from both 0 and 1 (or −1) indicates incomplete pleiotropy, meaning that the two traits are influenced to some extent by the same genes or sets of genes but that each trait also has a specific genetic basis. Finally, bivariate heritability, which is a measure of the extent to which shared genetic influences generates a correlation between two traits, is calculated as a function of the two univariate heritabilities and the genetic correlation [16].

Results

Characteristics of sample cohort

Table 1 shows characteristics of the study participants by sex. No difference in age distribution was observed between males and females; lower average values of AD-SoS, BTT, SDy and UBPI and higher values of FWA were observed in females ($p<0.0001$).

Precision was assessed for all 9 operators and RMS CV% ranges were 0.25–0.82 for AD-SoS, 0.0–3.0 for BTT, 0.9–8.9 for FWA, 0.0–35.3 for SDy and 1.23–3.20 for UBPI, whereas inter-operator precision was 0.91 for AD-SoS, 1.6 for BTT, 3.1 for FWA, 10.2 for SDy and 2.25 for UBPI.

Analysis of QUS determinants

With increasing age, a decreasing trend of all QUS measures in both females and males was observed; in particular, in women we noted a steeper decrease subsequently to the average menopausal age, which in our sample is 49.2 ± 4.6 years (Fig. 1).

Results of correlation analysis by sex are shown in Table 2. All QUS parameters except SDy were moderately to strongly correlated to anthropometric and bioimpedance measures: correlation with anthropometric variables was stronger in females than in males, whereas correlation with some of the bioimpedance variables (body cellular and muscular mass, extracellular water and phase angle) was stronger in males. Taller subjects show greater values of QUS parameters, while BMI, waist, hip and WHR are negatively correlated with QUS parameters. Lean, muscular and body cell masses, total body water and phase angle values showed a positive correlation with all QUS measures while fat mass and extracellular water a negative one.

Among blood serum parameters ALP showed a weak inverse relationship with QUS measurements; SCa and S-phosphorus had negligible correlations, even if occasionally statistically significant. Age at menarche, number of pregnancies and months since menopause were negatively correlated to QUS measures.

After evaluating simple correlations, we constructed multiple generalized models for all QUS measures considered as independent variables. Best models for all QUS parameters included sex, age, months since menopause (the variable "months since menopause" equals to zero in men and premenopausal women), fat mass, ALP and S-phosphorus. Further explanatory variables for AD-SoS were BMI, fat free, muscular and body cellular masses, exercise, spirits consumption, epilepsy, gastrointestinal disease and steroids assumption. Whereas for UBPI we found phase angle, age at menarche and steroids assumption as further predictors. As regards to the three parameters related to QUS attenuation, additional explanatory variables were muscular and body cellular masses, phase angle, waist circumference, exercise and epilepsy for BTT; BMI, muscular, fat free and body cellular masses, phase angle, waist circumference, age at menarche and number of pregnancies for FWA and finally number of pregnancies and age at menarche for SDy. On the whole, determination coefficient (adjusted R^2) of the best models fitting QUS data ranged from 40.5% for SDy to 63.5% for UBPI.

Quantitative genetic analysis

For estimating the unobserved genetic contribution to variation in QUS traits, we ran variance component models on 43 extended pedigrees adjusting for the related covariates (age, sex, months since menopause, BMI, fat and muscular mass, exercise, ALP and S-phosphorus). All QUS measures but SDy were normally distributed; to reduce excessive kurtosis of SDy, a modulus transformation was applied [17]. We performed separate heritability analyses for each QUS parameter in the 10 villages because each village represents a genetic isolate having a peculiar founder population with almost no migration or exchanges with all the others for many centuries up to 30 years ago. As sex may be a strong covariate, we preliminarily performed a sex-specific heritability analysis on QUS measurements (data not shown); results we obtained were not significantly different between men and women, so that we pooled together males and females in subsequent analysis. Heritability estimates for QUS measures varied from 23.8% (Talana) to 49.9% (Urzulei) for AD-SoS, from 33.0% (Seui) to 68.7% (Escalaplano) for BTT, from 35.7% (Loceri)

Table 1
Characteristics of the sample cohort (4166 women and 1890 men).

	Women				Men			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Age, years	55.76	14.78	30.00	99.00	55.06	15.12	30.00	103.0
Height, cm	151.8[a]	6.70	120.0	175.0	164.3[a]	7.21	120.0	188.5
Weight, kg	59.82[a]	11.50	32.00	133.0	72.72[a]	11.87	39.00	134.0
BMI, kg/m^2	26.00[a]	4.96	14.30	51.26	26.91[a]	3.91	16.42	48.91
Wrist, cm	16.06[a]	.95	12.77	21.00	17.65[a]	0.98	14.00	21.90
Waist, cm	87.22[a]	13.30	52.67	146.5	95.17[a]	10.38	62.00	135.7
Hip, cm	99.91[b]	10.33	65.67	151.3	100.7[b]	7.90	78.33	152.0
WHR	0.87[a]	0.09	0.62	1.40	0.94[a]	0.06	0.60	1.16
Menarche, years	13.70	1.83	8.00	25.00	–	–	–	–
No. pregnancies	2.33	2.04	0	13.00	–	–	–	–
Age at menopause, years	49.22	4.68	20.00	66.00	–	–	–	–
Months since menopause	200.1	124.2	12.00	660.0	–	–	–	–
Lean mass, kg	40.57[a]	5.34	24.30	134.4	56.35[a]	8.25	30.30	94.20
Cellular mass, kg	23.34[a]	5.35	7.20	205.9	31.49[a]	6.15	12.70	55.90
Fat Mass, kg	18.95[a]	8.27	0	64.40	16.51[a]	6.76	0.10	45.70
Muscular mass, kg	28.67[a]	6.08	10.60	234.1	38.60[a]	7.05	16.50	67.70
Total body water, L	30.68[a]	3.64	19.50	98.40	42.49[a]	5.66	24.30	69.00
Extracellular water, L	14.32[a]	1.89	0.80	24.90	18.77[a]	2.75	12.00	33.00
Phase angle	5.83[a]	0.79	2.5	11.6	6.39[a]	0.95	3.10	9.20
SCa, mg/dL	9.13	0.51	4.55	12.25	9.14	0.42	7.77	12.25
S-phosphorus, mg/dL	3.10[a]	0.48	0	6.90	2.91[a]	0.51	0	6.44
ALP, U/L	161.3	70.09	34.00	959.3	158.8	66.14	39.00	759.5
AD-SoS, m/s	2010[a]	113	1584	2313	2047[a]	86	1676	2258
BTT, μs	1.35[a]	0.26	0	2.20	1.75[a]	0.25	0	2.70
FWA, mV	2.64[a]	0.74	0	6.77	2.47[a]	0.55	0	5.23
SDy, mV/μs^2	−304.7	516	−1166	20774	−290.7	951.9	−1173	39928
UBPI	0.56[a]	0.24	0.03	1.00	0.63[a]	0.18	0.06	1.00

[a] Mean comparison *t*-test ($p < 0.00001$).
[b] Mean comparison *t*-test ($p < 0.05$).

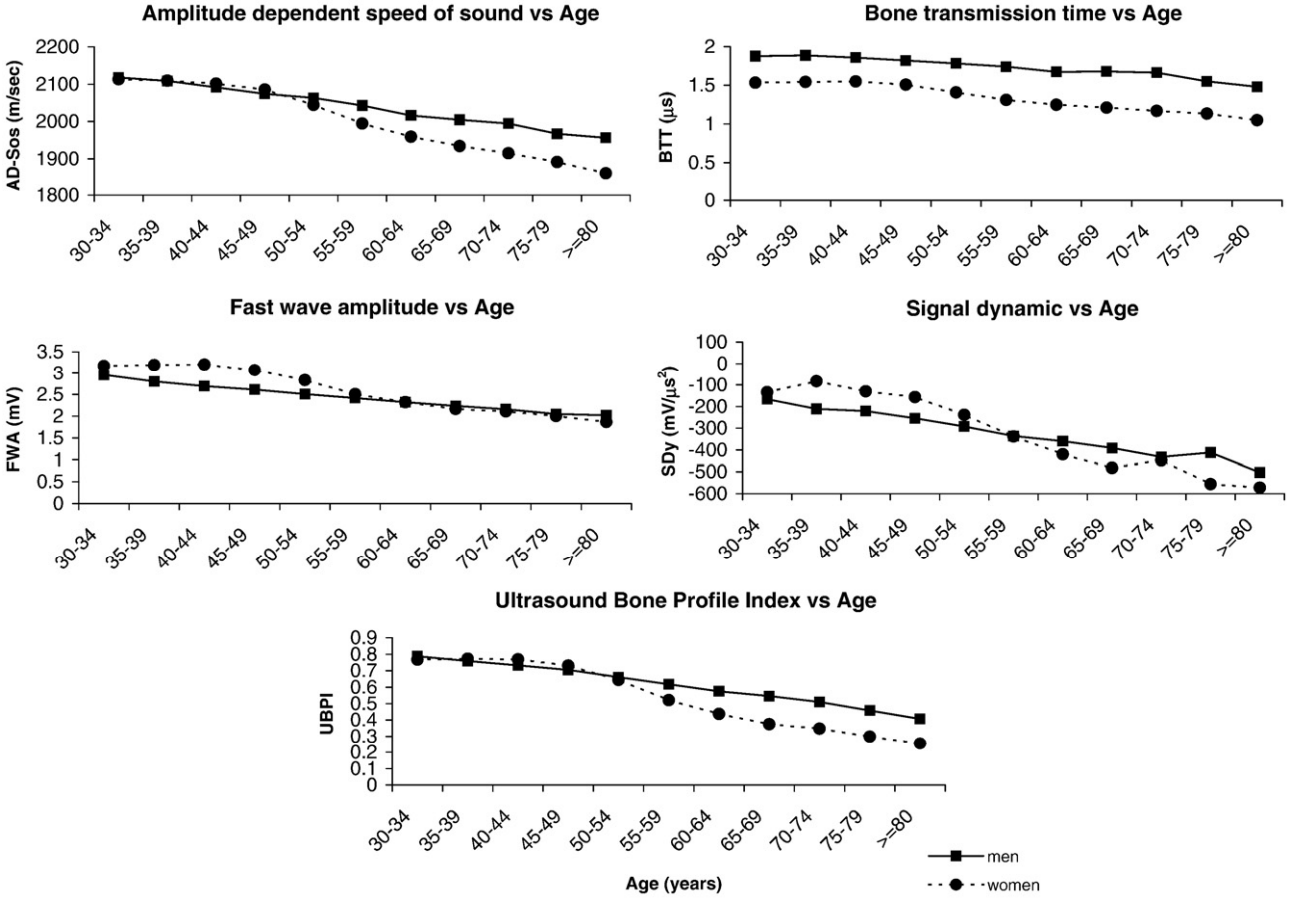

Fig. 1. Trends of QUS parameters by sex and age in Ogliastra.

Table 2
Correlation analysis of QUS parameters by sex.[a]

Variable	Women					Men				
	AD-SoS	BTT	FWA	SDy	UBPI	AD-SoS	BTT	FWA	SDy	UBPI
Age, years	−0.75[a]	−0.65[a]	−0.64[a]	−0.32[a]	−0.78[a]	−0.59[a]	−0.47[a]	−0.51[a]	−0.11[a]	−0.64[a]
Height, cm	0.42[a]	0.50[a]	0.26[a]	0.13[a]	0.39[a]	0.35[a]	0.42[a]	0.22[a]	0.07	0.36[a]
Weight, kg	−0.18[a]	−0.04	−0.13[a]	−0.03	−0.10[a]	−0.13[a]	0.09[a]	−0.01	−0.01	0.05
BMI, kg/m^2	−0.37[a]	−0.27[a]	−0.25[a]	−0.09[a]	−0.28[a]	−0.35[a]	−0.15[a]	−0.13[a]	−0.04	−0.15[a]
Wrist, cm	−0.28[a]	−0.10[a]	−0.22[a]	−0.21[a]	−0.22[a]	−0.24[a]	0.09	−0.19[a]	−0.04	−0.11[a]
Waist, cm	−0.45[a]	−0.35[a]	−0.30[a]	−0.31[a]	−0.37[a]	−0.40[a]	−0.19[a]	−0.22[a]	−0.04	−0.23[a]
Hip, cm	−0.36[a]	−0.25[a]	−0.24[a]	−0.23[a]	−0.28[a]	−0.29[a]	−0.11[a]	−0.14[a]	−0.04	−0.14[a]
WHR	−0.32[a]	−0.27[a]	−0.22[a]	−0.24[a]	−0.28[a]	−0.32[a]	−0.20[a]	−0.20[a]	−0.01	−0.23[a]
Menarche, years	−0.29[a]	−0.25[a]	−0.29[a]	−0.13[a]	−0.34[a]					
No. pregnancies	−0.33[a]	−0.24[a]	−0.30[a]	−0.14[a]	−0.34[a]					
Age at menopause, years	0.01	−0.01	−0.01	0.01	0.00					
Months since menopause	−0.69[a]	−0.59[a]	−0.58[a]	−0.29[a]	−0.72[a]					
Lean mass, kg	0.25[a]	0.39[a]	0.19[a]	0.10[a]	0.31[a]	0.13[a]	0.35[a]	0.12[a]	0.01	0.27[a]
Cellular mass, kg	0.17[a]	0.24[a]	0.18[a]	0.08[a]	0.25[a]	0.21[a]	0.34[a]	0.18[a]	0.03	0.33[a]
Fat mass, kg	−0.40[a]	−0.28[a]	−0.29[a]	−0.11[a]	−0.33[a]	−0.43[a]	−0.28[a]	−0.20[a]	−0.04	−0.27[a]
Muscular mass, kg	0.15[a]	0.24[a]	0.16[a]	0.08[a]	0.24[a]	0.19[a]	0.34[a]	0.17[a]	0.02	0.32[a]
Total body water, L	0.03	0.23[a]	0.00	0.02	0.08[a]	−0.02	0.27[a]	0.00	−0.01	0.12[a]
Extracellular water, L	−0.20[a]	−0.01	−0.2[a]	−0.08[a]	−0.19[a]	−0.28[a]	0.02	−0.20[a]	−0.05	−0.17[a]
Phase angle	0.36[a]	0.32[a]	0.33[a]	0.15[a]	0.41[a]	0.40[a]	0.35[a]	0.31[a]	0.07	0.45[a]
SCa, mg/dL	−0.09[a]	−0.11 [a]	−0.06[a]	−0.04	−0.09[a]	0.04	0.02	0.00	0.01	0.02
S-phosphorus, mg/dL	−0.04	−0.06 [a]	−0.03	−0.02	−0.05	0.07	0.02	0.09[a]	0.02	0.08
ALP, U/L	−0.31[a]	−0.26 [a]	−0.23[a]	−0.13[a]	−0.28[a]	−0.14[a]	−0.05	−0.13[a]	−0.01	−0.13[a]

[a] Significantly different from zero at the $p < 0.00025$ level.

to 67.8% (Baunei) for FWA, from 14.5% (Urzulei) to 42.9% (Baunei) for SDy and from 27.6% (Talana) to 62% (Baunei) for UBPI. They were not significantly different among villages so that we also carried out a further analysis pooling together pedigrees of all the villages. In Table 3, the calculation of the global heritability obtained for each QUS parameter when taking into account all the investigated population is reported: 40.9% for Ad-SoS, 54.1% for BTT, 54.7% for FWA, 28.7% for SDy and 51.6% for UBPI. The proportion of the phenotypic variance explained by covariates ranges from 35.8% for SDy to 59.1% for BTT.

Bivariate genetic analyses of QUS measures are presented in Table 4. Six out of 10 genetic correlations between pairs of QUS traits were significantly greater than zero and less than one, indicating incomplete pleiotropy ($\rho_G \neq 1$, $p < 0.0001$; $\rho_G \neq 0$, $p < 0.005$); genetic correlations of BTT with FWA and SDy were not different from zero, showing complete genetic independence; while genetic correlations between FWA and SDy, and between SDy and UBPI were not different from −1, indicating a state of complete negative pleiotropy. The environmental correlations of all but two pairs of QUS measures were greater than zero ($\rho_E \neq 0$, $p < 0.00001$). Finally, bivariate heritabilities of QUS traits pairs ranged from 2% (BTT–SDy) to 50% (FWA–UBPI), revealing varying degrees of genetic influences shared between pairs of QUS measures which are responsible for their phenotypic correlations.

Discussion

During the last decade, QUS applied to bone have been shown to provide more and different information on the physical properties of bone tissue, as compared to conventional X-ray-based techniques [18,19]. Bone density, structure and elasticity affect ultrasound transmission modulating velocity, absorption, scattering and signal characteristics [20,21]. Investigating such structural properties could be important for characterizing osteoporosis risk.

Table 3
Heritability of QUS measures in the whole investigated population.

Trait	Heritability	*P*-value	% explained by covariates
AD-SoS	0.41	1.57e−68	53.6
BTT	0.54	2.96e−101	59.1
FWA	0.55	1.62e−106	38.9
SDy	0.29	8.69e−34	35.8
UBPI	0.52	2.56e−94	58.9

In this study, genetic and environmental factors influencing bone tissue evaluated by QUS at the phalanges have been examined in genetic isolates of Ogliastra, a secluded area of Sardinia. The peculiar characteristics of high consanguinity and homogeneity in life style and eating habits found in these populations represent a unique favorable condition for reducing background variability, otherwise present in other studies.

Several studies have been performed on twins or nuclear families investigating the genetic and environmental contribution to the association between QUS and bone mineral density [8,22] or among calcaneal QUS phenotypes [23]. To our knowledge, this is the first study conducted on a large sample of healthy people spanning a wide range of ages to evaluate sources of variation of phalangeal QUS measures.

In our study, five QUS parameters were analyzed. Each one is supposed to carry out different information on bone structure properties: AD-SoS being related mostly to density; UBPI to bone fragility; and the other parameters of QUS attenuation to porosity, distribution and orientation of trabeculae [24–26].

As already noticed, SDy precision is worse than that of the other parameters [27]. SDy poor reproducibility likely relies on its mathematical definition, being a second derivative of amplitude versus time of the first two peaks of the ultrasound signal and ranging

Table 4
Bivariate genetic analysis of QUS measures.[a]

Traits	Phenotypic correlation	Bivariate heritability	Genetic correlation (SE)	Environmental correlation (SE)
AD-SoS–BTT	0.70[b]	0.30	0.63 (0.030)[c]	0.58 (0.024)[b]
AD-SoS–FWA	0.64[b]	0.28	0.59 (0.057)[c]	0.34 (0.035)[b]
AD-SoS–SDy	−0.58[b]	0.27	−0.78 (0.081)[c]	−0.14 (0.030)[b]
AD-SoS–UBPI	0.86[b]	0.38	0.83 (0.033)[c]	0.61 (0.023)[b]
BTT–FWA	0.18[b]	0.08	−0.14 (0.061)	−0.05 (0.046)
BTT–SDy	−0.21[b]	0.02	0.056 (0.060)	0.084 (0.035)
BTT–UBPI	0.57[b]	0.13	0.24 (0.058)[c]	0.30 (0.040)[b]
FWA–SDy	−0.78[b]	0.38	−0.94 (0.034)	−0.50 (0.025)[b]
FWA–UBPI	0.87[b]	0.50	0.93 (0.011)[c]	0.79 (0.014)[b]
SDy–UBPI	−0.79	0.38	−0.97 (0.029)	−0.54 (0.022)[b]

[a] Correlations are adjusted for age, sex and month since menopause.
[b] Parameter significantly different from zero $p < 0.00001$.
[c] Parameter significantly different from zero ($p < 0.0001$) and one ($p < 0.005$).

from minus infinity to plus infinity, assuming therefore negative and positive values in a non-linear fashion.

Higher values of FWA we noted in females may be due to their thinner phalanges: in men, who generally have thicker phalanges, the ultrasound signal is attenuated. The progressive decrease we observed in mean values of QUS parameters is related to ageing in both sexes and to menopause in females, as already noted in several studies [28,29]. Negative impact of weight, BMI and fat mass on QUS parameters can be ascribed to the effect of soft tissue, which in QUS measurements have been shown to lead to an underestimation of the velocity of transmission of the ultrasound pulse [1,30]. This observation has been made by other authors for ultrasound velocity only, whereas in our study we found negative association among BMI and all ultrasound parameters, even if AD-SoS revealed the highest correlation coefficient. To our knowledge, this is the first report on the association of several QUS parameters and BMI; this result can be ascribed to the different impact of soft tissue in ultrasound pulse transmission with respect to bone tissue alone. Regarding bioimpedance analysis parameters high fractions of lean, muscular and body cell masses and high values of phase angle seem to be protective factors, whereas fat mass and extracellular water appear as risk factors for osteoporosis. Laboratory data on blood serum parameters indicate a negative relationship of all investigated measures with ALP, pointing out an independent influence of such variable on QUS parameters. Similar results have been already obtained with phalangeal QUS measurement in different populations, such as dialyzed patients [31,32].

Reproductive and menopause history influence bone tissue in females: as expected, a marked decrease of all QUS parameters is observed with increasing months since menopause, even after age-adjusting; furthermore, an interesting influence of number of pregnancies and of age at menarche on AD-SoS, FWA and UBPI is observed. Late menarche as well as maternity and lactation have been already observed as a risk factor for low bone mass [33] even if some contrasting results have been reported [34]. The changes in bone tissue due to these factors, being clearly picked up by QUS parameters, may be more related to bone structure and geometrical distribution of the bone material rather than to bone density alone, but no studies can confirm this hypothesis.

Our data suggest that physical and sport activity play a positive role in preserving bone tissue, as highlighted by other researchers [5,35], whereas wine and spirit consumption revealed a weak negative effect on QUS parameters [36,37].

QUS phenotypes showed a considerable heritable component, ranging from 0.29 for SDy to 0.55 for FWA. The only study which assessed heritability of phalangeal QUS measures was performed on twins, but on the single speed of sound parameter, getting an heritability estimate of 0.74 [8]. The estimate of AD-SoS we obtained was lower (0.41), but twin designs can lead to an overestimation of the degree of genetic determination that can occur as a result of any greater sharing of environmental factors in monozygotic rather than in dizygotic twins. Furthermore, contrarily to other studies, all the covariates we considered in the variance component models accounted for a large portion of the phenotypic variance (from 36% to 59%), thus the indices of heritability we obtained are not inflated by their effect.

Genetic correlation analyses of QUS measures reveal that several traits share a common genetic basis while other traits show genetic independence from one another. Pairwise genetic correlations with AD-SoS range from −0.78 to 0.83, indicating that some loci for these traits pairs are either the same or closely linked. On the other hand, the incomplete pleiotropy observed between such measures suggests the existence of trait specific sets of genes controlling the single QUS measures. Similarly, they are influenced to a great extent by the same environmental factors, still preserving partially unique environmental determinants.

UBPI, which is supposed to best distinguish fractured from non-fractured subjects, represents the mathematical combination of three parameters of the graphic trace (SDy, FWA and BTT), for that reason it is expected that all pairwise phenotypic correlations with UBPI be from strong to very strong. Nonetheless from the genetic correlation analysis emerge that each trait preserve its own peculiarity, as shown by the wide range of trait pairs genetic correlations (from −0.97 to 0.24) and by the variability of their shared genetic influences (from 13% to 50%).

Interestingly between FWA and SDy, there is a complete negative pleiotropy, meaning that the same genes are affecting the two traits but in an opposite manner, so that while acting to increase the value of one trait they decrease the value of the other one, whereas between BTT and SDy and between BTT and FWA, phenotypic correlations are negligible and both environmental and genetic correlations are about zero, meaning that they are uncorrelated and different sets of genes influence them since they are all significantly heritable. Several studies demonstrated that AD-SOS correlates with cortical area, density and with the moment of inertia of the bone itself that BTT correlates with cortical area and with the moment of inertia while FWA correlates with the area of the medullary canal and with density [2,38–40]. The observed phenotypic correlations seem to reflect this relationships with a high correlation of AD-SoS-BTT and AD-SoS-FWA versus a negligible correlation of BTT–FWA.

There are some limitations that need to be acknowledged regarding the present study. One limitation is that in three of the 10 villages, we have not carried out quantitative ultrasonography on male population; nevertheless, data collected in the other villages allowed us to have a sufficiently large sample to perform an adequate description of QUS measures in men. Another limitation has to do with the lack of data on some important environmental factors related to bone tissue, like calcium intake, for example. Furthermore, the multigenerational structure of a pedigree could make it difficult to dissect the effect of environmental factors. Nevertheless, up till 50 years ago the villages we are studying were very isolated, so that the environment, nutrition and lifestyles were likely very homogeneous within and between villages; moreover, participants in the study are older than 30 years, so that the period of time influenced by a reduced environmental homogeneity is relatively short. An additional limitation is that the study population is rather isolated, and this could weaken the external validity of this study. Even so, once the 10 villages, that may be considered as 10 genetic isolates [41], are pooled together, they become more similar to an outbred population, thus legitimizing the generalization of results. Finally, in our study we did not consider the biomechanical aspects related to bone tissue, and we did not collect information on this aspect and therefore their relationships with QUS parameters.

In conclusion, this study highlights that risk factors usually associated to DXA-BMD and calcaneal QUS are also associated to phalangeal QUS parameters; AD-SoS is confirmed to be heritable as well as the other parameters of the graphic trace whose heritability was assessed for the first time; and finally this study also shows that QUS measures are under the influence of common as well as distinct sets of genetic factors, corroborating the hypothesis that they measure different properties of bone quality.

The substantial genetic component of QUS traits underlined by the heritability analysis and the advantageous characteristics of the study population are promising starting points in the search for bone genes through linkage analysis and genome-wide association.

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References

[1] Cadossi R, de Terlizzi F, Canè V, Fini M, Wuster C. Assessment of bone architecture with ultrasonometry: experimental and clinical experience. Horm Res 2000;54 (S1):9–18.
[2] Sakata S, Barkmann R, Lochmueller EM, Heller M, Gluer CC. Assessing bone status beyond BMD: evaluation of bone geometry and porosity by quantitative ultrasound of human finger phalanges. J Bone Miner Res 2004;19:924–30.
[3] Cavani F, Giavaresi G, Fini M, Bertoni L, de Terlizzi F, Barkmann R, et al. Influence of density, elasticity, and structure on ultrasound transmission through trabecular bone cylinders. IEEE Trans Ultrason Ferroelectr Freq Control 2008;55:1465–72.
[4] Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types of osteoporotic fracture in men and women: an observational study. Lancet 1999;353:878–82.
[5] Adami S, Giannini S, Giorgino R, Isaia G, Maggi S, Sinigaglia L, et al. The effect of age, weight, and lifestyle factors on calcaneal quantitative ultrasound: the ESOPO study. Osteoporos Int 2003;14:198–207.
[6] Lippuner K, von Overbeck J, Perrelet R, Bosshard H, Jaeger PH. Incidence and direct medical costs of hospitalizations due to osteoporotic fractures in Switzerland. Osteoporos Int 1997;7:414–25.
[7] Nguyen TV, Blangero J, Eisman JA. Genetic epidemiological approaches to the search for osteoporosis genes. J Bone Miner Res 2000;15:392–401.
[8] Howard GM, Nguyen TV, Harris M, Kelly PJ, Eiseman JA. Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. J Bone Miner Res 1998;13: 1318–27.
[9] Wright AF, Carothers AD, Pirastu M. Population choice in mapping genes for complex diseases. Nat Genet 1999;23:397–404.
[10] Varilo T, Peltonen L. Isolates and their potential use in complex gene mapping efforts. Curr Opin Genet Dev 2004;14:316–23.
[11] Cappello N, Rendine S, Griffo R, Mameli GE, Succa V, Vona G, et al. Genetic analysis of Sardinia: I. data on 12 polymorphisms in 21 linguistic domains. Ann Hum Genet 1996;60:125–41.
[12] Mancosu G, Cosso M, Marras F, Cappio Borlino C, Ledda G, Manias T, et al. Browsing isolated population data. BMC Bioinformatics 2005;6(Suppl 4):S17.
[13] Wigginton JE, Abecasis GR. PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 2005;21:3445–7.
[14] Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Gen 1998;62:1198–211.
[15] Lynch M, Walsh B. Genetics and Analysis of Quantitative Traits. Sunderland, MA: Sinauer; 1998. p. 638.
[16] Purcell S. Statistical methods in behavioural genetics. In: Plomin R, DeFries JC, McClearn McGuffin P, editors. Behavioural Genetics. New York: Worth; 2008. p. 397.
[17] John JA, Draper NR. An alternative family of transformations. Appl Stat 1980;29: 190–7.
[18] Hans D, Wu C, Njeh CF, Zhao S, Augat P, Newitt D, et al. Ultrasound velocity of trabecular bones reflects mainly bone density and elasticity. Calcif Tissue Int 1999;64:18–23.
[19] Gluer CC, for the International Quantitative Ultrasound Consensus Group. Quantitative ultrasound techniques for the assessment of osteoporosis: expert agreement on current status. J Bone Miner Res 1997;12:1280–8.
[20] Barkmann R, Gluer CC. Continuous amplitude dependent speed of sound: a new parameter improving the precision of phalanx ultrasound measurements. Bone 1998;23(Suppl) 5T496.
[21] Katz JL, Meunier A. The elastic anisotropy of bone. J Biomech 1987;20:1063–70.
[22] Deng FY, Lei SF, Li MX, Jiang C, Dvornyk V, Deng HW. Genetic determination and correlation of body mass index and bone mineral density at the spine and hip in Chinese Han ethnicity. Osteoporos Int 2006;17:119–24.
[23] Lee M, Czerwinski SA, Choh AC, Towne B, Demerath EW, Chumlea WC, et al. Heritability of calcaneal quantitative ultrasound measures in healthy adults from the Fels Longitudinal Study. Bone 2004;35:1157–63.
[24] Gluer CC, Wu CY, Genant HK. Broadband ultrasound attenuation signals depend on trabecular orientation: an in vitro study. Osteoporos Int 1993;3:185–91.
[25] Gluer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int 1994;55:46–52.
[26] Langton CM, Njeh CF, Hodgskinson R, Currey JD. Prediction of mechanical properties of the human calcaneus by broadband ultrasound attenuation. Bone 1996;18(6):495–503.
[27] Montagnani A, Gonnelli S, Cepollaro C, Mangeri M, Monaco R, Bruni D, et al. Quantitative ultrasound at the phalanges in healthy Italian men. Osteoporos Int 2000;11:499–504.
[28] Wuster C, Albanese C, De Aloysio D, Duboeuf F, Gambacciani M, Gonnelli S, et al. Phalangeal osteosonogrammetry study: age-related changes, diagnostic sensitivity, and discrimination power. J Bone Miner Res 2000;15:1603–14.
[29] Gluer CC, Eastell R, Reid DM, Felsenberg D, Roux C, Barkmann R, et al. Association of five quantitative ultrasound devices and bone densitometry with osteoporotic vertebral fractures in a population-based sample: the OPUS study. J Bone Miner Res 2004;19:782–93.
[30] Coin A, Perissinotto E, Enzi G, Zamboni M, Inelmen EM, Frigo AC, et al. Predictors of low bone mineral density in the elderly: the role of dietary intake, nutritional status and sarcopenia. Eur J Clin Nutr Jun 2008;62(6):802–9.
[31] Montagnani A, Gonnelli S, Cepollaro C, Martini S, Finato V, Di Paolo N, et al. Quantitative ultrasound in the assessment of skeletal status in uremic patients. J Clin Densitom 1999;2:389–95.
[32] Przedlacki J, Pluskiewicz W, Wieliczko M, Drozdzowska B, Matuszkiewicz-Rowińska J, Bogdańska-Straszyńska B, et al. Quantitative ultrasound of phalanges and dual-energy X-ray absorptiometry of forearm and hand in patients with end-stage renal failure treated with dialysis. Osteoporos Int 1999;10:1–6.
[33] Cooper GS, Sandler DP. Long-term effects of reproductive-age menstrual cycle patterns on peri- and postmenopausal fracture risk. Am J Epidemiol 1997;145: 804–9.
[34] Hassa H, Tanir HM, Senses T, Oge T, Sahin-Mutlu F. Related factors in bone mineral density of lumbal and femur in natural postmenopausal women. Arch Gynecol Obstet 2005;273:86–9.
[35] Farahmand BY, Persson PG, Michaelsson K, Baron JA, Alberts A, Moradi T, et al. Physical activity and hip fracture: a population-.based case-control study. Int J Epidemiol 2000;29:308–14.
[36] Lunt M, Masaryk P, Scheidt-Nave C, Nijs J, Poor G, Pols H, et al. The effects of lifestyle, dietary dairy intake and diabetes on bone density and vertebral deformity prevalence: the EVOS study. Osteoporos Int 2001;12:688–98.
[37] Stearns AT, Jaberoo MC, Ashraf R, Wheelwright EF, Maclean AD. Displaced intracapuslar hip fractures in the working age alcohol-abusing patient. Scott Med J 2009;54:16–20.
[38] Barkmann R, Lusse S, Stampa B, Sakata S, Heller M, Glüer CC. Assessment of the geometry of human finger phalanges using quantitative ultrasound in vivo. Osteoporos Int 2000;11:745–55.
[39] de Terlizzi F, Battista S, Cavani F, Canè V, Cadossi R. Influence of bone tissue density and elasticity on ultrasound propagation: an in vitro study. J Bone Min Res 2000;15:2458–66.
[40] Wuster C, de Terlizzi F, Becker S, Cadossi M, Cadossi R, Müller R. Usefulness of quantitative ultrasound in evaluating structural and mechanical properties of bone: comparison of ultrasound, dual-energy X-ray absorptiometry, micro-computed tomography, and mechanical testing of human phalanges in vitro. Technol Health Care 2005;13:497–510.
[41] Pistis G, Piras I, Pirastu N, Persico I, Sassu A, Picciau A, et al. High differentiation among eight villages in a secluded area of Sardinia revealed by genome-wide high density SNPs analysis. PLoS One 2009;4(2):e4654.