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Effects of the interaction between lean tissue mass and estrogen receptor α gene polymorphism on bone mineral density in middle-aged and elderly Japanese

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Abstract

Because both genetic and environmental factors influence bone mass, it is important to examine the effect of gene-environment interactions on bone mineral density (BMD) for the prevention of osteoporosis at an individual level. Estrogen receptor α (ERα) plays an important role in increasing BMD via mechanical strain and muscle mass is a reflection of the forces the muscle applies to the bone. The aim of this study is to investigate the effect of the interaction between lean tissue mass (LTM) and the ERα polymorphisms T→C (*PvuII*) [dbSNP: rs2234693] and A→G (*XbaI*) [dbSNP: rs9340799] on BMD in middle-aged and elderly individuals. Subjects were 2209 community-dwelling Japanese men and women, ages 40 to 79 years. ERα polymorphisms in the first intron, T→C and A→G were identified and lumbar spine and femoral neck BMD and LTM were measured by dual-energy X-ray absorptiometry. Both T→C and A→G polymorphisms were divided into two genotype groups (TT vs. TC/CC; AA vs. AG/GG). In postmenopausal women, the effect of LTM on femoral neck BMD was significantly larger for those with the TC/CC genotype than for those with the TT genotype for the T→C polymorphism, and larger for those with the AG/GG genotype than for those with the AA genotype for the A→G polymorphism. This gene–LTM interaction was observed at the femoral neck, but not at the lumbar spine. For men and premenopausal women, no gene–LTM interaction was found. In conclusion, there was an interaction between LTM and the ERα T→C and A→G polymorphisms with respect to their effect on femoral neck BMD in postmenopausal women and those with the TC/CC and AG/GG genotypes had larger effects of LTM than those with TT and AA genotypes.

Keywords: Single nucleotide polymorphism; Estrogen receptor alpha; BMD; Lean tissue mass; Postmenopausal women

Introduction

It is generally accepted that dynamic loading acts as an osteogenic stimulus [1] and that the forces applied to bone are primarily the result of muscular contraction [2]. Therefore, muscular weakness is an important factor contributing to osteoporosis [3]. The importance of skeletal muscle in preserving bone [4] and the relation between low skeletal mass and poor structural parameters of bone in elderly men [5] have been reported. A previous study suggested that physical exercise maintains bone mineral density (BMD) in postmenopausal women [6]. Vainionpaa et al. showed that the intensity of exercise was significantly correlated with BMD changes [7] and Kerr et al. reported that postmenopausal bone mass can be significantly increased by strength training, but not by endurance training [8].

Animal studies have suggested that mechanical strain stimulates osteoblast proliferation through estrogen receptor α (ERα) [9], and osteoblast-like cells from ERα knockout mice have deficient responses to mechanical strain [10]. Thus, it is thought that ERα plays an important role in increasing BMD via mechanical strain [11,12]. Although the association between ERα genotype and the risk of osteoporosis in humans remains controversial [13], many studies have suggested a

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relation between ERα polymorphism and BMD [14–16]. A study previously carried out in our laboratory also showed that the ERα gene was a susceptibility locus for reduced bone mass, especially at the femoral neck, in elderly Japanese women [17].

Because the effects of environment on individuals might differ in accordance with individuals' different genetic make-ups, it is important to examine the effects of the gene-environment interaction on BMD, particularly for the prevention of osteoporosis at an individual level. Some studies have investigated the effect of ERα polymorphism on the relationship between exercise and BMD. These studies have shown an effect of the ERα gene (*Pvu*II)–exercise interaction on BMD in middle-aged men [18] and prepubertal and early pubertal girls [19].

Because magnetic resonance imaging (MRI)-measured muscle area correlates with muscle strength [20], and the differences between MRI-measured and dual-energy X-ray absorptiometry (DXA)-predicted skeletal muscle mass are small [21], DXA-predicted total body lean mass can be legitimately used as an index of skeletal load. As mentioned above, a few studies have investigated the effects of the ERα gene–exercise interaction on BMD. However, the effects of the ERα gene–lean tissue mass (LTM) interaction were unknown. Furthermore, these previous studies involved single-sex populations within a limited age range. This study investigated for the first time the effects of the interaction between LTM and the typical ERα polymorphisms T→C (*Pvu*II) and A→G (*Xba*I) on BMD in both men and women in a large population.

Materials and methods

Subjects

Study subjects were 1119 men and 1090 women, ages 40–79 years, who participated in the first wave (from April 1998 to March 2000) of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), which is a population-based prospective cohort study of aging and age-related diseases. Participants in the NILS-LSA were randomly selected age and sex stratified individuals selected from the pool of independent residents in the NILS neighborhood, Obu city and Higashiura town, Aichi Prefecture, central Japan. Details of the NILS-LSA have been given elsewhere [22]. The study protocol was approved by the Committee of Ethics of Human Research of the National Center for Geriatrics and Gerontology. Written informed consent was obtained from all subjects.

Anthropometric variables

Body weight was measured to the nearest 0.01 kg using digital scales, height was measured to the nearest 0.1 cm using a stadiometer, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Menstrual status

Menopause was confirmed as the absence of menses by a questionnaire.

Dual-energy X-ray absorptiometry

Whole-body fat mass, LTM, bone mineral content (BMC), and BMD of the femoral neck and lumbar spine (L2–4) were assessed by DXA (QDR-4500; Hologic, Madison, OH, USA). Lean tissue mass is equal to the fat-free mass minus BMC, and is assumed to be an index of the amount of muscle mass.

ERα genotype analysis

DNA was extracted from peripheral blood lymphocytes by using a standard procedure. ERα genotypes were determined in accordance with a study by Yamada et al. [17] The ERα genotypes were analyzed by using an automated fluorescent allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan). To determine the T→C (*Pvu*II) genotype, the polymorphic region of the gene was amplified by polymerase chain reaction (PCR) using allele-specific sense primers labeled at the 5′ end either with fluorescein isothiocyanate (5′-AGTTCCAAATGTCCCAGXTG-3′) or with Texas red (5′-AGTTCCAAATGTCCCAGXCG-3′) and an antisense primer labeled at the 5′ end with biotin (5′-TCTGGGAAACAGAGACAAAGC-3′). The reaction mixture (25 μl) contained 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgCl_2$, and 1U DNA polymerase (rTaq; Toyobo, Osaka, Japan) in rTaq buffer. The amplification protocol consisted of initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 62.5 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 2 min. For determination of the A→G (*Xba*I) genotype, the polymorphic region of the gene was amplified by PCR using a sense primer labeled at the 5′ end with biotin (5′-CTGTTTCCCA-GAGACCCTGAG-3′) and allele-specific antisense primers labeled at the 5′ ends either with fluorescein isothiocyanate (5′-CCAATGCTCAT-CCCAACTXTA-3′) or with Texas red (5′-CCAATGCTCATCCCAACTXCA-3′). The reaction mixture (with the exception of the primers) and the amplification protocol (with the exception that the annealing temperature was 65 °C) were identical to those used for genotyping of the T→C polymorphism.

Amplified DNA was incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature. The plate was placed on a magnetic stand and the supernatant was discarded. After two washings, 0.01 M NaOH was added to the wells and mixed well. The plate was placed on a magnetic stand again and the supernatants were transferred to the wells of a new 96-well plate. The fluorescence was measured by using a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 nm and 538 nm, respectively, for fluorescein isothiocyanate, and 584 nm and 612 nm, respectively, for Texas red.

Haplotype analysis

The haplotype distribution was calculated by using Haplotyper, a software program for haplotype inference, with the Bayesian algorithm [23,24].

Statistical analysis

Values are expressed as the mean±standard error (SE). The chi-squared test was used to identify significant departures from Hardy-Weinberg equilibrium. Both T→C and A→G polymorphisms were divided into two genotype groups (TT vs. TC/CC; AA vs. AG/GG). The differences between genotype groups were analyzed using one-way analysis of variance and the Tukey–Kramer post hoc test. A general linear model was employed to evaluate the effect of the LTM–genotype interaction on BMD (adjusted for age and BMI). When the effect of the interaction on BMD was significant for both T→C and A→G polymorphisms, further analysis (in accordance with haplotype groups) was

Table 1
Distribution of T→C and A→G genotypes of the ERα gene

	AA		AG		GG		Total	
	n	%	*n*	%	*n*	%	*n*	%
TT	787	35.6	1	0.1	0	0.0	788	35.7
TC	584	26.4	465	21.1	5	0.2	1054	47.7
CC	120	5.4	174	7.9	73	3.3	367	16.6
Total	1491	67.5	640	29.0	78	3.5	2209	100.0

Table 2
Physical characteristics of subjects with reference to the T→C and A→G genotypes of the ERα gene

	Men (n=1119)		Premenopausal women (n=278)		Postmenopausal women (n=812)	
	TT (n=398)	TC/CC (n=721)	TT (n=98)	TC/CC (n=180)	TT (n=292)	TC/CC (n=520)
Age (years)	58.9±0.6	59.3±0.4	46.2±0.5	46.2±0.3	62.8±0.5	64.6±0.4*
Weight (kg)	62.9±0.5	62.2±0.3	53.9±0.8	54.7±0.6	52.5±0.5	51.7±0.4
BMI (kg/m2)	23.2±0.1	22.9±0.1	22.5±0.3	22.9±0.2	23.1±0.2	23.0±0.2
LTM (kg)	47.2±0.3	46.6±0.2	36.3±0.4	36.5±0.3	33.9±0.2	33.7±0.2
L2–4 BMD (kg/cm^2)	0.99±0.01	0.98±0.01	1.03±0.01	1.02±0.01	0.82±0.01	0.80±0.01
Femoral neck BMD (g/cm^2)	0.76±0.01	0.75±0.004	0.78±0.01	0.77±0.01	0.66±0.01	0.64±0.004*
	AA (n=769)	AG/GG (n=350)	AA (n=192)	AG/GG (n=86)	AA (n=530)	AG/GG (n=282)
Age (years)	59.2±0.4	59.1±0.5	46.3±0.3	46.0±0.5	63.7±0.4	64.2±0.5
Weight (kg)	62.7±0.3	61.9±0.5	53.5±0.5	56.4±1.0	51.9±0.3	52.2±0.5
BMI (kg/m^2)	23.1±0.1	22.8±0.1	22.3±0.2	23.7±0.4**	22.9±0.1	23.3±0.2
LTM (kg)	47.0±0.2	46.5±0.3	36.1±0.3	37.0±0.5	33.8±0.2	33.7±0.2
L2–4 BMD (kg/cm^2)	0.99±0.01	0.97±0.01	1.03±0.01	1.02±0.01	0.81±0.01	0.81±0.01
Femoral neck BMD (g/cm^2)	0.75±0.004	0.75±0.01	0.77±0.01	0.78±0.01	0.65±0.004	0.64±0.01

Data are mean±SE. *p<0.05 vs. TT genotype, **p<0.01 vs. AA genotype.

carried out. Values of p<0.05 were considered to indicate statistical significance. Data were analyzed with the Statistical Analysis System (SAS) release 8.2 (SAS Institute Inc., Cary, NC, USA).

Results

Distribution of ERα genotypes

The distribution of genotype combinations was examined (Table 1). The distributions of ERα T→C and A→G genotypes were both in Hardy–Weinberg equilibrium. There were no subjects with the TT and GG genotypic combination and few with the TT/AG or TC/GG genotypic combination.

Physical characteristics

Physical characteristics of the subjects were compared with reference to the ERα T→C and A→G genotype groups (Table 2). For men and premenopausal women, age, weight, BMI, LTM, L2–4 BMD, and femoral neck BMD did not differ between subjects with the TT and TC/CC genotypes. In contrast, in postmenopausal women, age was significantly higher and femoral neck BMD was significantly lower in individuals with the TC/CC genotype than in those with the TT genotype. After adjusting for age, statistical significance was not achieved for the difference in femoral neck BMD in postmenopausal women (data not shown). In men and postmenopausal women, there were no differences in age and physical characteristics between subjects with the AA and AG/GG genotypes. In premenopausal women, age, weight, LTM, and BMD did not differ between subjects with the AA and AG/GG genotypes, whereas BMI was significantly greater in those with the AG/GG genotype than in those with the AA genotype. After adjusting for BMI, the relationship of L2–4 and femoral neck BMD between AA and AG/GG genotypes still did not show a significant difference in premenopausal women (data not shown).

ERα genotype and association between LTM and BMD

To investigate whether an interaction between ERα genotype and LTM had an effect on L2–4 and femoral neck BMDs, general linear models for BMD were analyzed using LTM, ERα

Table 3
General linear model for bone mineral density (BMD) with interaction between the ERα genotype and LTM

Dependent variables	Independent variables	Men		Premenopausal women		Postmenopausal women	
		F	p value	F	p value	F	p value
L2–4 BMD	LTM	45.65	<0.0001	24.73	<0.0001	25.53	<0.0001
	T→C genotype	0.91	ns	1.36	ns	2.41	ns
	LTM×(T→C genotype)	0.83	ns	1.29	ns	2.55	ns
Femoral neck BMD	LTM	63.90	<0.0001	15.07	<0.0001	25.35	<0.0001
	T→C genotype	0.03	ns	0.13	ns	8.15	0.004
	LTM×(T→C genotype)	0.03	ns	0.06	ns	7.48	0.007
L2–4 BMD	LTM	45.27	<0.0001	24.36	<0.0001	25.41	<0.0001
	A→G genotype	0.10	ns	0.16	ns	2.20	ns
	LTM×(A→G genotype)	0.05	ns	0.26	ns	2.14	ns
Femoral neck BMD	LTM	64.07	<0.0001	14.95	<0.0001	24.95	<0.0001
	A→G genotype	0.38	ns	0.07	ns	8.15	0.004
	LTM×(A→G genotype)	0.45	ns	0.05	ns	8.03	0.005

ns=not significant. Adjusted for age and BMI.

genotype, and the interaction between ERα genotype and LTM as independent variables, adjusting for age and BMI (Table 3). Lean tissue mass was significantly associated with L2–4 and femoral neck BMDs in both sexes and irrespective of menstrual status. In postmenopausal women, genotype and the interaction between genotype and LTM were significantly associated with femoral neck BMD for both the T→C and A→G genotypes, but not with L2–4 BMD. In men and premenopausal women, the effects of genotype and the interaction between genotype and LTM on BMD were not significant.

To clarify the influence of LTM on femoral neck BMD for T→C and A→G ERα genotypes in postmenopausal women, a general linear model for BMD was analyzed by each genotype, using LTM as an independent variable, adjusting for age and BMI. Fig. 1 shows the regression lines between femoral neck BMD and LTM for the ERα T→C and A→G genotype groups in postmenopausal women. For the T→C genotype (Fig. 1A), the slope was significantly larger ($p<0.01$) for TC/CC (slope=0.0071, $p<0.0001$) than for TT individuals (slope=0.0015, not significant). For the A→G genotype (Fig. 1B), the slope was significantly larger ($p<0.01$) for AG/GG (slope=0.0081, $p<0.0001$) than for AA individuals (slope=0.0033, $p=0.012$).

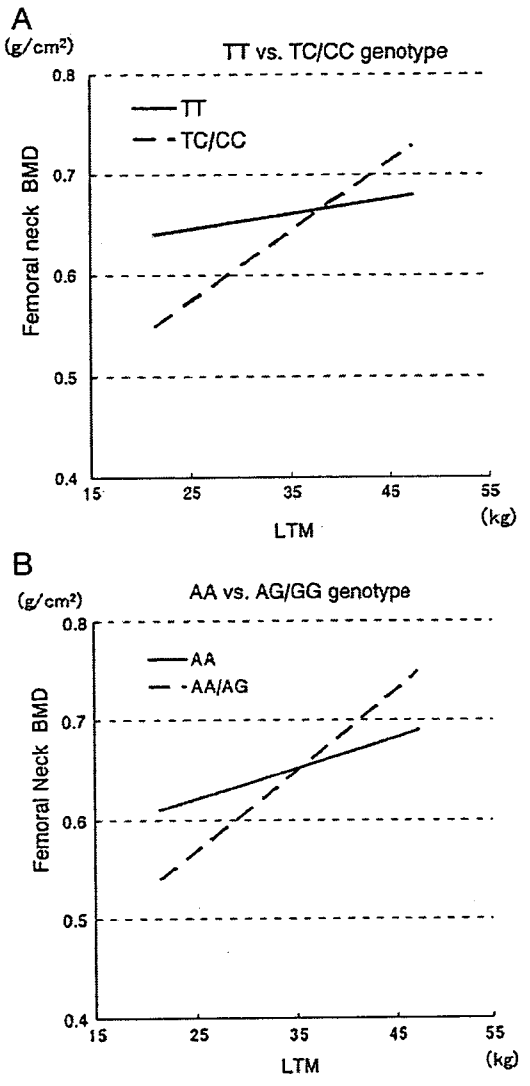

Fig. 1. Relationship between femoral neck BMD and LTM with reference to ERα T→C and A→G genotypes in postmenopausal women, adjusted for age and BMI. (A) T→C genotype. Solid line, TT genotype; Dotted line, TC/CC genotype. (B) A→G genotype. Solid line, AA genotype; Dotted line, AG/GG genotype.

Table 4
Physical characteristics of postmenopausal women with reference to ERα T→C and A→G haplotype

	Haplotype		
	TA	CA	CG
N	965	349	307
Age (years)	63.4±0.3	64.8±0.5*	64.3±0.5
Weight (kg)	52.2±0.3	51.6±0.4	51.8±0.5
BMI (kg/m²)	23.0±0.1	22.9±0.2	23.2±0.2
LTM (kg)	33.8±0.1	33.7±0.2	33.6±0.2
BMD L2–4 (g/cm²)	0.81±0.004	0.80±0.01	0.80±0.01
BMD femoral neck (g/cm²)	0.65±0.003	0.64±0.01	0.64±0.01

Data are mean±SE. *$p<0.05$ vs. TA.

ERα haplotype and association between LTM and BMD in postmenopausal women

Because there were significant genotype-LTM interactions on femoral neck BMD for both T→C and A→G polymorphisms in postmenopausal women, further analysis was carried out to evaluate the effect of the haplotype–LTM interaction. The distribution of haplotypes is shown in Table 4. The possible haplotype combinations for the ERα T→C and A→G polymorphisms were TA, CA, TG, and CG, but very few subjects had the TG haplotype. For postmenopausal women, the number of TA haplotype was 965; CA was 349; and CG was 307. Physical characteristics and BMD were compared with reference to these three haplotypes (Table 4). Age was significantly higher for those with the CA haplotype than for those with the TA haplotype. Weight, BMI, LTM, and L2–4 and femoral neck BMDs did not differ among haplotypes.

To clarify the influence of the interaction between ERα haplotype and LTM on femoral neck BMD in postmenopausal women, general linear models for BMD were analyzed using LTM, ERα haplotype, and the interaction between ERα haplotype and LTM as independent variables, adjusting for age and BMI (Table 5). Lean tissue mass, haplotype, and the interaction between haplotype and LTM were significantly

Table 5
General linear model for femoral neck BMD with interactions between ERα haplotype and LTM in postmenopausal women

Dependent variables	Independent variables	Postmenopausal women	
		F	*p*
Femoral neck BMD	LTM	49.80	<0.0001
	haplotype	6.63	0.001
	LTM×haplotype	6.23	0.002

Adjusted for age and BMI.

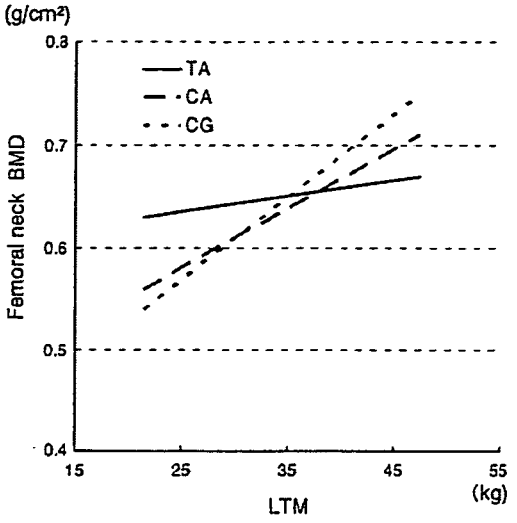

Fig. 2. Relationship between femoral neck BMD and LTM with reference to ERα haplotype in postmenopausal women, adjusted for age and BMI. Solid line, TA haplotype; Dotted line, CA haplotype; Fine dotted line, CG haplotype.

associated with femoral neck BMD. To evaluate the extent of the influence of LTM on BMD with respect to different haplotypes, general linear models were analyzed by each haplotype, using LTM as an independent variable, adjusting for age and BMI. Fig. 2 presents the relationship between femoral neck BMD and LTM with respect to the ERα haplotypes in postmenopausal women. The slope was significantly larger ($p<0.01$) for subjects with the CG (slope=0.0081, $p<0.0001$) and CA (slope=0.0063, $p<0.0001$) haplotypes than for those with the TA haplotype (slope=0.0035, $p=0.0003$), but there was no difference between the CG and CA haplotypes.

Discussion

We found that ERα polymorphisms influence the relationship between LTM and femoral neck BMD in postmenopausal women and that the effect of LTM on BMD was significantly larger in individuals with the TC/CC genotype of the T→C polymorphism than in those with the TT genotype, and larger in those with the AG/GG genotype of the A→G polymorphism than in those with the AA genotype. Haplotype analysis revealed that the effect was significantly larger for those with the CG and CA haplotypes than for those with the TA haplotype. This is the first study to investigate the effect of ERα gene–LTM interaction on BMD and to reveal the significant interaction in postmenopausal women.

In this study, a significant gene–LTM interaction in postmenopausal women was found at the femoral neck, but not at the lumber spine. It has been reported that hip joint compression forces reach 2.5 to 3 times body weight during walking [25,26]. The significant results found only at the femoral neck could be explained by the fact that high loading occurs at this site even in ordinary daily life.

We also analyzed data for different combinations of genotypes (TC/TT vs. CC; AG/AA vs. GG) (data not shown). In premenopausal women, no significant gene–LTM interaction was found. In postmenopausal women, significant interaction was found at femoral neck between TT/TC and CC genotype groups; however, no significant genotype–LTM interaction was found between GG and AA/AG genotype groups. This might be due to the small number in the GG genotype group ($n=28$). In men, when divided into TT/TC and CC genotypes, significant interaction was found at L2–4 and the femoral neck (L2–4, $p=0.04$; femoral neck, $p=0.02$) and the effect of LTM on BMD was larger for those with CC genotype than for those with TC/TT genotype. However, these significant interactions in men were weak in spite of the large number and the coefficients of determination (R^2) in the analysis model were low in men compared with postmenopausal women (men at the femoral neck, 0.28; men at L2–4, 0.18; postmenopausal women at the femoral neck, 0.38). Therefore, there might be other related factors in men and we considered that these results in men are insufficient to draw a clear conclusion about the effect of ERα gene–LTM interaction between TT/TC and CC genotype groups. We will examine this problem by adding other factors and analyzing the data longitudinally.

There have been some human studies investigating the effects of ERα gene polymorphism on exercise-induced effects on BMD. In a 4-year exercise intervention study, Remes et al. [18] reported that middle-aged Finnish men with the Pp (TC) or PP (CC) ERα genotype had increased lumbar spine BMD values. In the study of Remes et al., the subjects were middle-aged men, and the exercise intervention group spent 45–60 min on prescribed aerobic exercise five times a week for 4 years. Because we did not intervene as in the research of Remes et al., there might not have been a significant interaction between TT and TC/CC genotype groups in men.

Suuriniemi et al. [19] found that prepubertal and early pubertal Finnish girls with the Pp (TC) ERα genotype and high levels of physical activity had significantly higher bone mass and BMD at loaded bone sites. The subjects were 10- to 13-year-old prepubertal and early pubertal girls, whose estrogen concentrations were low, like those of postmenopausal women. A previous report indicated that estrogen can affect bone strength and mass by lowering the remodeling threshold, and that loss of estrogen would raise the threshold and help cause postmenopausal bone loss [27]. ERα expression in osteoblasts and osteocytes depends on estrogen concentration [28]. The increase in the potential of mechanical loading to stimulate bone gain in the peripubertal period is associated with marked increases in serum estrogen [11]. Thus, in the peripubertal period, when estrogen concentrations are high, the response to mechanical loading might be greater than in the prepubertal and postmenopausal periods, when estrogen concentrations are low. However, in the present study, significant interactions were found for postmenopausal women and in the study of Suuriniemi et al. [19] significant interactions were found for prepubertal and early pubertal girls. Accordingly, the effect of the gene–LTM interaction on BMD, that is, differences between individuals with different single nucleotide polymorphisms, might more readily appear in groups with low estrogen concentrations than in those with high concentrations.

In the study of Suuriniemi et al. [19], the interaction was found only for individuals with the Pp (TC) genotype (heterozygotes). This was not in agreement with our results. In this previous study, girls with low levels of physical activity bearing the Pp genotype had lower values for bone parameters compared with other groups. Because there are differences in the subject characteristics, age, sex, lifestyle, and study design, it is difficult to simply compare the results of the present study with those of the study by Suuriniemi et al. Further investigations are necessary to clarify these differences.

The mechanisms by which the ERα T→C and A→G polymorphisms might affect the femoral neck BMD are not clear, because the affected regions lie in an intronic and non-functional area of the gene. However, single nucleotide polymorphisms are usually linked to each other, so the two polymorphisms in intron 1 may be in linkage disequilibrium with causal polymorphisms elsewhere in the ERα gene or in genes nearby. In this regard, it is known that the T→C and A→G polymorphisms are in linkage disequilibrium with an upstream TA repeat polymorphism in the promoter region of the ERα gene [29]. An association between the TA repeat polymorphism and BMD has been shown in postmenopausal Japanese [30] and Italian women [29]. The number of TA repeats could be important in ERα gene transcription [31].

There are conflicting results regarding the association between ERα genotype and BMD in previous studies. Gene–LTM interactions might be one of the reasons for these differing results. That is, differences in the amount of muscle mass might change the association between ER genotype and BMD. For example, as shown in Fig. 1A, it is considered that in the group with low LTM, postmenopausal women with the TT genotype for T→C polymorphism have higher femoral neck BMD than those with the CC genotype. Conversely, in the group with high LTM, postmenopausal women with the CC genotype have higher BMD than those with the TT genotype.

In the present study, we evaluated the relationship between LTM and BMD with reference to ERα genotype, but we did not evaluate cause and effect directly. A previous exercise intervention study [18] showed an interaction between ERα genotype and exercise (i.e. the effect of mechanical loading) on BMD in men. Because LTM correlates with muscle strength [20] and it can be used as an index of skeletal load, the result of this previous exercise intervention study supports our present results regarding the gene–LTM interaction.

The strengths of the present study are the large sample size, the inclusion of both sexes, and the wide range of ages. Previous research has evaluated only one sex and a limited age range [18,19]. So far, the ERα gene–environment interaction concerning mechanical loading has not been investigated in postmenopausal women, who are particularly susceptible to osteoporosis. In the present study, both T→C and A→G ERα polymorphisms were examined and haplotype analysis was carried out.

As already described, previous studies have investigated the effects of ERα gene polymorphism on exercise-induced effects on BMD, but there has been no study that has evaluated the effect of the ERα gene–LTM interaction on BMD. The well-known phenomenon of reduction in muscle mass with aging is known as sarcopenia. Recent studies have reported a high prevalence of sarcopenia in postmenopausal woman with osteoporosis [32]. It would be very useful to identify individuals in this group who would experience a marked effect from increasing muscle mass, and the results of this study might assist in developing this process.

ERα plays an important role in the increase of BMD via mechanical strain [9–11]. A recent study has suggested that the effect of chronic immobility might be more marked on bone formation than bone resorption [33]. On the basis of our results, we can speculate that the influence of the mechanical loading increase on BMD via ER is different according to the ERα T→C and A→G polymorphisms in postmenopausal women.

In conclusion, there was an interaction between LTM and the T→C and A→G ERα polymorphisms with respect to their effect on femoral neck BMD in postmenopausal women and those with the TC/CC and AG/GG genotypes had larger effects of LTM than those with TT and AA genotypes.

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Brief Genetic Analysis

No Association between rs7566605 Variant and Being Overweight in Japanese

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Abstract

KUZUYA, MASAFUMI, FUJIKO ANDO, AKIHISA IGUCHI, AND HIROSHI SHIMOKATA. No association between rs7566605 variant and being overweight in Japanese. *Obesity*. 2007;15:2531–2534.

It has recently been demonstrated that a common single-nucleotide polymorphism (rs7566605) upstream of the transcription start site for the insulin-induced gene 2 is associated with obesity in several European/European origin or African-American cohorts. We tested whether this variant is also linked to overweight among Asian populations. Our sample included 2233 randomly selected, community-dwelling, middle-age and older Japanese people (men, 1128; women, 1105; age, 40 to 79 years; C allele frequency, 0.32). We observed that there were no differences in BMI levels [men, 22.9 ± 0.3 (mean ± standard error) vs. 22.9 ± 0.1, $p = 0.820$; women, 22.8 ± 0.3 vs. 22.9 ± 0.1, $p = 0.792$], waist circumferences and hip circumferences, waist-to-hip ratio, and fat mass between rs7566605 GG/GC and CC genotypes in both genders. In addition, logistic regression analysis, using age and sex as covariates, revealed no association of the single-nucleotide polymorphism with overweight (BMI ≥25) between rs7566605 genotypes in the Japanese cohort (CC vs. CG/GG, odds ratio = 1.18; 95% confidence interval = 0.84 to 1.65, $p = 0.333$; CC vs. GG, odds ratio = 1.19, 95% confidence interval = 0.84 to 1.69, $p = 0.325$). No significant associations were observed between polymorphism and glucose or insulin levels. These results suggested no association of the rs7566605 variant with overweight in Japanese people.

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Key words: glucose metabolism, genotype, BMI, body weight, insulin resistance

Recently, Herbert et al. (1) demonstrated that a common genetic single-nucleotide polymorphism (rs7566605) upstream of the transcription start site for insulin-induced gene 2 was associated with obesity in 694 individuals of the National Heart, Lung, and Blood Institute-Framingham Heart Study. Analysis suggests that rs7566605 CC homozygotes (C allele frequency, 0.37) have higher BMI levels than individuals with GC or GG genotypes, regardless of sex or age. This finding was replicated in four of five populations studied. A meta-analysis of all four case-control samples showed that CC homozygosity was also significantly associated with obesity (BMI ≥30 kg/m^2), with an odds ratio (OR)[1] of 1.22 [95% confidence interval (CI), 1.05 to 1.42]. However, more recent studies do not support the association of the rs7566605 polymorphism with obesity for the different samples (2–4). There may be many reasons that the association is not seen in these studies, including those related to study design, underlying genetic heterogeneity of populations, and different environmental exposures. However, most of the samples studied were of European/European origin or African Americans. We tested whether this variant is also linked to obesity/overweight among Asian populations. Our sample consisted of 2233 randomly selected, community dwelling, middle-age and older Japanese people.

The genotype frequencies for rs7566605 polymorphism were: GG, 0.465; GC, 0.432; and CC, 0.104 (C allele frequency, 0.32), which are similar frequencies to those reported by Herbert et al. (1) (Table 1). These frequencies are consistent with those expected under Hardy-Weinberg equilibrium (1 *df*). There were no significant differences in the genotype distributions of rs7566605 polymorphism between men and women or among the different age groups (Table 1). Table 2 shows the mean (standard error) of BMI, other anthropometric variables, and glucose metabolic variables tested in the GG/GC and CC genotypes. There were

[1] Nonstandard abbreviations: OR, odds ratio; CI, confidence interval; NILS, National Institute for Longevity Sciences; LSA, Longitudinal Study of Aging.

Table 1. Distribution of rs7566605 genotypes

		CC		GC		GG		GC/GG	
	n	*n*	%	*n*	%	*n*	%	*n*	%
Total	2233	233	10.4	962	43.1	1038	46.5	2000	89.6
Men*	1128	121	10.7	470	41.7	537	47.6	1007	89.3
Women	1105	112	10.1	492	44.5	501	45.3	993	89.9
Age (yrs)†									
40 to 49	563	61	10.8	224	39.8	278	49.4	502	89.2
50 to 59	556	56	10.1	265	47.7	235	42.3	500	89.9
60 to 69	561	64	11.4	241	43.0	256	45.6	497	88.6
70 to 79	553	52	9.4	232	42.0	269	48.6	501	90.6

* CC, GC, GG, men vs. women, $\chi^2 = 1.863$, $p = 0.394$; CC, GC/GG, men vs. women, $\chi^2 = 0.209$, $p = 0.648$.
† CC, GC, GG, age groups, $\chi^2 = 9.306$, $p = 0.157$; CC, GC/GG, age groups, $\chi^2 = 1.373$, $p = 0.712$.

no differences in BMI levels between these genotypes. In addition, no significant differences in waist and hip circumferences, waist-to-hip-ratio, and fat mass were observed between these genotypes in either gender (Table 2). No significant association was observed between these genotypes and fasting glucose, insulin, hemoglobin A_{1c}, or homeostasis model assessment for insulin resistance levels in men or women (Table 2). The rs7566605 genotypes showed similar allele frequencies in diabetic individuals and in non-diabetic controls (data not shown).

In our cohort, only a small number of participants had a BMI ≥30 kg/m^2 (0.97% in men, 3.37% in women). When logistic regression was performed to calculate the OR for the CC genotype compared with the CG/GG genotypes or the GG genotype, defining overweight as a BMI ≥25 kg/m^2 (23.6% in men, 22.0% in women), using age and sex as covariates, the CC genotype showed no association of the single-nucleotide polymorphism with overweight (vs. CG/GG, OR = 1.18, 95% CI, 0.84 to 1.65, $p = 0.333$; vs. GG, OR = 1.19, 95% CI, 0.84 to 1.69, $p = 0.325$). The CC/CG

Table 2. Anthropometric and glucose metabolic variables according to rs7566605 genotypes

	Men						Women					
		CC		GG/GC				CC		GG/GC		
	n	Mean	SE	Mean	SE	*p*	*n*	Mean	SE	Mean	SE	*p*
Weight (kg)	1127	62.0	0.8	62.1	0.3	0.804	1104	52.8	0.8	52.4	0.3	0.612
Height (cm)	1127	164.3	0.5	164.6	0.2	0.695	1104	151.9	0.5	151.2	0.2	0.176
BMI (kg/m^2)	1127	22.9	0.3	22.9	0.1	0.820	1104	22.8	0.3	22.9	0.1	0.792
Waist circumference (cm)	1127	82.0	0.8	82.4	0.3	0.585	1104	75.2	0.9	75.1	0.3	0.969
Hip circumference (cm)	1127	90.9	0.4	91.1	0.2	0.622	1104	90.6	0.5	90.6	0.2	0.985
Waist-to-hip ratio	1127	0.9	0.01	0.9	0.002	0.641	1104	0.8	0.01	0.8	0.002	0.877
Fat mass (kg)	1125	21.0	0.4	21.4	0.1	0.335	1096	31.4	0.5	31.5	0.2	0.864
Glucose (mM)*	1056	5.8	0.1	5.7	0.03	0.233	1049	5.4	0.1	5.5	0.03	0.304
Insulin (μU/mL)*	1071	8.0	0.5	8.2	0.2	0.691	1069	7.8	0.5	8.4	0.2	0.210
Hemoglobin A_{1c} (%)*	1055	5.3	0.1	5.2	0.02	0.373	1048	5.1	0.05	5.2	0.02	0.252
HOMA-IR*	1055	2.2	0.2	2.2	0.1	0.959	1048	1.9	0.2	2.1	0.1	0.190

SE, standard error; HOMA-IR, homeostasis model assessment of insulin resistance.
* Analysis of subjects who were not on oral hypoglycemic agents or insulin. Data were adjusted for age.

genotype also showed no significant association of the single-nucleotide polymorphism with overweight under a recessive model (vs. GG, OR = 1.05, 95% CI, 0.86 to 1.28, $p = 0.614$).

These results suggested no association of the rs7566605 variant with overweight in Japanese people. An ethnic difference may have contributed to the lack of this association. Another possibility is that the lower BMI levels and fewer obese individuals in our cohort may have affected our results. It is possible that due to the low number of obese individuals in our population, we lacked sufficient power to attempt replication of the previously reported association between rs7566605 and obesity in this study. In fact, it has been reported that excluding the upper quartile from the analysis in the Kooperative Gesundheitsforschung in der Region Augsburg (KORA) population in the study by Herbert et al. (1) eliminated the evidence for association of rs7566605 CC genotype with obesity, indicating that an association with obesity is strongest in those who are more obese. However, similar genotype frequency for this variant between our cohort and others may indicate that environmental influences can overcome the genetic influence on the anthropometric measurement.

Research Methods and Procedures

The present study consisted of a cross-sectional analysis of 1105 women and 1128 men who participated in the first wave of examinations in the National Institute for Longevity Sciences (NILS)-Longitudinal Study of Aging (LSA) from April of 1998 to March of 2000. The subjects of the NILS-LSA were male and female residents, 40 to 79 years old. The population of Obu city and Higashiura town in the Aichi prefecture in central Japan was stratified by both age and gender and randomly selected from resident registrations in cooperation with the local governments. The number of men and women was to be the same to test gender difference. Age at baseline was to be 40 to 79 years, and the number of participants in each decade (40s, 50s, 60s, 70s) was to be the same. The examinations include various areas of gerontology and geriatrics such as medical examinations, anthropometry, body composition, physical functions, physical activities, psychological assessments, nutritional analysis, and molecular epidemiology. The subjects will be followed up every 2 years. The details of the NILS-LSA have been described elsewhere (5,6). Randomly selected men and women were invited by mail to attend an explanatory meeting. At that meeting, the procedures for each examination and the follow-up schedule were fully explained. Written, informed consent for the entire procedure was obtained from each participant. The study was approved by the Ethics Committee of the NILS.

Anthropometric Variables

Body weight was measured to the nearest 0.01 kg using a digital scale, height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and BMI was calculated as weight (kilograms) divided by height squared (meters squared). Waist circumference and waist-to-hip ratio were used as the indices for body fat distribution in this study. Waist-to-hip ratio was calculated as the ratio of waist circumference measured at the midpoint between the anterior superior iliac crest and the lowest rib-to-hip circumference. Whole-body fat mass, assessed by DXA (QDR-4500; Hologic, Madison, WI), was used as an index for determining body composition.

Biochemical Assays of Blood

An antecubital blood sample was drawn from each subject after an overnight fast. Fasting plasma glucose was assayed by the glucose oxidase method (7). Plasma insulin was measured by radioimmunoassay (8). Coefficients of variation of glucose and insulin were 16.3% and 64.3%, respectively. The homeostasis model assessment for insulin resistance was calculated as fasting serum insulin (microunits per milliliter) × fasting plasma glucose (millimolar)/22.5 (9).

Determination of rs7566605 Genotypes

Genotypes were determined using a fluorescence-based allele-specific DNA primer assay system (Toyobo Tsuruga Gene Analysis, Tsuruga, Japan). The polymorphic regions of rs7566605 were amplified by polymerase chain reaction with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-TCATTGCAATAGCCACTGCCAAGTAC-3′) or Texas red (5′-GGATATTTGATCGTGGTCCTTTA-3′) as allele-specific hybridization probe and with an antisense primer labeled at the 5′-end with biotin (5′-AAAAACTGAAAACCACCCTGGTACAGAC-3′). The reaction mixtures (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mM deoxynucleoside triphosphate, 2.0 mM $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo Co., Ltd.) in polymerase buffer. The amplification protocol consisted of initial denaturation at 95 °C for 5 minutes, followed by 45 cycles of denaturation at 95 °C for 30 seconds, annealing at 65 °C for 30 seconds, and extension at 72 °C for 30 seconds; a final extension was conducted at 72 °C for 2 minutes. Our genotyping error rate was ~0.1%.

Data Analysis

Quantitative data adjusted for age were compared between the two groups by unpaired Student's t test. Allele frequencies were estimated by the gene counting method, and the χ^2 test was used to identify any significant departure from Hardy-Weinberg equilibrium. Logistic regression was performed to calculate the OR for the CC allele genotype compared with CG/GG genotypes or the GG genotype, defining overweight as BMI ≥25 kg/m^2, using age and sex as covariates. In this study, the significant difference in BMI

by genotype should be >0.76 kg/m^2 in men and >0.92 kg/m^2 in women with a power $(1 - \beta)$ of 0.8 and an α of 0.05. In the analyses to examine the association between genotypes and glucose metabolisms, participants who were being treated with oral hypoglycemic agents or insulin were excluded. The general linear model was applied to control for age. In the model, each quantitative variable was the dependent variable, and age and genotype were the independent variables. Least square means of the dependent variable by genotype were compared and tested by Student's t test. A p value <0.05 was considered to be statistically significant. The data were analyzed with SAS, release 8.2 (SAS Institute, Inc., Cary, NC).

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Cognitive impairment and frontal-subcortical geriatric syndrome are associated with metabolic syndrome in a stroke-free population

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Abstract

Background: Metabolic syndrome (Met.S) consists of a conglomeration of obesity, hypertension, glucose intolerance, and dislipidemia. Frontal-subcortical geriatric syndrome (FSCS) is caused by ischemic disruption of the frontal-subcortical network. It is unknown if Met.S is associated with FSCS.

Methods: We evaluated 422 community-dwelling elderly (≥60) in Brazil. FSCS was defined as the presence of at least one frontal release sign (grasping, palmomental, snout, or glabellar) plus coexistence of ≥3 the following criteria: (1) cognitive impairment, (2) late-onset depression, (3) neuromotor dysfunction, and (4) urgency incontinence. All values were adjusted to age and gender.

Results: Met.S was present in 39.3% of all subjects. Cases without any of the FSCS components represented 37.2% ('successful neuroaging' group). People with 1–3 of the FSCS components ('borderline pathological neuroaging' group) were majority (52.6%), whereas those with 4–5 of these components (FSCS group) were minority (10.2%). Met.S was significantly associated with FSCS (OR = 5.9; CI: 1.5–23.4) and cognitive impairment (OR = 2.2; CI: 1.1–4.6) among stroke-free subjects. Number of Met.S components explained 30.7% of the variance on the number of FSCS criteria ($P < 0.001$). If Met.S were theoretically removed from this population, prevalence of FSCS would decline by 31.6% and that of cognitive impairment by 21.4%.

Conclusions: Met.S was significantly associated with a 5.9 and 2.2 times higher chance of FSCS and cognitive impairment, respectively. Met.S might be a major determinant of 'successful' or 'pathological' neuroaging in western societies.

Keywords: Frontal-subcortical; Metabolic syndrome; Successful aging; Cognitive impairment; Vascular depression; Executive dysfunction; Neuromotor dysfunction; Urgency-type incontinence; Elderly; Brazil

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1. Introduction

1.1. Metabolic syndrome and the frontal-subcortical syndrome

More than 100 years ago, Biswanger was the first to describe a syndrome of subcortical atherosclerotic encephalopathy (SAE) among the elderly, neuropathologically characterized by diffuse WML [76]. This was followed by the report of the 'lacunes of cerebral disintegration' syndrome by Marie [61] and Ferrand [27], when they described neuropathological findings in 50 patients who died in a nursing home. Clinical features included small-stepped gait ('marche à petits pas' of Dégerine), dysartria, psedobulbar palsy, dementia, incontinence and emotional lability; most of these symptoms denoting advanced frontal-subcortical network damage. Advanced cases evolved to the syndrome of apathia-akinesia and abulia, and terminal state was characterized by akinetic mutism. Multi-infarct dementia, a term coined by Hachinski [38], often coexists with SAE and represents an advanced state of cognitive deficit caused by such neuropathological lesions.

The clinical picture above described, though common in nursing homes, are rarely seen in the community [27,38,61,76]. Instead, a milder form characterized by cognitive impairment, late-onset depression, lower neuromotor dysfunction and urgency incontinence are often seen both in the geriatric outpatient clinic and in the community-dwelling elderly [54,82].

Just recently evidences accumulated that SAE disrupts the frontal network and promote frontal atrophy, leading to a 'frontal-subcortical syndrome' (FSCS) which is much more common among community-dwelling elderly than classical vascular dementia [54,82]. FSCS has been linked to a series of geriatric disorders, such as cognitive decline, late-onset depression, dysexecutive syndrome, gait disorders, falls, and urgency incontinence [54]. There is increasing evidence that these are manifestations of a single geriatric syndrome, namely, the FSCS [54,82]. FSCS is extremely common among otherwise neurologically normal elderly subjects, but it is usually underappreciated, and its prevalence in the community has not yet been investigated [82].

Several aspects of the FSCS have been independently linked to the metabolic syndrome (Met.S) or its individual components, yet no study has comprehensively evaluated the independent association between these two syndromes [5–9,16–19,53–57,81,82,84] as well as if these associations are dependent of clinical stroke.

Metabolic syndrome (Met.S) is defined as a cluster of obesity, glucose intolerance, hypertension, low HDL and/or high triglycerides [47]. Most of above items have been shown to be independent risk factors for stroke. Met.S itself was already evidenced to be an independent risk factor for cardiovascular disease, including stroke [6,63,71]. Met.S increases the risk of both clinical and asymptomatic stroke by 2–3 times [43,63]. Moreover, in patients with stroke the coexistence of Met.S is associated with a more advanced atherosclerotic process [71]. Met.S has also been previously shown to increase the risk of overall dementia [49], Alzheimer's disease (AD) [36], and cognitive decline [98].

Insulin resistance and hyperinsulinemia are usually considered to be the underlying common pathophysiological mechanism [30]. Prevalence of Met.S among the American elderly was shown to be around 24% [30].

Met.S, but not its conventional risk factors, was recently shown to be independently associated with intracranial atherosclerosis and lacunar stroke [6]. Hyperinsulinemia was also associated with cerebral small-vessel disease, with lesions in the white matter and basal ganglia [99]. Coexistence of DM and hypertension (a situation in which Met.S is often present) is associated with a three times higher chance of having silent infarct(s) in elective MRI, when compared with the group with HT but without DM [25].

Increasing evidences support a role for cerebral small-vessel lesions as a cause for dysfunction in frontal-subcortical systems [82]. It is unknown whether FSCS is associated with Met.S in a stroke-free population.

1.2. 'Successful', 'usual', and 'pathological' neural aging

'Successful' aging is defined as aging without major chronic-debilitating diseases and keeping independence for the activities of daily life (ADL) to a maximum extend before death [86].

Neural aging (here the term 'neuroaging' will be used) refer to the progressive deterioration of the nervous system capacity to promptly and adequately respond to a determined stimulus, be it cognitive, affective, motor or sensitive [11,65].

Recent evidences suggest that neural changes occurring during normal aging are more subtle and selective than once believed [11,65]. Among the brain regions affected by aging, the frontal-subcortical and hippocampus networks seem to be particularly vulnerable [11]. Nonetheless, even within these networks interindividual differences on the impact of chronological aging exist, with some individuals showing little age-related decline [42]. In fact, many healthy elderly, including those above 84 years, did not seem to experience measurable declines in cognitive functioning when followed for a period of 4 years [42].

Most individuals, however, usually show considerable age-related changes in cognitive, affective, and neuromotor functions; notably those functions which rely heavily on the medial-temporal and prefrontal networks, such as learning, memory, humor and executive function [11].

Cerebral small-vessel disease, a very common cause of pathological neuroaging, is associated with a steeper decline in information processing speed, executive function and memory [81].

Disruption of the frontal-subcortical network sufficient to cause concomitant cognitive deficit, vascular-type depression, executive/neuromotor dysfunction, and loss of urinary

inhibitory effect are common features of (pathological) neuroaging [82]. Disruptions in this network are mainly caused by accumulation of lacunar infarcts and white matter lesions (WML) that often occurs with 'unsuccessful' aging. Because circuits that control cognitive, affective, executive, and motor functions are in close proximity, multiple small vascular lesions may simultaneously cause dysfunction in the entire circuit [82].

1.3. Metabolic syndrome and risk for cerebrovascular disease in Brazil

Brazilian life expectancy at birth reached 71.7 years in 2004 [68] and those who are 60 years old can now expect to live another 19.78 years [31].

Latin America (LA) is the world's region with the average highest number of years (8.3) lived with disability [66]. Non-communicable diseases account for most of this disability as well as for most of the difference on this index between LA and the developed world (6.3 years) [66]. Stroke is the most powerful disabling disease of older ages in both Western [35] and Eastern societies [15].

Prevalence of stroke goes in line with that of silent stroke and WML [93], the pathological markers of FSCS. Stroke is also the leading cause of mortality in Brazil [59]. Different international comparisons have shown that stroke rates in Brazil are among the highest in LA [59] and one of the highest in the world [24]. Stroke, especially ischemic stroke, strongly correlates with the degree of vascular aging [90].

Besides its usual association with overfeeding and obesity, Met.S seems to accelerate biological aging also by promoting protein glycation [96], insulin resistance and telomere attrition [32]. Obesity is the most important component of the Met.S [32] and it is growing in prevalence among the elderly in both the USA [45,46] and Brazil [45,64]. In fact, together with American and European elderly, Latin American older people have already one of the highest body mass index (BMI) among all the world regions [45]. A study done in the Brazilian southernmost state showed that nearly 30% of all adult population was obese [91].

Following the obesity upsurge [39], Met.S is also becoming a global epidemic [62]. In Brazil, there is a paucity of data on Met.S for international comparisons. However, LA is the world region with the highest mortality and morbidity burden attributable to both obesity and DM [66] and, therefore, it is reasonable to cogitate that it may also be so for Met.S. BMI index physiologically declining after the sixties, just in LA the prevalence of obesity keeps increasing after the age 60 and 70 among men [39].

1.4. Working hypothesis: metabolic syndrome, pathological neuroaging, and the frontal-subcortical syndrome

Many associations have been reported between obesity, metabolic (hyperinsulinism, NIDDM, low HDL-c and hypertriglyceridemia) and hypertensive disease (components of the Met.S), in one side, and cognitive deficit, depression, lower executive, neuromotor, functional status and incontinence (components of FSCS), in another side [5–9,16–19,53–57,81,82,84].

However, an area that has drawn surprisingly little, if any, attention is the direct relationship between Met.S and FSCD. Met.S, by promoting pathological neuroaging, might be a central explanation for the high prevalence of dependence among Latin American and, specially, Brazilian elderly [74].

We hypothesize that, among a stroke-free elderly population, Met.S may be a major discriminative factor between those who experience a 'successful' neuroaging and those who show evidences of the FSCS.

2. Methods

2.1. Population and setting

We investigated 434 older people (≥60 years) living in two towns of the southernmost Brazilian State, Rio Grande do Sul, Brazil is a heterogeneous society, constituted predominantly by Whites (53.4%) and Mestizos (40.4%) inhabitants [31]. Two different tows, Estancia Velha and Charqueadas were selected in order to better ethnically represent the southern Brazilian elderly. Estancia Velha has a predominantly White population, whereas Charqueadas is largely a Mestizo town [31]. A preliminary analysis did not evidence any major differences in terms of prevalence of Met.S and FSCS, or their association, between these two towns. Data were, therefore, pooled and analyzed together as to account for a reliable sample of the southern Brazilian elderly.

A randomized sample was selected from a list provided by the 'Department of Social Assistance' of each City Hall, which contained virtually all people aged ≥60 years in the town.

A phone call to 10% of the respective samples confirmed that all randomly chosen individuals were invited to participate in the research. Dependent individuals were brought to the research site and taken home by an appropriate vehicle.

2.2. Variables

The interviews and the battery of neurogeriatric tests were performed by trained (one full day) medical students. At the end of the questionnaire all elderly were submitted to blood exam, to a battery of geriatric assessment scales, and to independent geriatric and neurologic evaluations. Among the blood exams, analysis of fasting glucose, haemoglobin, albumin, total cholesterol, HDL-cholesterol (HDL), triglycerides, and creatinine levels were included.

For diagnosis of the Met.S, an adapted form of the ATP III [30] criteria was applied for use in the elderly, whereby BMI ≥30 kg/m^2 and blood pressure ≥140/90 mmHg were used to diagnose the obesity and hypertensive component of

the syndrome, respectively. The diagnosis required three or more of the following five criteria: obesity (BMI $\geq$30 kg/m^2), HDL-c <40 mg/dl in men and <50 mg/dl in women, triglycerides $\geq$150 mg/day, blood pressure $\geq$140 mmHg for systolic or $\geq$90 mmHg for diastolic, and fasting glucose $\geq$110 mg/dl. People without Met.S were considered as control group. Additionally, the use of drugs for hypertension, elevated serum glucose or triglyceride, and low HDL-c was also considered as scoring positive for each respective Met.S component.

Positive frontal release reflexes (FRR) are associated with small-vessel cerebrovascular disease and atrophy of the frontal-subcortical network [23,83]. Presence of atleast one out of four FRR correlates better with frontal-subcortical network functions, such as executive control, neuromotor function, and attention than presence of either sign alone [23]. These same four FRR were also evaluated in this study, namely: hand grasping, palmomental, snout, and glabellar reflexes. Presence of FRR was considered positive if at least one of them was bilaterally positive.

There is no clear consensus in the diagnosis of FSCS [54,82]. FSCS was arbitrary defined by presence of atleast one FRR plus presence of three or more of the following four criteria [54,82]: (1) cognitive impairment as defined below by the mini-mental state evaluation (MMSE) [29], (2) late-onset depression as diagnosed by a psychiatrist (DSM-IV-R criteria [4]), (3) lower limbs neuromotor dysfunction (as below), and (4) urgency incontinence [8]. Absence of all the above conditions was considered as 'successful neuroaging', and presence of 1–3 of them was considered as 'borderline pathological aging'.

Cognitive impairment was defined as a 0.67 standard deviations (S.D.) (three points) below the predicted score from the linear regression formula (including all stroke-free population), where MMSE was the predicted variable and age and education were the independent variables.

Depression was diagnosed by a psychiatrist according to the DSM-IV-R [4]. Cases were screened using two questions: (1) Have you dropped many of your activities and interests? (2) Do you often feel sad or depressed? Cases with positive answers in either question were selected for the psychiatry interview. Both major and minor (dysthymia) depression were considered as a single 'depression' variable. Additionally, depressive symptomatology was graded using the 15-item geriatric depression scale (GDS), Brazilian validated version [3].

Lower limbs neuromotor dysfunction was considered positive if either 'fear of falling' or history of falls was positive [89]. The question on 'fear of falling' was: "are you often afraid to fall and hurt yourself"? "Very afraid" and "afraid" answers were considered positive and 'not so much' and 'not at all' were considered negatives for the sake of binary logistic regression analysis. An individual was considered to have history of falls if he/she has fallen once or more in a period of 1 year, without significant immediate reasons for that fall. Positive cases for either question (combined variable on falls) were considered as having neuromotor dysfunction. Those unable to walk were, together with those with a diagnosis of stroke or dementia, excluded from the final analysis.

Additionally, balance as measured by the 'Functional Reach' test [77], and gait speed as evaluated by the timed 'Up&Go' [80] walking test, were evaluated. 'Functional Reach' test $\leq$16 cm and time to perform the 'Up&Go' test $\geq$15 s were used as cutoff points for categorizing both variables. Detailed methodology on these two tests was reported elsewhere [77,80].

A pre-analysis has shown a stronger association between the combined question on falling and both the 'Up&Go' and 'Functional Reach' tests ($R = 0.211$ and -0.193, respectively; $P < 0.001$ for both) than either questions on falls alone. Therefore, this combined variable on falls was used as a clinical surrogate for lower limb neuromotor dysfunction.

Urgency incontinence was diagnosed if the elderly had clear symptoms, including urge, loss of urine in the way to the toilet, increase in the urinary frequency, feeling of incomplete urination, and no positive history or symptoms of effort-related incontinence symptoms (especially for women), prostatism/overflow incontinence, or suspicion of neurogenic bladder, (especially in cases with advanced DM) [8].

Executive control function (ECF) is the ability to independently perform complex, goal-oriented and self-serving activity [1]. ECF was measured using two methods. First, the performance on the back-7 and backward spelling items of the MMSE (the highest score in either task) was considered a measure of abstract executive performance. These two questions tests tap attention, load heavily on working memory, and have been reported to be impaired in patients with dysexecutive syndrome [34]. This score ranges from 0 to 5 and here was named ECF-working memory (ECF-WM). A score lower than three was considered as impaired ECF-WM. Second, ECF-related questions of the Tokyo Metropolitan Institute of Gerontology (TMIG) scale [52] were utilized to evaluate ECF using a more practical approach, and for this reason were called ECF-ADL. It contains questions related to ideation, planning, and execution acts on taking bus, shopping, paying bills, banking, filling forms, visiting friends, and managing properties. The ECL-ADL score ranged from 0 to 7. Difficulty or incapacity in performing one or more of the above scale's items was considered as impairment for ECL-ADL. To control for confusion between Met.S and the physical components of these activities, values were adjusted to ADL and to the non-ECL questions of the TMIG.

Stroke was defined and diagnosed in consonance with the World Health Organization (WHO) [97]. Stroke diagnosis was performed on the basis of clinical history, findings in the neurological examination, previous brain CT scans or MRI, and medical records. Presence of stroke was diagnosed by a neurologist, and Met.S was diagnosed by a geriatrician. Both agreed in a diagnosis of FSCS. Dementia was diagnosed according to the DSM-IV-R.

2.3. Inclusion criteria

Inclusion criteria were absence of dementia and independence for walking. Individuals with poor scores in the MMSE associated with low educational level were included if they did not fit DSM-IV-R criteria for dementia. Initial baseline analysis included stroke distributions and relationships between stroke and FSCS variables. In a second step, cases with stroke were removed from the analysis involving the relationship between Met.S, FSCS and its individual components.

2.4. Population attributable risk (PAR)

PAR is defined as "the fraction of total disease experience in the population that would not have occurred if the effect associated with the risk factor of interest is removed" [10]. To calculate the estimate for the PAR, it was utilized the formulation of Bruzzi et al. [10], which substitutes the relative risk used in the formula for longitudinal studies for the odds ratio in crossectional designs. The utilized formula was: $PAR = (OR - 1/OR) \times P$, were OR is the odds ratio of the risk factor for the disease in question and P is the prevalence of the disease in the population. OR was adjusted for age and gender. Adjusted OR was used to calculate the PAR.

2.5. Statistical analysis

For statistical analyses SPSS, version 11.5 (SPSS Inc., Chicago, IL) was used. Multivariate logistic regression analysis was used to assess relationships between categorical variables. Independent t-test and analysis of variance (ANOVA) were performed in order to compare continuous variables means between two or more groups, respectively. Multivariate linear regression was used to evaluate the relationship between two numeric variables. A 95% confidence intervals (CI) was utilized and calculated on the basis of the binomial distribution, being a P-value <0.05 considered as statistically significant. All values were adjusted for age, gender, and other relevant confounders.

This project was approved by the Ethical Committee of the Catholic University of Rio Grande do Sul State, Brazil.

3. Results

Twelve individuals had dementia and/or inability to walk, and were excluded from the analysis. In the included sample of 422 elderly, mean age was 68.3 years (60–91) and percentage of females was 64.8%. Presence of at least one FRR was observed in 91 (21.6%) subjects. Cognitive impairment was observed in 85 (20.1%) individuals. Late-onset depression was observed in 68 (16.1%) elderly. History of falls was present in 120 (28.4%) cases, 'fear of falling' in 97 (23%) individuals, and urgency incontinence in 10 (2.4%) subjects.

Cases free from any of the above items (157) represented 37.2% ('successful neuroaging' group). People with 1–3 of the above items ('borderline pathological neuroaging' group) were majority (222 cases, 52.6%), whereas those with 4–5 of these items (FSCS group) were minority (43 cases, 10.2%).

Met.S was present in 166 subjects (39.3%). Those who did not present any of the five components of the Met.S were 56 (13.3%). Those who presented 1 or 2 components were 104 (24.6%) and 96 (22.7%), respectively.

FSCS was strongly associated with Met.S (OR = 6.9; CI: 2.0–23.8; $P = 0.002$). Adjustment to stroke decreased the power of the association by 16.1% without changing the significance of the association (OR = 5.8; CI: 1.7–20.3; $P = 0.006$). Analogously, removing the stroke cases did not significantly modified the association (OR = 5.9; CI: 1.5–23.4; $P = 0.011$). Number of Met.S components explained 30.7% of the variance on the number of FSCS criteria ($P < 0.001$, adjusted to age and gender). When both variables were considered as dichotomies, i.e. having or not Met.S and FSCS, this value was significantly reduced (14.6%; $P = 0.002$). Among the stroke-free population, prevalence of FSCS would be reduced by 33.6% if Met.S were theoretically eliminated (PAR = 33.6%).

Stroke cases were included in this preliminary analysis, including Table 1, but were excluded hereafter from the analysis (see Section 2). Forty subjects (9.5%) presented a diagnose compatible with previous stroke episode. Clinical stroke tended to be associated with Met.S (CI: 0.88–3.3; $P = 0.109$), but did not reached significance. Stroke was strongly associated with FSCS (OR = 4.2; CI: 1.4–12.4; $P = 0.01$).

Table 1 shows the baseline characteristics according to the presence or absence of Met.S. BMI and rates/values of hypertension, pulse rate, glucose intolerance, DM, LDL-c, total cholesterol/HDL-c ratio, and triglycerides were higher in the Met.S group than in the control group. Lack of regular exercise, not to be actively working, and HDL <40/45 were significantly more common in the Met.S group.

Among the 382 remaining elderly without stroke, 153 (40.0%) did not present any FSCS component ('successful' neuroaging group), 195 (50.1%) had 1–3 features of the FSCS ('borderline pathological' neuroaging group), and 34 (8.9%) had 4–5 components of the syndrome (FSCS group). Mean age of these groups was 66.5, 68.4, and 70.9 years, respectively ($P < 0.001$). Thirty-three age and gender matched cases were randomly selected from the first and second groups above for the sake of case-control comparison with the FSCS group.

The predicted (linear regression) MMSE formula as a function of age and education was: MMSE = 37.1 + (Educ × 0.778) − (age × 0.228), where 'Educ' is years of schooling and age is in years ($P < 0.001$ for formula's significance).

Table 2 depicts the odds ratio and PAR of Met.S as a significant associated factor for lower scores in all neurofunctional variables but the Functional Reach test. Most of the FSCS

Table 1
Baseline characteristics between Met.S and control groups

	Metabolic syndrome		
	No	Yes	*P*-value[a]
N (%)	256 (60.7)	166 (39.3)	–
Age	68.3	67.9	0.455
Gender (%)	166 (64.8)	97 (58.4)	0.186
White/Mestizo	1.21:1.0	1.23:1.0	0.592
Income (US$)	712	662	0.342
Education (years)	3.11	2.88	0.199
Live alone (%)	39 (15.2)	23 (13.9)	0.677
Anemia (%)	66 (25.8)	35 (21.1)	0.307
Albumin	4.23	4.28	0.372
Systolic BP (mmHg)	152.3	159.4	0.003
Diastolic BP (mmHg)	88	91	0.004
Mean arterial BP (mmHg)	106.5	112.0	0.001
Hypertension (%)	207 (80.9)	157 (94.6)	<0.001
Pulse pressure (mmHg)	65.5	69.4	0.085
Pulse rate (min)	73.3	74.8	0.036
BMI (kg/m^2)	26.4	30.4	<0.001
Obesity (BMI > 30 kg/m^2)	33 (12.9)	143 (86.2)	<0.001
Total cholesterol (mg/dl)	188.1	189.8	0.627
HDL-C (mg/dl)	48.9	34.8	<0.001
HDL-C < 40 (M) < 45 (F) mg/dl (%)	69 (27.0)	153 (92.2)	<0.001
T-Cho/HDL ratio	3.9	5.6	<0.001
LDL-c (mg/dl)	115.2	121.4	0.04
Triglycerides (mg/dl)	119.6	168.2	<0.001
Triglycerides < 150 mg/dl (%)	22 (8.6)	115 (69.3)	<0.001
Glucose (mg/dl)	110.7	144.1	<0.001
Glucose intolerance (%)	19 (7.4)	59 (35.6)	<0.001
Diabetes mellitus 2 (%)	30 11.7	73 (44.0)	<0.001
Number of metabolic syndrome components	1.48	3.52	<0.001
Sleep (hours)	7.28	7.32	0.874
Taking drugs (%)	230 (89.8)	148 (89.2)	0.786
Alcohol (>once a week)	63 (24.6)	31 (18.7)	0.293
Smoking past (%)	61 (23.8)	43 (25.9)	0.364
Smoking present (%)	32 (12.5)	20 (12.0)	0.851
Bone fracture (%)	78 (30.5)	46 (27.7)	0.574
Osteoarthrosis (%)	119 (46.5)	82 (49.4)	0.552
Heart diseases (%)	73 (28.5)	52 (31.3)	0.602
Ischemic heart disease (%)	23 (9.0)	17 (10.2)	0.656
Stroke (%)	21 (8.2)	27 (13.6)	0.109
Actively working (%)	171 (66.8)	91 (54.8)	0.008
Regular exercise[b] (%)	106 (41.4)	32 (19.3)	0.003

BP, blood pressure; BMI, body mass index; HDL-c, HDL-cholesterol; T-Cho, total cholesterol; LDL-c, LDL-cholesterol.
[a] *t*-Test for numeric and Chi-square for categorical variables.
[b] Greater than or equal to three times a week.

individual features presented a consistent association with the other components of the syndrome, except for the association between cognitive impairment and the Functional Reach test, which was of borderline significance.

Age alone explained 47% of all MMSE variance in the Met.S group, but just 12.8% in the control group (difference = 34.2%; $P < 0.001$). Analogously, the difference on the GDS variation according to age was 18.7% between the Met.S and the control groups. For all evaluated neurofunctional variables there was a significant trend for the control group to keep a more homogeneous score through the different ages when compared with the Met.S group, suggesting a faster (pathological) neuroaging process in this last group ($P < 0.001$ for all differences).

There was a significant association between Met.S and incontinence (OR = 4.8; CI: 1.0–21.1), but not between stroke and incontinence (CI: 0.5–11.9).

Fig. 1A depicts the cognitive (MMSE), affective (GDS), neuromotor (Up&Go and Functional Reach), executive (ECF-WM and ECF-ADL), and physical function (ADL) scores according to the number of Met.S risk factors. There was a consistent and significant worsening on these respective scores with increasing number of Met.S components. There were no significant differences on average age or gender distribution in these different groups.

Fig. 1B shows the respective OR for the investigated neurofunctional variables. All variables were associated with a significant higher risk for lower performance when the num-

Table 2
Metabolic syndrome (Met.S) as an associated factor for dysfunction in several neurofunctional variables and consistency of associations among the diverse variables utilized to assess the FSCS

	Met.S	Met.S PAR (%)	Frontal-subcortical geriatric syndrome components (consistency of their associations)							
			Cognitive impairment	Depression	Up&Go test	Functional Reach	Fear of falling	Falls	Urgency incontinence	ECF-WM
Met.S	–	–								
Cognitive impairment	2.23 1.1–4.6	22.5	–							
Depression	2.93 1.8–4.9	20.6	2.9 1.8–4.9	–						
Up&Go test	3.8 1.6–9.0	28.4	2.3 1.5–3.5	1.83 1.1–3.1	–					
Functional Reach	NS	NS	1.44 0.95–2.2	1.77 1.0–3.4	1.6 1.0–2.6	–				
Fear of falling	2.0 1.1–3.7	19.7	3.1 1.9–4.9	4.1 2.4–7.0	2.1 1.3–3.4	2.1 1.3–3.4	–			
Falls	2.2 1.1–4.4	21.4	2.9 1.9–4.4	2.54 1.5–4.3	2.3 1.5–3.6	1.6 1.0–2.4	3.71 2.3–3.0	–		
Urgency incontinence	4.6 1.0–21.1	30.8	3.1 2.7–3.5	5.2 1.5–18.3	2.0 1.8–2.3	4.2 1.0–17.6	5.3 1.5–19.2	3.9 1.1–14.1	–	
ECF-WM	2.2 1.1–4.5	27.1	NA	2.2 1.3–3.9	4.0 2.3–6.9	1.7 1.1–2.5	3.1 1.9–5.0	2.4 1.5–3.7	4.04 1.0–16.3	–
ECF-ADL	2.4 1.1–5.2	22.9	6.1 3.8–9.7	7.0 3.6–13.7	3.4 2.0–5.8	1.8 1.2–2.7	4.3 2.5–7.1	1.8 1.2–2.8	11.0 4.1–29.5	4.3 2.8–6.5

PAR, population attributable risk (see Section 2); ECF-WK, executive control function-working memory; ECF-ADL, executive control function-related activities of daily living; NS, not significant (if $P > 0.05$); NA, not applicable. See Section 2 for cut points of continuous variables.

ber of Met.S components was equal or higher than three ($P < 0.05$ for all).

Fig. 1C illustrates the proportion of cases considered to present "successful neuroaging", 'borderline pathological neuroaging', and FSCS. From zero to three or more Met.S components, there was a significant decrease in the percentage of 'successful neuroaging' cases, along with increasing prevalence of FSCS ($P < 0.001$, adjusted to age and gender), whereas cases with 'borderline pathological neuroaging' did not present significant differences in its distribution. The additional risk of 1 Met.S component for coexisting FSCS was 1.59 ($P = 0.017$, adjusted for age and gender).

Met.S was associated with a 2.2 (CI: 1.0–4.6; $P = 0.035$) higher likelihood of having a low ECL-WM and a 2.4 (CI: 1.1–5.2; $P = 0.021$) higher chance of impairment in the ECF-ADL (adjusted for non-ECF-ADL, IADL, age and gender). Correlation between ECF-WM and ECF-ADL scores ($R = 0.419$; $P < 0.001$) was much stronger than that between ECF-WM and the non-ECF-ADL scores ($R = 0.177$).

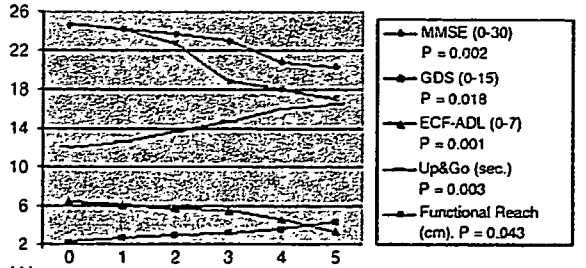
Obesity was associated only with falls (OR = 1.67; CI: 1.0–2.6) but this association disappeared once the Met.S cases were removed (CI: 0.44–2.5). Neither the other variables related to neuromotor function, nor those reflecting cognitive, affective, executive or urinary function were associated with obesity. Obesity was also not associated with FSCS itself (CI: 0.71–3.1). Moreover, adjustment for BMI did not significantly alter the associations between FSCS features and Met.S shown above.

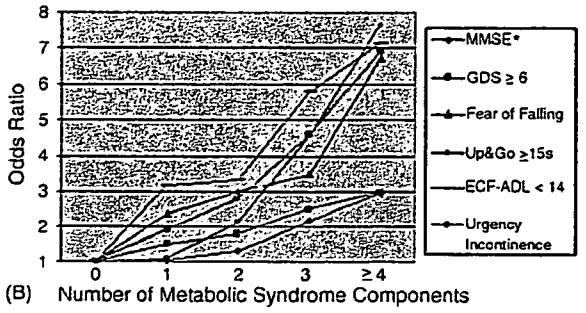
4. Discussion

Both Met.S [30] and FSCS [82] are relatively newly 'reborn' nosological concepts. Insulin resistance increases with age in most subjects [17,18]. The relationship between insulin resistance, cerebrovascular disease and neurodegenerative diseases is tantalizing in its potential to offer an integrated model for aging of the body and of the brain [18].

In the present study, 37.2% of all elderly presented no component of the FSCS and were considered as making the 'successful' neuroaging group specifically for this regard. Interestingly, a very recent metanalysis on successful aging included 29 studies and has shown that, in average, 35.8% of the investigated elderly were considered as presenting 'successful' aging [22].

In contrast, 8.6% of all clinical stroke-free elderly had a diagnosis compatible with FSCS. The Rotterdam Study has found that among community-dwelling elderly, taking 70 years as mean age (similar to our population), prevalence of silent brain infarct was nearly 17% [93]. The same study has also reported that silent brain infarcts were five times as prevalent as symptomatic ones in the general elderly popu-





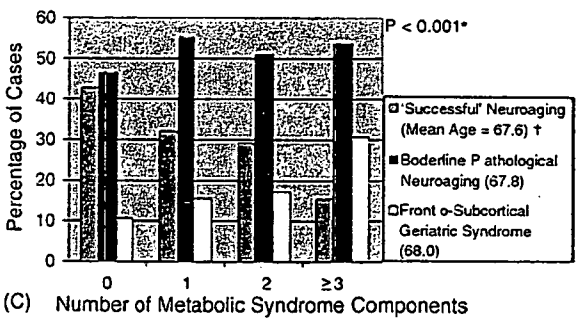


Fig. 1. (A) Cognitive, neuromotor, affective, executive, and functional variables scores according to the number of metabolic syndrome components. (B) Odds ratio for lower performance in the different neurofunctional tests according to the number of metabolic syndrome components. (C) Groups by degree of 'neuroaging' vs. number of metabolic syndrome components. MMSE, mini-mental state examination; GDS, geriatric depression scale; ECF-WK, executive control function-working memory. ECF-ADL, executive control function-related activities of daily living.

lation [93]. If this is true also for our population we would expected a prevalence of silent brain infarctions of roughly 47% (9.5% × 5), which is well above the 10.2% prevalence of FSCS here found. Therefore, among those 51.1% who had 1–3 features of the FSCS and were classified as belonging to the borderline 'pathological' neuroaging group, many subjects might still have silent strokes. This implies that our definition of FSCS had possibly a high specificity but might have excluded many milder cases in the spectrum from normality (successful neuroaging) to the FSCS. Indeed, because just 10 (2.4%) cases in our population had urgency incontinence, FCSC diagnosis was highly dependent upon the concomitant presence of cognitive impairment, depression and gait disorder. In fact, just 3 (7%) cases out of 43 depended on urgency incontinence for a diagnosis of FSCS.

As we have excluded cases with stroke episodes, and WML/lacunar strokes were already shown to be associated with FSCS and its individual compounds [53,82], we believe that frontal-subcortical small-vessel disease may be the one important mediating factor for the association between Met.S and FSCS found in this study.

The present study included just people aged 60 years and over. This moment coincides with a sharper acceleration of the decline in the cognitive function and functional status for a large proportion of individuals [75]. Rates of polio- and leuko-araiosis also accelerate geometrically after age 60, correlating with cortical and subcortical atrophy, ventricular enlargement and decreased synaptic density during aging [75,82]. However, even though leukoaraiosis is age-related, it is accelerated by hypertension, DM and oligaemia [82]. Indeed, there are evidences that Met.S and hyperinsulinemia: (1) accelerate the aging process [33], (2) are strongly associated with lacunar strokes and WML [6,50,63,71], and (3) are a risk factor for dementia [49].

FSCS was strongly associated with Met.S (OR = 6.9). Removing stroke cases decreased the power of the association by 14.5% (OR = 5.9), without changing the significance of the association. Moreover, stroke presented just a weak trend towards an association with Met.S (CI: 0.88–3.3; $P = 0.109$). Taken together these results suggest that asymptomatic lacunar strokes and WML might be responsible for an appreciable part of this association. These results may also imply that Met.S might be more closely related to microvascular cerebrovasculopathy (FSCS etiology) than to major stroke episodes.

Among the stroke-free population, prevalence of FSCS would be reduced by 32.6% if Met.S were theoretically eliminated.

Met.S was also individually associated with lower cognitive and neuromotor functions, depressive symptoms, fear of falling, falls, functional dependence and urgency incontinence. Because Met.S was associated with FSCS, hyperinsulinism and the other four major components of Met.S (obesity, HT, glucose intolerance, and dislipidemia) are probably still actuating to promote vascular disease at older age. Indeed, the number of Met.S components explained 30.7% of the variance on the number of FSCS criteria. When both variables are considered as dichotomies, i.e. having or not Met.S and FSCS, this value is significantly reduced (14.6%), suggesting the effect to be incremental. However, due to the crossectional nature of this research, these values might account for just a fraction of all the cumulative variance on FSCS attributable to Met.S. In fact, for a given cerebrovas-

cular risk factor the maximum explanatory variance upon outcomes might be found some 10–20 years, or even more, before this outcome [14].

Diagnosis of previous stroke was strongly associated with FSCS (OR = 4.2). Unfortunately, diagnose of ischemic stroke subtype was not available in this sample. However, considering that: (1) in LA lacunar strokes are often more common than atherothrombotic ones [87]; (2) silent lacunar strokes often precede clinical stroke and increase its risk by 4–10 times [51]; (3) subjects with clinical stroke have a three-fold higher chance for coexisting subcortical silent lacunar strokes [93], the association between clinical stroke and FSCS was not surprising.

Interestingly, FSCS was even more strongly associated with Met.S (OR = 5.9) than with stroke (OR = 4.2), suggesting that Met.S might have a preference for small-vessel disease, lacunar infarction and WML, all neuropathological characteristics of FSCS. Indeed, there is evidence that Met.S is less associated with large atherothrombotic stroke than with small, lacunar strokes and WML [6].

Met.S was responsible for nearly 20% of cases with 'fear of falling'. This is not surprising since gait disorders are common in cerebrovascular diseases and vascular dementia, and even predicts the development of the later [94]. Walking is generally viewed as an automated, over-learned, rhythmic motor task. New evidences suggest, however, that walking is a complex motor task. Walking was shown to be associated with higher-level cognitive resources, specifically executive function, which is dependent upon the frontal lobes [40]. Frontal gait is common in the elderly, increases the number of necessary steps, and requires longer walking an ascertained distance [40]. Frontal gait in the elderly is most often the result of cerebrovascular disease [40].

There was a significant association between Met.S and incontinence (OR = 4.8), but absence of association between stroke and incontinence (CI: 0.5–11.9). This phenomenon suggests that Met.S may impair urinary continence not through major strokes but mainly due to small-vessel disease and WML in the frontal-subcortical network. Indeed, there is evidence that, both urinary inhibition and lower motor function depend on neural fibers that pass through periventricular white matter [92], which are generally compromised by multiple WML and lacunes in the FSCS. Upper motor function is usually spared because fibers descending to the upper limbs are located further to the ventricle, being better irrigated and, hence, disturbed less frequently [44].

All individual criteria for FSCS presented a consistent association with the other features of the syndrome. This finding provides further evidence that the concept of FSCS, besides having a common etiology [54,82], is 'statistically consistent'.

Age alone explained as much as 47% of all MMSE variance in the Met.S group, but just 12.8% in the control group (difference = 34.2%). Analogously, the difference on the GDS variation according to age was 18.7% between the Met.S and the control groups. For all neurofunctional variables evaluated there was a significant trend for the control group to keep a more homogeneous score through the different ages as compared with the Met.S group, suggesting a faster (pathological) neuroaging process in this last group.

There was a consistent and significant worsening in the neurofunctional scores with the increase in the number of individual Met.S components. Moreover, with the increasing number of Met.S components there was a significant decrease in the percentage of 'successful neuroaging' cases, along with an increase in the prevalence of FSCS cases. Mean additional risk of 1 Met.S component for coexisting FSCS was 1.59.

The decrease in performance with age for each neurofunctional variable was significantly lower in the non-Met.S group than in the Met.S group ($P < 0.001$ for all). In the case of GDS there was no change at all in its mean across ages among those without Met.S. The strength of the inverse relationship between the MMSE and GDS scores was much stronger in the Met.S group ($R = -0.38$; $P < 0.001$) than in the non-Met.S group ($R = -0.11$; $P = 0.064$; $P < 0.05$ for difference), where it did not reach significance, suggesting a less important vascular relationship between MMSE and GDS in the non-Met.S group than in the Met.S group. Moreover, there were no cases of GDS ≥ 12 in the non-Met.S group, whereas the Met.S group presented three cases where GDS ≥ 12 (5.6%), in despite of the higher number of subjects in the former group (60.7%). This suggests that not just risk of depression is higher among the Met.S group, but also that depressive cases tended to be more severe in this group. Indeed, mean GDS was higher among Met.S depressed subjects (10.1) than among non-Met.S ones (8.0; $P = 0.047$).

The strong inverse relationship between MMSE and GDS scores found in this population ($R = -0.353$; $P < 0.001$) completely disappeared after adjusting for the presence of FSCS ($R = 0.206$). We believe this is not merely an effect of grouping both cognitive impairment and depression cases together when using these variables as criteria for FSCS. Indeed, among those with 'successful neuroaging' alone there was no significant correlation between MMSE and GDS ($P = 0.22$). Taken together, these results suggest that the usual association between cognitive impairment and depression in the general elderly population seems to be mainly due to a common vascular cause among those experiencing 'pathological' aging.

Elderly with Met.S were 2.2 and 2.4 times more likely to present lower ECL-WM and ECF-ADL scores, respectively, than controls. Furthermore, correlation between ECF-WM and ECF-ADL scales ($R = 0.419$; $P < 0.001$) was much stronger than that between ECF-WM and the non-ECF-ADL scale ($R = 0.177$), possibly indicating the expected shared ECF measurement between both. This is in accord with the finding that, among older people, insulin resistance is independently associated with poor performance in frontal cortex neuropsychological tests related to ECF [34].

Lack of regular exercise was significantly more common in the Met.S group. Physical activity has been shown to reduce both the risk of Met.S and stroke [78]. Reaven himself