velopment, is usually random, resulting in the generation of tissues with approximately equal numbers of cells in which the active X chromosome is of maternal or paternal origin [38]. Deviation from such an equal distribution of the two cell types can occur, however. A skewed pattern of X chromosome inactivation affecting the CAG repeat polymorphism of *AR* has been associated with other hormone-related diseases in women [38, 39, 40]. Given that no information is available on the relative extents of inactivation of the *S* and *L* alleles of *AR* in the present study, the evaluation of BMD in individuals with the *SL* genotype requires caution.

The −395G→A and 1818C→T polymorphisms of *KL* have previously been associated with BMD for the total body in white women aged 65 years or older and with that for the distal radius in Japanese women of the same age group, with BMD decreasing according to the rank orders of genotypes *GG* >*GA* >*AA* for the −395G→A polymorphism and *CC* >*CT* >*TT* for the 1818C→T polymorphism [12]. In the present study we examined the relationship of BMD at various sites to the −395G→A polymorphism but not to the 1818C→T polymorphism, since the latter is a synonymous polymorphism (His→His) and appears not to have a functional effect. We found that the −395G→A polymorphism of *KL* is significantly associated with BMD for the lumbar spine in all women, with the *GG* genotype representing a risk factor for reduced BMD. However, when premenopausal and postmenopausal women were analyzed separately, this polymorphism was not significantly related to BMD in either group, although there was a trend for the *GG* genotype to be associated with low BMD in postmenopausal women. The alleles of the −395G→A polymorphism associated with reduced BMD thus differ between the present study (*G* allele) and the previous study (*A* allele) [12]. Although the reason for this discrepancy is unclear, there are two major differences between the two studies: (a) The number of subjects in which the association was detected was greater in our study (n=1,110) than in the previous study (n=55 for white women, n=215 for Japanese women). (b) BMD was compared among *KL* genotypes with adjustment for age, height, and body weight in our study, but BMD was not adjusted in the previous study. However, it is possible that the −395G→A polymorphism of *KL* is in linkage disequilibrium with other polymorphisms of *KL* or of nearby genes that are actually the determinants of BMD. Although we adopted a strict criterion of statistical significance ($P \leq 0.005$) for the association of genotypes with BMD, we cannot completely exclude the possibility of statistical errors such as false positives.

Evidence suggests that the −395G→A polymorphism of *KL* affects promoter function [12]. Electrophoretic mobility-shift analysis revealed that the amount of DNA-protein complex formed by the *G* allele of the promoter was greater than that formed by the *A* allele, suggesting that the binding of one or more proteins to the promoter is impaired by the G→A substitution, which may affect the expression of *KL*. The effect of this polymorphism on the transcriptional activity of *KL*, however, remains to be determined.

There were no subjects with clinical vitamin D deficiency such as osteomalacia in the present population. However, National Nutrition Survey in 2001 suggested that in approximately 25% of Japanese individuals, the amount of vitamin D taken was smaller than that of daily requirement (100 IU). Serum concentrations of free thyroxine in three subjects (0.3%) slightly exceeded the normal range (0.77–1.93 ng/dl). It is thus possible that subclinical vitamin D deficiency or thyrotoxicosis affected the results obtained in the present study.

In conclusion, our present results suggest that *AR* is a determinant of BMD in premenopausal Japanese women, with the $(CAG)_{n \geq 23}$ allele representing a risk factor for reduced BMD. *KL* is also a determinant for bone mass in Japanese women, with the *G* allele being a risk factor for reduced BMD. The effects of both polymorphisms on BMD were statistically independent.

Acknowledgements This work was supported in part by Research Grants for Health and Labor Sciences Research Grants for Comprehensive Research on Aging and Health (H15-Chojyu-014) from the Ministry of Health, Labor, and Welfare of Japan.

References

1. Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
2. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S (1987) Genetic determinations of bone mass in adults: a twin study. J Clin Invest 80:706–710
3. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G (1995) Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 10:2017–2022
4. Howard GM, Nguyen TV, Harris M, Kelly PJ, Eisman JA (1998) Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. J Bone Miner Res 13:1318–1327
5. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB (1997) Linkage of a gene causing high bone mass to human chromosome 11 (11q12–13). Am J Hum Genet 60:1326–1332
6. Devoto M, Shimoya K, Caminis J, Ott J, Tenenhouse A, Whyte MP, Sereda L, Hall S, Considine E, Williams CJ, Tromp G, Kuivaniemi H, Ala-Kokko L, Prockop DJ, Spotila LD (1998) First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4p. Eur J Hum Genet 6:151–157
7. Econs MJ, Koller DL, Hui SL, Fishburn T, Conneally PM, Johnston CC Jr, Peacock M, Foroud TM (2004) Confirmation of linkage to chromosome 1q for peak vertebral bone mineral density in premenopausal white women. Am J Hum Genet 74:223–228
8. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA (1994) Prediction of bone density from vitamin D receptor alleles. Nature 367:284–287
9. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA, Grant SFA, Hofman A, van Leeuwen JPTM, Pols HAP, Ralston SH (1998) Relation of alleles of the collagen type Iα1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. N Engl J Med 338:1016–1021

10. Yamada Y, Ando F, Niino N, Shimokata H (2001) Transforming growth factor-β1 gene polymorphism and bone mineral density. JAMA 285:167–168
11. Sowers M, Willing M, Burns T, Deschenes S, Hollis B, Crutchfield M, Jannausch M (1999) Genetic markers, bone mineral density, and serum osteocalcin levels. J Bone Miner Res 14:1411–1419
12. Kawano K, Ogata N, Chisano M, Molloy H, Kleyn P, Spector TD, Uchida M, Hosoi T, Suzuki T, Orimo H, Inoue S, Nabeshima Y, Nakamura K, Kuro-o M, Kawaguchi H (2002) Klotho gene polymorphisms associated with bone density of aged postmenopausal women. J Bone Miner Res 17:1744–1751
13. Vanderschueren D, Bouillon R (1995) Androgens and bone. Calcif Tissue Int 56:341–346
14. Pederson L, Kremer M, Judd J, Pascoe D, Spelsberg TC, Riggs BL, Oursler MJ (1999) Androgens regulate bone resorption activity of isolated osteoclasts in vitro. Proc Natl Acad Sci USA 96:505–510
15. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E (2000) Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 92:2009–2017
16. Hsing AW, Gao Y-T, Wu G, Wang X, Deng J, Chen Y-L, Sesterhenn IA, Mostofi FK, Benichou J, Chang C (2000) Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. Cancer Res 60:5111–5116
17. Chamberlain NL, Driver ED, Miesfeld RL (1994) The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 22:3181–3186
18. Kazemi-Esfarjani P, Trifiro MA, Pinsky L (1995) Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG) n-expanded neuronopathies. Hum Mol Genet 4:523–527
19. Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 82:3777–3782
20. Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nat Genet 5:184–188
21. Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E (2001) The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. Clin Endocrinol (Oxf) 55:649–657
22. Langdahl BL, Stenkjaer L, Carstens M, Tofteng CL, Eriksen EF (2003) A CAG repeat polymorphism in the androgen receptor gene is associated with reduced bone mass and increased risk of osteoporotic fractures. Calcif Tissue Int 73:237–243
23. Chen H-Y, Chen W-C, Wu M-C, Tsai F-J, Tsai C-H (2003) Androgen receptor (AR) gene microsatellite polymorphism in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. Eur J Obstet Gynaecol Reprod Biol 107:52–56
24. Tofteng CL, Kindmark A, Brändström H, Abrahamsen B, Petersen S, Stiger F, Stilgren LS, Jensen JEB, Vestergaard P, Langdahl BL, Mosekilde L (2004) Polymorphisms in the CYP19 and AR genes—relation to bone mass and longitudinal bone changes in postmenopausal women with or without hormone replacement therapy: the Danish Osteoporosis Prevention Study. Calcif Tissue Int 74:25–34
25. Salmén T, Heikkinen A-M, Mahonen A, Kröger H, Komulainen M, Pallonen H, Saarikoski S, Honkanen R, Mäenp PH (2003) Relation of androgen receptor gene polymorphism to bone mineral density and fracture risk in early postmenopausal women during a 5-year randomized hormone replacement therapy trial. J Bone Miner Res 18:319–324
26. Remes T, Väisänen SB, Mahonen A, Huuskonen J, Kröger H, Jurvelin JS, Penttilä IM, Rauramaa R (2003) Aerobic exercise and bone mineral density in middle-aged Finnish men: a controlled randomized trial with reference to androgen receptor, aromatase, and estrogen receptor α gene polymorphisms. Bone 32:412–420
27. Irvine RA, Yu MC, Ross RK, Coetzee GA (1995) The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 55:1937–1940
28. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima Y (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390:45–51
29. Kawaguchi H, Manabe N, Miyaura C, Chikuda H, Nakamura K, Kuro-o M (1999) Independent impairment of osteoblast and osteoclast differentiation in klotho mouse exhibiting low-turnover osteopenia. J Clin Invest 104:229–237
30. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y (1998) Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. Biochem Biophys Res Commun 242:626–630
31. Ogata N, Matsumura Y, Shiraki M, Kawano K, Koshizuka Y, Hosoi T, Nakamura K, Kuto-o M, Kawaguchi H (2002) Association of klotho gene polymorphism with bone density and spondylosis of the lumbar spine in postmenopausal women. Bone 31:37–42
32. Shimokata H, Ando F, Niino N (2000) A new comprehensive study on aging—the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10:S1–S9
33. Yamada Y, Ando F, Niino N, Shimokata H (2003) Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. J Clin Endocrinol Metab 88:3372–3378
34. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M, Yokota M (2002) Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 347:1916–1923
35. Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA (2000) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet 9:267–274
36. Hsiao P-W, Lin D-L, Nakao R, Chang C (1999) The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem 274:20229–20234
37. Westberg L, Baghaei F, Rosmond R, Hellstrand M, Landén M, Jansson M, Holm G, Björntorp P, Eriksson E (2001) Polymorphisms of the androgen receptor gene and the estrogen receptor β gene are associated with androgen levels in women. J Clin Endocrinol Metab 86:2562–2568
38. Buller RE, Sood AK, Lallas T, Buekers T, Skilling JS (1999) Association between nonrandom X-chromosome inactivation and BRCA1 mutation in germline DNA of patients with ovarian cancer. J Natl Cancer Inst 91:339–346
39. Vottero A, Stratakis CA, Ghizzoni L, Longui CA, Karl M, Chrousos GP (1999) Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation. J Clin Endocrinol Metab 84:1091–1095
40. Kristiansen M, Langerød A, Knudsen GP, Weber BL, Børresen-Dale A-L, Ørstavik KH (2002) High frequency of skewed X inactivation in young breast cancer patients. J Med Genet 39:30–33

ELSEVIER

Cholecystokinin A Receptor Gene Promoter Polymorphism and Intelligence

HIROSHI SHIMOKATA, MD, PHD, FUJIKO ANDO, MD, PHD, NAOAKIRA NIINO, MD, PHD, KYOKO MIYASAKA, MD, PHD, AND AKIHIRO FUNAKOSHI, MD, PHD

PURPOSE: To study the association between Cholecystokinin A receptor (CCKAR) genotypes and intelligence in community-living men and women.
METHOD: Subjects were 2251 community-dwelling Japanese men and women aged 40 to 79 years. The CCKAR gene promoter polymorphisms A-81G and G-128T were determined. Intelligence was assessed by Japanese Wechsler Adult Intelligence Scales – Revised Short Forms (JWAIS-R SF). The difference in intelligence between wild type and mutation was tested.
RESULTS: There were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations. Both A-81G and G-128T genotypes were related to intelligence quotient (IQ) estimated by JWAIS-R SF. The mean and SE of IQ levels of subjects with the wild-type allele and the mutation allele at nucleotide -128 were 103.4 ± 0.3 and 101.6 ± 0.6, respectively. There was a significant difference in IQ for G-128T ($p = 0.008$). The difference in IQ for A-81G was also significant ($p = 0.011$). The IQ level was 103.6 ± 0.4 in the subjects with the wild-type allele and 102.0 ± 0.5 in the subjects with the mutation. Differences in IQ levels by haplotypes for combinations of A-81G/G-128T were examined. IQ significantly decreased with an increasing number of mutation alleles ($p = 0.018$).
CONCLUSION: There were statistically significant differences in IQ for CCKAR gene promoter polymorphisms A-81G and G-128T in community-living Japanese.
Ann Epidemiol 2005;15:196–201.

KEY WORDS: Cholecystokinin, Intelligence, Genotype, Epidemiology.

INTRODUCTION

It is suspected that various genes influence intelligence, but the association between gene polymorphism and intelligence is still unclear. Cholecystokinin (CCK) is one of the major physiologic substances of gallbladder contraction and pancreatic enzyme secretion. CCK also plays an important role in the central nervous system (CNS) by interacting with dopamine and other neurotransmitters (1). CCK receptors have been classified into two subtypes, CCK type-A receptor (CCKAR) and type-B receptor (CCKBR). CCKAR has been found in the CNS (2). Associations with feeding disorders (3), anxiety (4), and schizophrenia (5) have been reported. It was also reported that learning and memory functions were impaired in CCKAR gene-knockout (OLETF) rats (6, 7). The CCKAR gene may be related to intelligence in humans. We examined the association between CCKAR gene promoter polymorphisms and intelligence in a group of 2251 community-dwelling Japanese men and women.

METHODS

Subject Selection

The subjects in this study were participants in the National Institute for Longevity Sciences – Longitudinal Study of Aging (NILS-LSA) (8). The NILS-LSA started in November 1997. The first phase of examinations was finished by the end of March 2000, and followed-up every 2 years. Participants in the NILS-LSA were independent residents in Obu city and Higashiura town in Aichi prefecture, central Japan. Data on all residents in the area are maintained in a Resident Registration System by local governments. Residents aged 40 to 79 years old were selected using Resident Registration. Samples of 7790 males and females were selected by age and gender stratified random sampling and invited to an explanatory meeting by mail. The number of replies was 3434. Of these, 881 refused to attend the meeting, 2553 agreed to attend, and 2513 actually attended. After the meeting, 2267 participated in the first phase examination. At the meeting, the procedures

From the Department of Epidemiology, National Institute for Longevity Sciences, Obu Aichi, Japan (H.S., F.A., N.N.); Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (K.M.); and Department of Gastroenterology, National Kyushu Cancer Center, Fukuoka, Japan (A.F.).

Address correspondence to: Hiroshi Shimokata, M.D., Ph.D., Department of Epidemiology, National Institute for Longevity Sciences, 36-3 Gengo, Morioka-cho, Obu Aichi 474-8522, Japan. Tel.: +81-562-46-2311, ext. 815; Fax: +81-562-44-6593. E-mail: hiroshi@nils.go.jp

This work was supported in part by Research Grants for Longevity Sciences (12C-01) from the Ministry of Health and Welfare of Japan to Drs. H. Shimokata and A. Funakoshi.

Received June 6, 2004; accepted June 14, 2004.

360 Park Avenue South, New York, NY 10010

1047-2797/05/$–see front matter
doi:10.1016/j.annepidem.2004.06.007

Selected Abbreviations and Acronyms

BMI = body mass index
CCK = cholecystokinin
CCKAR = cholecystokinin A receptor
CNS = central nervous system
DNA = deoxyribonucleic acid
GLM = general linear model
IQ = intelligence quotient
JWAIS-R-SF = Japanese Wechsler Adult Intelligence Scales – Revised Short Forms
NILS-LSA = National Institute for Longevity Sciences – Longitudinal Study of Aging
PCR-RFLP = polymerase chain reaction – restriction fragment length polymorphism
OLETF = Otsuka Long-Evans Tokushima Fatty
SE = standard error
WAIS-R = Wechsler Adult Intelligence Scales – Revised

for each examination and follow-up schedule were fully explained. Written informed consent to participate in all procedures was obtained from each subject. All persons in the Resident Registration list had Japanese nationality, and there were no persons who had a foreign name among the subjects. The subjects in this study were supposed to be ethnically homogenous Japanese.

Among the 2267 participants in the first phase examination, 2251 men and women were evaluated for CCKAR genotypes and intelligence. These subjects were analyzed for cross-sectional associations between genotype and intelligence. The number of the subjects by gender and age was almost equal (Table 1). The mean and standard deviation for age was 59.2 ± 10.9 years. Among the subjects, 26.7% had an educational background of college or greater. The Ethical Committee of Chubu National Hospital approved all procedures of the NILS-LSA.

Evaluation of Intelligence and Other Variables

The Wechsler Adult Intelligence Scales – Revised (WAIS-R) is one of the most popular tools used to assess intelligence (9). A Japanese version of the WAIS-R (JWAIS-R) has been developed and is widely used in Japan (10). In this study, intelligence was assessed by the Japanese Wechsler Adult Intelligence Scales - Revised - Short Forms (JWAIS-R-SF) (11). The JWAIS-R-SF consists of the following four subtests: Information, Similarities, Picture Completion, and Digit Symbol. Scaled scores of subtests were used in the analysis. The intelligence quotient (IQ) was estimated from the combination of these four subtests. Psychologists conducted the interviews and JWAIS-R-SF tests. Height and weight were measured while wearing lightweight clothes, and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Life-style and medical history including annual income, education, and smoking status were checked by questionnaires. The questionnaires were checked by a physician at the medical examination. All drugs used during the previous 2 years were to be documented by participants; the physician confirmed them at an interview and coded the drugs used during the last 2 weeks. Among the 2251 subjects in the study, 213 had used drugs acting on the CNS, that is, hypnotic sedative agents, antianxiety agents, antiepileptic agents, stimulant drugs, antihypnotic drugs, anti-Parkinson drugs, and anti-psychotic drugs during the previous 2 weeks. The IQ was less than 70 in 11 subjects, and only one of them used drugs acting on the CNS.

TABLE 1. Distribution of the subjects by gender and age

	Age (years)				
Gender	40–49	50–59	60–69	70–79	Total
Males	291	282	281	280	1134
Females	278	278	283	278	1117
Total	569	560	564	558	2251

CCKAR Genotype Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by a standard procedure. A mismatch PCR-RFLP method was used to analyze polymorphisms in the upstream region of the CCKAR gene [GenBank Accession No. U23427 (5)]. One pair of primers, sense primer = 5′-GCATATGTACACATGTGTGTAAAAAGCAGCCAGAC-3′, anti-sense primer = 5′-GCCCTTTCCTGGGCCAGACT-3) was designed to amplify a 103-base pair product, digested with restriction enzyme Hinf I, and analyzed by 3% agarose gel electrophoresis. Two sequence changes were detected: a G to *T* change at nucleotide -128, and an A to G change at nucleotide -81 (12).

Statistical Analysis

All values were expressed as the mean ± SE, if not specified. Both polymorphisms at nucleotides -128 and -81 were divided into two groups; as wild-type and mutation. Hetero groups were classified as mutation. The difference between wild-type and mutation groups was tested by the *t*-test for continuous variables and the 2 × 2 chi-square test for categorical variables. The difference in IQ and JWAIS-R subtests score by genotype was also tested by the *t*-test excluding subjects who had used drugs acting on the CNS or subjects with IQ less than 70. The trend among the three groups was tested by the general linear model (GLM) and the probability for trend (p for trend) was shown. Statistical analyses were performed using the SAS system (SAS Institute Inc., Cary, NC). All p-values were two-tailed.

AEP Vol. 15, No. 3
March 2005: 196–201

RESULTS

Distribution of CCKAR Promoter Genotypes

The distributions of CCKAR promoter single nucleotide polymorphisms A-81G and G-128T were both in Hardy-Weinberg equilibrium. The distribution of genotype combination was examined (Table 2). These polymorphisms were in linkage disequilibrium. There were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations. Thus, subjects with a mutation at -128 always had a mutation at -81.

Background Characteristics and CCKAR Genotype

Figure 1 shows the IQ distribution. The distribution was slightly skewed to the left (lower IQ) and close to a normal distribution. The mean value of the IQ of the all subjects was 103.0, and the median was also 103. The difference between the mean and median was very small. The lowest IQ was 43 and the highest IQ was 142 among the subjects. The number of subjects with IQ less than 70 was 11, and those with IQ 135 or over was 13. Background characteristics were compared by CCKAR G-128T and A-81G genotypes (Table 3). Age, body weight, body mass index, annual income, education, and smoking status did not differ between wild-type (GG) and mutation (*GT* or *TT*) for the CCKAR G-128T genotype. These variables also did not differ for the CCKAR A-81G genotype except for education status. Education status in the wild-type (*AA*) group was significantly higher than that in the mutation-type (*AG* or GG) group ($p = 0.009$). The IQ was significantly different by education status ($p < 0.001$). The IQ for the low education group was 100.3 ± 0.3 and that for the high education group was 110.6 ± 0.5.

Intelligence and CCKAR Genotype

The IQ levels in subjects with wild-type and mutation alleles at nucleotide -128 were 103.4 ± 0.3 and 101.6 ± 0.6, respectively. There was a significant difference in IQ for the G-128T genotype ($p = 0.008$). The score of Digit Symbol was lower in subjects with a mutation ($p = 0.003$). There was no difference in the scores of Information, Picture Completion, and Similarities subtests for polymorphism G-128T. The IQ level was 103.6 ± 0.4 in subjects with wild-type (AA) and 102.0 ± 0.5 in subjects with mutation (AG or GG) at nucleotide -81. The difference in IQ for the A-81G polymorphism was significant ($p = 0.011$). The Picture Completion and Digit Symbol subtest scores were significantly lower in subjects with the mutation ($p = 0.043$ and $p = 0.008$, respectively). The Similarities subtest score was marginally lower for a mutation at nucleotide -81 ($p = 0.051$).

In the low education group, IQ was 100.5 ± 0.4 in the -128 wild-type group and 99.5 ± 0.6 in the -128 mutation-type group. There was no significant difference in IQ between the wild- and mutation-type of G-128T genotype. However, the IQ for the -81 wild-type group was 100.8 ± 0.4, which was significantly higher than that for the mutation group (99.4 ± 0.4) ($p = 0.038$). In the high education group, the IQ was 111.5 ± 0.6 in the -128 wild-type group and 107.9 ± 1.1 in the -128 mutation-type group. There was a significant difference between the wild and mutation groups ($p = 0.004$). The IQ in the -81 wild-type group (111.1 ± 0.7) did not differ from that in the mutation group (109.8 ± 0.9).

Intelligence was compared excluding subjects who had used drugs acting on the CNS and subjects with IQ less than 70 (Table 4). The number of excluded subjects was 223. Differences in IQ between in the wild-type and mutation groups were still significant both for A-81G and G-128T

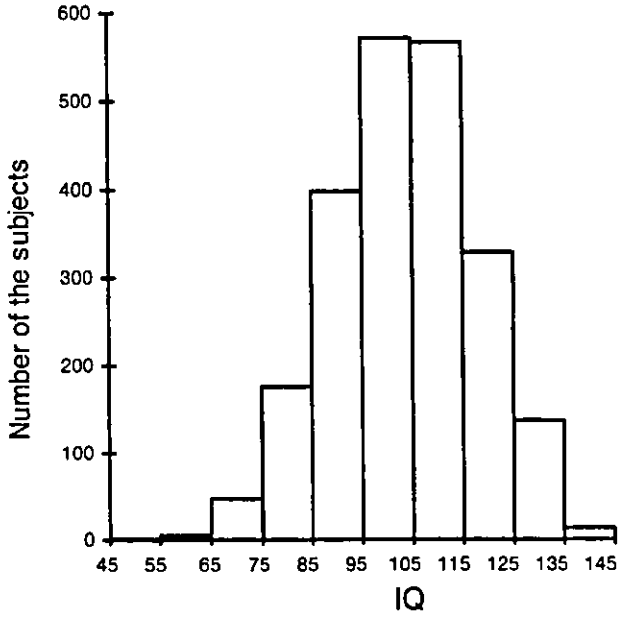

FIGURE 1. Distribution of IQ levels in the subjects.

TABLE 2. Distribution of CCKAR G-81T and A-128G genotypes

CCKAR G-128T	CCKAR A-81G AA	AG	GG	Total
GG	1317 (58.5%)	307 (13.6%)	26 (1.2%)	1650 (73.3%)
GT	0 (0.0%)	491 (21.8%)	61 (2.7%)	552 (24.5%)
TT	0 (0.0%)	0 (0.0%)	49 (2.2%)	49 (2.2%)
Total	1317 (58.5%)	798 (35.5%)	136 (6.0%)	2251 (100.0%)

TABLE 3. Comparison of variables between wild-type and mutation alleles in CCKAR G-81T and A-128G genotypes

	CCKAR G-128T			CCKAR A-81 G		
	Wild type GG	Mutation GT or TT	p*	Wild type AA	Mutation AG or GG	p
n	1650	601		1317	333	
Age (years)	59.2 ± 0.3†	59.3 ± 0.4	NS‡	59.1 ± 0.3	59.5 ± 0.4	NS
Weight (kg)	57.5 ± 0.2	57.0 ± 0.4	NS	57.6 ± 0.3	57.0 ± 0.3	NS
BMI (kg/m^2)	22.9 ± 0.1	22.9 ± 0.1	NS	22.9 ± 0.1	22.9 ± 0.1	NS
Annual income (%; 54,000 US$ or over)	57.5	58.3	NS	58.3	57.0	NS
Education (%; college or over)	26.9	26.0	NS	27.4	25.6	0.009
Smoking (%; smoker)	22.8	22.8	NS	23.6	21.8	NS
JWAIS-R-SF						
IQ	103.4 ± 0.3	101.6 ± 0.6	0.008	103.6 ± 0.4	102.0 ± 0.5	0.011
Information	9.9 ± 0.1	9.7 ± 0.1	NS	9.9 ± 0.1	9.8 ± 0.1	NS
Picture Completion	10.2 ± 0.1	10.0 ± 0.1	NS	10.2 ± 0.1	10.0 ± 0.1	0.043
Similarities	10.3 ± 0.1	10.1 ± 0.1	NS	10.3 ± 0.1	10.1 ± 0.1	0.051
Digit Symbol	11.7 ± 0.1	11.3 ± 0.1	0.003	11.7 ± 0.1	11.4 ± 0.1	0.008

†Mean ± SE.
‡NS = not significant.
*p-value tested by the t-test or χ^2 test.

genotypes. The IQ levels of subjects with wild-type and mutation alleles at nucleotide -128 were 104.1 ± 0.4 and 102.0 ± 0.6, respectively. There was a significant difference in IQ ($p = 0.002$). The scores of Information and Digit Symbol were significantly lower in subjects with a mutation ($p = 0.012$ and $p = 0.003$, respectively). There were no differences in the scores of Picture Completion and Similarities subtests for polymorphism G-128T. The IQ level was 104.2 ± 0.4 in the subjects with wild-type and 102.6 ± 0.5 in the subjects with mutation at nucleotide -81. Difference in IQ by A-81G polymorphism was significant ($p = 0.008$). Similarities and Digit Symbol subtest scores were significantly lower in subjects with the mutation ($p = 0.033$ and $p = 0.013$, respectively). The Information subtest score was marginally lower with mutation of nucleotide -81 ($p = 0.078$). However, there was no significant difference in the score of Picture Completion subtest.

Haplotype Analysis

Possible haplotypes in the combinations of polymorphism A-81G/G-128T were GA, GG, TG, and TA. However, there were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations (Table 2). The common haplotype of *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations was TA. It was considered that no subject had a TA haplotype. The distribution of haplotypes GA, GG, and TG is shown in Table 5. The number of GA haplotypes was 3432; GG was 420; and TG was 650. There was a significant difference in IQ among haplotypes GA, GG, and TG. The IQ for haplotype GA was the highest and the IQ for haplotype TG was the lowest. With an increase in the number of mutation alleles, the IQ level decreased ($p = 0.018$). Digit Symbol scores also significantly decreased with an increasing number of mutation alleles ($p = 0.012$).

TABLE 4. Comparison of intelligences between wild-type and mutation alleles in CCKAR G-81T and A-128G genotypes. Subjects who had used drugs acting on the CNS or subjects with IQ less than 70 were excluded

	CCKAR G-128T			CCKAR A-81G		
	Wild type GG	Mutation GT or TT	p*	Wild type AA	Mutation AG or GG	p
n	1489	539		1178	850	
JWAIS-R-SF						
IQ	104.1 ± 0.4†	102.0 ± 0.6	0.002	104.2 ± 0.4	102.6 ± 0.5	0.008
Information	10.0 ± 0.1	9.6 ± 0.1	0.012	10.0 ± 0.1	9.8 ± 0.1	0.078
Picture Completion	10.2 ± 0.1	10.1 ± 0.1	NS‡	10.3 ± 0.1	10.1 ± 0.1	NS
Similarities	10.4 ± 0.1	10.2 ± 0.1	NS	10.4 ± 0.1	10.2 ± 0.1	0.033
Digit Symbol	11.8 ± 0.1	11.4 ± 0.1	0.003	11.8 ± 0.1	11.5 ± 0.1	0.013

†Mean ± SE.
‡NS = not significant.
*p-value tested by the t-test.

AEP Vol. 15, No. 3
March 2005: 196–201

TABLE 5. Comparison of intelligences between wild-type and mutation alleles in CCKAR G-81T and A-128G genotypes

	Haplotype			
	GA	GG	TG	p for trend*
n	3432	420	650	
JWAIS-R-SF				
IQ	103.2 ± 0.2†	103.0 ± 0.7	101.7 ± 0.6	0.018
Information	10.0 ± 0.1	9.8 ± 0.1	9.7 ± 0.1	NS‡
Picture Completion	10.2 ± 0.1	10.1 ± 0.1	10.0 ± 0.1	NS
Similarities	10.3 ± 0.1	10.1 ± 0.1	10.1 ± 0.1	NS
Digit symbol	11.6 ± 0.1	11.6 ± 0.1	11.3 ± 0.1	0.012

†Mean ± SE.
‡NS = not significant.
*Trend of the three groups was tested by the general linear model.

DISCUSSION

Accumulating data support the involvement of the dopaminergic system in cognitive processing. It is known that CCKAR modulates CCK-stimulated dopamine release in the brain, and mutations in the CCKAR gene may influence the dopaminergic system (5). Considerable preclinical and clinical evidence indicate that inhibitory effects on dopaminergic systems by antipsychotic medications may account for cognitive impairment. A report showed sustained activation of the human mesolimbic dopaminergic system during the performance of cognitive tasks (13). It was also reported that systemic administration of the CCKAR selective antagonist, devazepide, impaired the development of conditioned incentive learning in rats (14). From these data, it is suspected that mutation in the CCKAR gene may influence intelligence.

The CCKAR promoter genotypes were significantly related to IQ. The IQ levels of subjects with the mutant allele were significantly lower than those of subjects with the wild-type allele both for G-128T and A-81G genotypes. A difference in IQ by CCKAR promoter gene polymorphisms was seen in both middle-aged and elderly people. In analyses excluding the subjects who had used drugs acting on the CNS and subjects with IQ less than 70, there was also a significant difference in IQ between the wild and mutation genotypes. We carried out association studies of quantitative traits with haplotypes, and found that the IQ became lower with an increase in the number of mutation alleles.

The CCKAR gene polymorphisms of G-128T and A-81G are located in the promoter region of the gene. It is suspected that mutation of these genotypes is related to the amount of CCKAR production. However, it is still unclear whether these CCKAR polymorphisms are functional or if they are in linkage disequilibrium with other as yet unknown polymorphisms in the CCKAR gene or in a neighboring gene.

In the studies on intelligence in the general population, investigation of genetic factors is an important issue (15). However, at the present time, gene polymorphism has infrequently been reported to be associated with cognition (16). It is suspected that there are many genes associated with individual differences in intelligence, and intelligence is determined from interactions of these gene polymorphisms. However, the contribution of each gene to intelligence may be small as indicated by the results of this study. Testing of thousands of subjects is required to detect small but significant differences. A detailed assessment of IQ requires interviews by psychologists. Assessment of IQ in a large-scale community-dwelling population is generally difficult. It is also difficult to obtain DNA specimens from community-dwelling populations. Because of this, studies on the association between genotype and intelligence have not progressed. In the present study, we showed the relationship between intelligence and CCKAR promoter mutations G-128T and A-81G in community-living middle-aged and elderly Japanese. CCKAR-promoter genotyping may provide useful information for assessing intelligence and preventing cognitive impairment.

REFERENCES

1. Hansen TV. Cholecystokinin gene transcription: Promoter elements, transcription factors, and signaling pathways. Peptides. 2001;22:1201–1211.
2. Hill DR, Shaw TM, Graham W, Woodruff GN. Autoradiographical detection of cholecystokinin-A receptors in primate brain using 125I-Bolton Hunter CCK-8 and 3H-MK-329. J Neurosci. 1990;10:1070–1081.
3. Brenner LA, Ritter RC. Type A CCK receptors mediate satiety effects of intestinal nutrients. Pharmacol Biochem Behav. 1996;54:625–631.
4. Lydiard RB. Neuropeptides and anxiety: Focus on cholecystokinin. Clin Chem. 1994;40:315–318.
5. Tachikawa H, Harada S, Kawanishi Y, Okubo T, Shiraishi H. Novel polymorphisms of the human cholecystokinin A receptor gene: An association analysis with schizophrenia. Am J Med Genet. 2000;96:141–145.
6. Nomoto S, Miyake M, Ohta M, Funakoshi A, Miyasaka K. Impaired learning and memory in OLETF rats without cholecystokinin (CCK)-A receptor. Physiol Behav. 1999;66:869–872.
7. Li XL, Aou S, Hori T, Oomura Y. Spatial memory deficit and emotional abnormality in OLETF rats. Physiol Behav. 2002;75:15–23.
8. Shimokata H, Ando F, Niino N. A new comprehensive study on aging—The National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol. 2000;10:S1–S9.
9. Wechsler D. Wechsler Adult Intelligence Scale – Revised manual. New York: Psychological Corporation; 1981.
10. Shinagawa F, Kobayashi S, Fujita K, Maekawa HH. Japanese Wechsler Adult Intelligence Scale–Revised [in Japanese]. Tokyo: Nihon-Bunka-Kagaku-Sha (Japan Culture and Science Company); 1990.
11. Kobayashi S, Fujita K, Maekawa H, Dairoku H. Short form method in Japan [in Japanese]. In: Misawa G, ed. Japanese Wechsler Adult Intelligence Scale-Revised Short Forms. Tokyo: Nihon-Bunka-Kagaku-Sha (Japan Culture and Science Company); 1993:23–55.
12. Funakoshi A, Miyasaka K, Matsumoto H, Yamamori S, Takiguchi S, Kataoka K, et al. Gene structure of human cholecystokinin (CCK) type-A receptor: Body fat content is related to CCK type-A receptor gene promoter polymorphism. FEBS Lett. 2000;466:264–266.

13. Fried I, Wilson CL, Morrow JW, Cameron KA, Behnke ED, Ackerson LC, et al. Increased dopamine release in the human amygdala during performance of cognitive tasks. Nat Neurosci. 2001;4:201–206.
14. Josselyn SA, Franco VP, Vaccarino FJ. Devazepide, a CCKA receptor antagonist, impairs the acquisition of conditioned reward and conditioned activity. Psychopharmacology. 1996;123:131–143.
15. Bouchard TJ Jr, McGue M. Familial studies of intelligence: A review. Science. 1981;212:1055–1059.
16. Payton A, Holland F, Diggle P, Rabbitt P, Horan M, Davidson Y, et al. Cathepsin D exon 2 polymorphism associated with general intelligence in a healthy older population. Mol Psychiatry. 2003;8:14–18.

Association of a −1997G → T Polymorphism of the Collagen Iα1 Gene with Bone Mineral Density in Postmenopausal Japanese Women

YOSHIJI YAMADA,[1] Fujiko Ando,[2] Naoakira Niino,[2] and Hiroshi Shimokata[2]

Abstract Genetic variants that affect collagen Iα1 metabolism may be important in the development of osteoporosis or osteoporotic fractures. A −1997G → T polymorphism in the promoter of the collagen Iα1 gene (COL1A1) was shown to be associated with bone mineral density (BMD) for the lumbar spine in postmenopausal Spanish women. The relation of this polymorphism to BMD in Japanese women or men has now been examined in a population-based study. The subjects (1,110 women, 1,126 men) were 40 to 79 years of age and were randomly recruited for a population-based prospective cohort study of aging and age-related diseases. BMD for the lumbar spine, right femoral neck, right trochanter, and right Ward's triangle was measured using dual-energy x-ray absorptiometry. Genotypes for the −1997G → T polymorphism of COL1A1 were determined with a fluorescence-based allele-specific DNA primer assay system. When all women were analyzed together, BMD for the lumbar spine and trochanter was significantly lower in subjects with the *COL1A1*G/*G* genotype than in those in the combined group of *COL1A1*G/*T* and *COL1A1*T/*T* genotypes. When postmenopausal women were analyzed separately, BMD for the femoral neck and trochanter was also significantly lower in those with the *COL1A1*G/*G* genotype than in those with the *COL1A1*G/*T* genotype or those in the combined group of *COL1A1*G/*T* and *COL1A1*T/*T* genotypes. BMD was not associated with −1997G → T genotype in premenopausal women or in men. Multivariate regression analysis revealed that −1997G → T genotype affected BMD at various sites with a variance of 0.46–0.62% for all women and 0.61–1.01% for postmenopausal women. The −1997G → T genotype was not related to the serum concentration of osteocalcin, the serum activity of bone-specific alkaline phosphatase, or the urinary excretion of deoxypyridinoline or cross-linked N-telopeptides of type I collagen in men or in premenopausal or postmenopausal women. These results suggest that COL1A1 is a susceptibility locus for reduced BMD in postmenopausal Japanese women.

[1]Department of Human Functional Genomics, Life Science Research Center, Mie University, 1515 Kamihama, Tsu, Mie 514-8507, Japan.
[2]Department of Epidemiology, National Institute for Longevity Sciences, Obu, Aichi, Japan.

Human Biology, February 2005, v. 77, no. 1, pp. 27–36.

KEY WORDS: BONE MINERAL DENSITY, COLLAGEN Iα1, COLA1A, OSTEOPOROSIS, JAPAN.

Osteoporosis, a major health problem of the elderly, is characterized by a reduction in bone mineral density (BMD) and a deterioration in the microarchitecture of bone, both of which result in predisposition to fractures (Kanis et al. 1994). Although several environmental factors, including diet, smoking, and physical exercise, influence BMD, a genetic contribution to this parameter has been recognized (Peacock et al. 2002). Genetic linkage analyses (Morrison et al. 1994; Johnson et al. 1997; Devoto et al. 1998; Koller et al. 1998, 2000; Niu et al. 1999) and candidate gene association studies (Morrison et al. 1994; Kobayashi et al. 1996; Uitterlinden et al. 1998; Yamada et al. 2001; Ishida et al. 2003) have implicated several loci and candidate genes in the regulation of bone mass and the prevalence of osteoporosis or osteoporotic fractures. However, the genes that contribute to genetic susceptibility to osteoporosis remain to be identified definitively.

Type I collagen is the most abundant protein of bone matrix. Mutations in the coding regions of the genes for the two type I collagen chains (COL1A1 and COL1A2) result in a severe autosomal dominant pediatric condition known as osteogenesis imperfecta (Sykes 1990). A G → T single nucleotide polymorphism (SNP) at the first base of a consensus binding site for the transcription factor Sp1 in the first intron of COL1A1 was associated not only with BMD in white women (Grant et al. 1996) but also with osteoporotic fractures in postmenopausal women (Langdahl et al. 1998; Uitterlinden et al. 1998). The *COL1A1*T* allele of this polymorphism affects collagen gene regulation in such a manner that it increases the production of the α1(I) collagen chain relative to that of the α2(I) chain and leads to reduced bone strength by a mechanism that is partly independent of bone mass (Mann et al. 2001). These observations thus implicate genetic variants that affect collagen Iα1 metabolism as important determinants of the development of osteoporosis and osteoporotic fractures. Other studies, however, have shown only a weak association of the Sp1 binding site polymorphism with BMD or osteoporotic fractures in premenopausal French women (Garnero et al. 1998) or a lack of association in postmenopausal women in Sweden (Liden et al. 1998), in American women (Hustmyer et al. 1999), or in postmenopausal Danish women (Heegaard et al. 2000).

A −1997G → T SNP in the promoter of COL1A1 was also associated with BMD for the lumbar spine in postmenopausal Spanish women, and this SNP and the G → T SNP of the Sp1 binding site of COL1A1 were shown to be in linkage disequilibrium (Garcia-Giralt et al. 2002). Given the ethnic divergence of gene polymorphisms, it is important to examine polymorphisms potentially related to BMD in each ethnic group. We have now examined whether the −1997G → T SNP of COL1A1 is associated with BMD in Japanese women or men in a population-based study.

Materials and Methods

Study Population. The National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA) is a population-based prospective cohort study

of aging and age-related diseases (Shimokata et al. 2000). The subjects of the NILS-LSA are stratified by both age and sex and are randomly selected from resident registrations in the city of Obu and the town of Higashiura in central Japan (Yamada et al. 2003a, 2003b). Individuals with disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, renal diseases, rheumatoid arthritis, and thyroid, parathyroid, and other endocrinologic diseases, were excluded from the study. Women who had taken drugs such as estrogen, progesterone, glucocorticoids, and bisphosphonates were also excluded.

We examined the relation of BMD at various sites to the −1997G → T SNP of COL1A1 in 2,236 participants (1,110 women, 1,126 men). All analyses were performed separately for men and for women. In addition, to uncover potential differences between women according to menopausal status, we conducted all analyses separately for premenopausal and postmenopausal women. Menopausal status was evaluated by a detailed questionnaire, and menopause was defined as complete cessation of menstruation. Furthermore, the relation of biochemical markers of bone turnover to −1997G → T genotype of COL1A1 was examined for men or premenopausal or postmenopausal women separately. The study protocol was approved by the Committee on Ethics of Human Research of National Chubu Hospital and the NILS, and written informed consent was obtained from each subject.

Measurement of BMD. BMD for the lumbar spine (L2–L4), right femoral neck, right trochanter, and right Ward's triangle was measured using dual-energy x-ray absorptiometry (DXA) (QDR 4500; Hologic, Bedford, Mass.). The coefficients of variation (CVs) of the DXA instrument were 0.9% (L2–L4), 1.3% (femoral neck), 1.0% (trochanter), and 2.5% (Ward's triangle); these values were determined by measurement of BMD three times at each site in 10 healthy subjects (mean age ± SE, 38.7 ± 2.4 years).

Determination of Genotypes. Genotypes were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (Yamada et al. 2002). The polymorphic region of COL1A1 was amplified using the polymerase chain reaction with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-TGGGTCAGTTCCAAGAGXCC-3′) or Texas red (5′-TGGGTCAGTTCCAAGAGXAC-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-TCTAAATGTCTGTTCCCTCCAA-3′). The reaction mixture (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 3.5 mmol/L $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol was initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 2 min.

The 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 then placed on a magnetic stand, and the supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/L NaOH and measured for fluorescence with 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 of 584 nm and 612 nm, respectively, for Texas red.

Measurement of Biochemical Markers of Bone Turnover. Venous blood and urine samples were collected in the early morning after the subjects had fasted overnight. Blood samples were centrifuged at 1,600 × *g* for 15 min at 4°C, and the serum fraction was separated and stored at −80°C until analysis. The serum concentration of intact osteocalcin was measured with an immunoradiometric assay kit (Mitsubishi Chemical, Tokyo, Japan). The activity of bone-specific alkaline phosphatase in serum was measured with an enzyme immunoassay kit (Metra Biosystems, Mountain View, Calif.). Urine samples were collected in plain tubes and stored at −80°C. Urinary deoxypyridinoline was measured with an enzyme immunoassay kit (Metra Biosystems); the values were corrected for urinary creatinine and expressed as picomoles per micromole of creatinine. The urinary concentration of cross-linked N-telopeptides of type I collagen (NTx) was measured with an enzyme-linked immunosorbent assay kit (Mochida Pharmaceutical, Tokyo, Japan); the values were expressed as picomoles of bone collagen equivalents per micromole of creatinine. Urinary creatinine was enzymatically measured with a creatinine test kit (Wako Chemical, Osaka, Japan).

Statistical Analysis. Quantitative data were compared among the three groups using one-way analysis of variance and the Tukey-Kramer post hoc test and between two groups using the unpaired Student's *t* test. BMD values were analyzed with adjustment for age and body mass index (BMI) using the least-squares method in a general linear model. The effect of −1997G → T genotype on BMD at various sites was evaluated using multivariate regression analysis; R^2 and *P* values were calculated from the analysis including age, BMI, and COL1A1 genotype (0 = *COL1A1*G/*G*, 1 = *COL1A1*G/*T* = *COL1A1*T/*T*) . Allele frequencies were estimated using the gene-counting method, and the chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. A *P* value less than 0.05 was considered statistically significant.

Results

The distribution of −1997G → T genotypes was in Hardy-Weinberg equilibrium, and age and BMI did not differ among genotypes for all women (Table 1). BMD for the lumbar spine and trochanter was significantly lower in women with the **G/*G* genotype than in those in the combined group of **G/*T* and

Table 1. BMD and Other Characteristics of All Women (n = 1,110) According to the −1997G → T Genotype of COL1A1

Characteristic	**G/*G*	**G/*T*	**T/*T*	**G/* T + *T/*T*
Number (%)	407 (36.7)	526 (47.4)	177 (15.9)	703 (63.3)
Age (years)	60.0 ± 0.5	58.9 ± 0.5	58.4 ± 0.8	58.8 ± 0.4
BMI (kg/m^2)	22.9 ± 0.2	22.8 ± 0.1	23.0 ± 0.2	22.9 ± 0.1
BMD values (g/cm^2)				
L2–L4	0.855 ± 0.006	0.870 ± 0.006	0.878 ± 0.010	0.872 ± 0.005[a]
Femoral neck	0.672 ± 0.004	0.681 ± 0.004	0.680 ± 0.007	0.681 ± 0.003
Trochanter	0.564 ± 0.004	0.575 ± 0.004	0.574 ± 0.006	0.575 ± 0.003[b]
Ward's triangle	0.500 ± 0.006	0.512 ± 0.005	0.508 ± 0.009	0.511 ± 0.004

Data are means ± SE. BMD values are adjusted for age and BMI.
a. P = 0.039 vs. **G/*G*.
b. P = 0.033 versus **G/*G*.

**T/*T* genotypes; the difference in BMD between the **G/*G* genotype and the combined group of **G/*T* and **T/*T* genotypes (expressed as a percentage of the corresponding larger value) was 1.9% for both the lumbar spine and the trochanter.

We also analyzed BMD and other characteristics for premenopausal and postmenopausal women independently. The distributions of −1997G → T genotypes were in Hardy-Weinberg equilibrium, and age and BMI did not differ among genotypes for premenopausal or postmenopausal women (Table 2). For postmenopausal women there was no difference in years after menopause among genotypes. For premenopausal women BMD was not associated with −1997G → T genotype. In contrast, BMD for the femoral neck or trochanter was significantly lower in postmenopausal women with the **G/*G* genotype than in those with the **G/*T* genotype or those in the combined group of **G/*T* and **T/*T* genotypes; the differences in BMD between the **G/*G* genotype and the combined group of **G/*T* and **T/*T* genotypes were 2.5% for the femoral neck and 2.2% for the trochanter.

The distribution of −1997G → T genotypes was in Hardy-Weinberg equilibrium, but BMD did not differ among these genotypes in men (Table 3).

The effect of −1997G→T genotype on BMD was evaluated using multivariate regression analysis (Table 4). The analysis revealed that the −1997G → T genotype affected BMD at various sites with a variance of 0.46–0.62% for all women and of 0.61–1.01% for postmenopausal women.

The relation of biochemical markers of bone turnover to −1997G → T genotype of COL1A1 was also examined. No association of −1997G → T genotype with the serum concentration of intact osteocalcin, serum activity of bone-specific alkaline phosphatase, or urinary excretion of deoxypyridinoline or NTx was apparent for men or premenopausal or postmenopausal women (Table 5).

Table 2. BMD and Other Characteristics of Women (n = 1,093) According to Menopausal Status and the −1997G → T Genotype of COL1A1

	Premenopausal Women (n = 278)				*Postmenopausal Women* (n = 815)			
Characteristic	**G/*G*	**G/*T*	**T/*T*	**G/*T + *T/*T*	**G/*G*	**G/*T*	**T/*T*	**G/*T + *T/*T*
Number (%)	94 (33.8)	140 (50.4)	44 (15.8)	184 (66.2)	306 (37.5)	377 (46.3)	132 (16.2)	509 (62.5)
Age (years)	45.9 ± 0.4	46.3 ± 0.4	45.8 ± 0.6	46.2 ± 0.3	64.6 ± 0.5	63.8 ± 0.4	62.8 ± 0.7	63.5 ± 0.4
Years after menopause					15.4 ± 0.5	15.1 ± 0.5	13.7 ± 0.8	14.7 ± 0.4
BMI (kg/m^2)	22.8 ± 0.3	22.9 ± 0.3	22.4 ± 0.5	22.8 ± 0.2	23.0 ± 0.2	22.8 ± 0.2	23.2 ± 0.3	22.9 ± 0.1
BMD values (g/cm^2)								
L2–L4	1.018 ± 0.012	1.026 ± 0.010	1.044 ± 0.018	1.030 ± 0.009	0.798 ± 0.007	0.813 ± 0.007	0.821 ± 0.011	0.815 ± 0.006
Femoral neck	0.782 ± 0.009	0.767 ± 0.008	0.771 ± 0.014	0.768 ± 0.007	0.634 ± 0.005	0.650 ± 0.004[a]	0.647 ± 0.007	0.650 ± 0.004[b]
Trochanter	0.656 ± 0.009	0.661 ± 0.007	0.656 ± 0.013	0.660 ± 0.006	0.532 ± 0.005	0.544 ± 0.004	0.545 ± 0.007	0.544 ± 0.004[c]
Ward's triangle	0.663 ± 0.012	0.657 ± 0.010	0.658 ± 0.018	0.657 ± 0.009	0.444 ± 0.007	0.459 ± 0.006	0.455 ± 0.010	0.458 ± 0.005

Data are means ± SE. BMD values are adjusted for age and BMI.

a. P = 0.034 vs. **G/*G*.

b. P = 0.011 vs. **G/*G*.

c. P = 0.033 vs. **G/*G*.

Table 3. BMD and Other Characteristics of Men (n = 1,126) According to the −1997G → T Genotype of COL1A1

Characteristic	**G/*G*	**G/*T*	**T/*T**	*G/*T + *T/*T*
Number (%)	457 (40.6)	511 (45.4)	158 (14.0)	669 (59.4)
Age (years)	58.5 ± 0.5	59.7 ± 0.5	59.2 ± 0.9	59.6 ± 0.4
BMI (kg/m^2)	22.9 ± 0.1	23.0 ± 0.1	22.9 ± 0.2	22.9 ± 0.1
BMD values (g/cm^2)				
L2–L4	0.990 ± 0.007	0.975 ± 0.007	0.983 ± 0.012	0.977 ± 0.006
Femoral neck	0.754 ± 0.005	0.754 ± 0.004	0.744 ± 0.008	0.751 ± 0.004
Trochanter	0.672 ± 0.005	0.665 ± 0.004	0.667 ± 0.008	0.665 ± 0.004
Ward's triangle	0.557 ± 0.006	0.552 ± 0.005	0.540 ± 0.010	0.549 ± 0.005

Data are means ± SE. BMD values are adjusted for age and BMI.

Discussion

The −1997G → T SNP of the COL1A1 promoter has previously been associated with BMD for the lumbar spine and, to a lesser extent, with BMD for the femoral neck in postmenopausal Spanish women, and with the **T/*T* genotype, which represents a risk factor for reduced BMD (Garcia-Giralt et al. 2002). We have now shown that the −1997G → T SNP is associated with BMD for the femoral neck and trochanter in postmenopausal Japanese women and with the **G/*G* genotype, which represents a risk factor for reduced BMD. The −1997G → T genotype affected BMD at various sites with a variance of 0.61–1.01% for postmenopausal women, although this SNP was not associated with biochemical markers of bone turnover.

The alleles of the −1997G → T polymorphism associated with reduced

Table 4. Effects of the −1997G → T Genotype of COL1A1 on BMD for All Women (n = 1,110) or Postmenopausal Women (n = 815)

Site	R^2	P
All women		
L2–L4	0.0061	0.0102
Femoral neck	0.0047	0.0243
Trochanter	0.0062	0.0093
Ward's triangle	0.0046	0.0262
Postmenopausal women		
L2–L4	0.0061	0.0263
Femoral neck	0.0101	0.0044
Trochanter	0.0076	0.0137
Ward's triangle	0.0071	0.0172

The R^2 and P values were derived from multivariate regression analysis including age, BMI, and COL1A1 genotype (0 = **G/*G*, 1 = **G/*T* = **T/*T*).

Table 5. Biochemical Markers of Bone Turnover for Women or Men According to the −1997G → T Genotype of COL1A1

Marker	**G/*G*	**G/*T*	**T/*T*
Premenopausal women			
Osteocalcin (ng/mL)	6.35 ± 0.29	6.46 ± 0.24	6.93 ± 0.42
Bone-specific alkaline phosphatase (U/L)	19.6 ± 0.5	20.3 ± 0.5	19.0 ± 0.8
dPyr (pmol/μmol Cr)	5.54 ± 0.15	5.35 ± 0.12	5.50 ± 0.22
NTx (pmol BCE/μmol Cr)	33.5 ± 1.5	33.6 ± 1.3	38.3 ± 2.3
Postmenopausal women			
Osteocalcin (ng/mL)	10.53 ± 0.21	10.30 ± 0.19	10.25 ± 0.32
Bone-specific alkaline phosphatase (U/L)	31.6 ± 0.6	31.5 ± 0.6	30.5 ± 0.9
dPyr (pmol/μmol Cr)	4.08 ± 0.06	4.01 ± 0.05	3.99 ± 0.10
NTx (pmol BCE/μmol Cr)	60.4 ± 1.6	60.2 ± 1.5	59.4 ± 2.5
Men			
Osteocalcin (ng/mL)	7.67 ± 0.11	7.64 ± 0.11	7.64 ± 0.20
Bone-specific alkaline phosphatase (U/L)	26.3 ± 0.4	25.6 ± 0.4	26.2 ± 0.7
dPyr (pmol/μmol Cr)	4.08 ± 0.06	4.01 ± 0.05	3.99 ± 0.10
NTx (pmol BCE/μmol Cr)	36.6 ± 0.7	36.2 ± 0.7	36.4 ± 1.2

Data are means ± SE. dPyr, deoxypyridinoline; Cr, creatinine; NTx, cross-linked N-telopeptides of type I collagen; BCE, bone collagen equivalents.

BMD thus differ between the present study (**G* allele) and the previous study (**T* allele) (Garcia-Giralt et al. 2002). Although the reason for this apparent discrepancy is unclear, there are three major differences between the two studies. First, the subjects were older in our study (mean age of 64 years for postmenopausal women) than in the previous study (mean age, 51 years), and years since menopause were significantly greater in our study (mean, 15.0 years) than in the previous study (mean, 3.6 years). Given that bone resorption markedly increases during 10 years after menopause, genetic effects on BMD might differ between women for short and long time after menopause. Second, the number of subjects in which the association was detected was greater in our study ($n = 815$ for postmenopausal women) than in the previous study ($n = 256$). The results of association studies with small sample sizes are prone to bias compared with those with large sample sizes. Finally, the distribution of −1997G → T genotypes differed significantly ($P < 0.0001$; chi-square test) between our study (postmenopausal women: **G/*G*, 38%; **G/*T*, 46%; **T/*T*, 16%) and the previous study (**G/*G*, 76%; **G/*T*, 22%; **T/*T*, 2%), possibly reflecting the difference in ethnicity. The difference in genetic influences on BMD between different ethnic groups might be attributable, at least in part, to the difference in the distribution of genotypes. It is also possible that the −1997G → T SNP of COL1A1 is in linkage disequilibrium with other polymorphisms of COL1A1 or with polymorphisms of other nearby genes that are actually responsible for the observed association with BMD. Given the multiple comparisons of genotype performed, we cannot completely exclude the possible occurrence of statistical errors such as

false positives, although we observed a significant association of this SNP with BMD at different sites.

Evidence suggests that the −1997G → T SNP of COL1A1 may affect promoter function (Garcia-Giralt et al. 2002). A double-stranded oligonucleotide containing the −1997G → T site bound osteoblast nuclear factors; however, the extent of factor binding was even more pronounced with a single-stranded antisense DNA probe, suggesting the involvement of a protein selective for single-stranded DNA. The extent of factor binding observed with a probe corresponding to the **G* allele was greater than that apparent with a probe based on the **T* allele. The effect of this SNP on COL1A1 transcription, however, remains to be determined.

In conclusion, our present results suggest that the −1997G → T SNP of COL1A1 is associated with BMD for the femoral neck and trochanter in postmenopausal Japanese women and that the alleles associated with reduced BMD differ between postmenopausal Japanese (**G* allele) and Spanish (**T* allele) women, although the contribution of this SNP to bone mass appears relatively small.

Acknowledgments This study was supported in part by a Research Grant for Longevity Sciences (grant 12C-01 awarded to Y. Yamada and H. Shimokata) and by a Health and Labor Sciences Research Grant for Comprehensive Research on Aging and Health (grant H15-Chojyu-014 awarded to Y. Yamada, F. Ando, N. Niino, and H. Shimokata) from the Ministry of Health, Labor, and Welfare of Japan.
Received 22 March 2004; revision received 16 July 2004.

Literature Cited

Devoto, M., K. Shimoya, J. Caminis et al. 1998. First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p, and 4p. *Eur. J. Hum. Genet.* 6:151–157.

Garcia-Giralt, N., X. Nogués, A. Enjuanes et al. 2002. Two new single-nucleotide polymorphisms in the COL1A1 upstream regulatory region and their relationship to bone mineral density. *J. Bone Miner. Res.* 17:384–393.

Garnero, P., O. Borel, S. F. A. Grant et al. 1998. Collagen Iα1 Sp1 polymorphism, bone mass, and bone turnover in healthy French premenopausal women: The OFELY Study. *J. Bone Miner. Res.* 13:813–817.

Grant, S. F. A., D. M. Reid, G. Blake et al. 1996. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type Iα1 gene. *Nat. Genet.* 14:203–205.

Heegaard, A., H. L. Jorgensen, A. W. Vestergaard et al. 2000. Lack of influence of collagen type Iα1 Sp1 binding site polymorphism on the rate of bone loss in a cohort of postmenopausal Danish women followed for 18 years. *Calcif. Tissue Int.* 66:409–413.

Hustmyer, F. G., G. Liu, C. C. Johnston et al. 1999. Polymorphism at an Sp1 binding site of COL1A1 and bone mineral density in premenopausal female twins and elderly fracture patients. *Osteoporosis Int.* 9:346–350.

Ishida, R., M. Emi, Y. Ezura et al. 2003. Association of a haplotype (196Phe/532Ser) in the interleukin-1-receptor associated kinase (IRAK1) gene with low radial bone mineral density in two independent populations. *J. Bone Miner. Res.* 18:419–423.

Johnson, M. L., G. Gong, W. Kimberling et al. 1997. Linkage of a gene causing high bone mass to human chromosome 11 (11q12–13). *Am. J. Hum. Genet.* 60:1,326–1,332.

Kanis, J. A., L. J. Melton III, C. Christiansen et al. 1994. The diagnosis of osteoporosis. *J. Bone Miner. Res.* 9:1,137–1,141.

Kobayashi, S., S. Inoue, T. Hosoi et al. 1996. Association of bone mineral density with polymorphism of the estrogen receptor gene. *J. Bone Miner. Res.* 11:306–311.

Koller, D. L., M. J. Econs, P. A. Morin et al. 2000. Genome screen for QTLs contributing to normal variation in bone mineral density and osteoporosis. *J. Clin. Endocrinol. Metab.* 85:3,116–3,120.

Koller, D. L., L. A. Rodriguez, J. C. Christian et al. 1998. Linkage of a QTL contributing to normal variation in bone mineral density to chromosome 11q12–13. *J. Bone Miner. Res.* 13:1,903–1,908.

Langdahl, B. L., S. H. Ralston, S. F. Grant et al. 1998. An Sp1 binding site polymorphism in the COLIA1 gene predicts osteoporotic fractures in both men and women. *J. Bone Miner. Res.* 13:1,384–1,389.

Liden, M., B. Wilen, S. Ljunghall et al. 1998. Polymorphism at the Sp1 binding site in the collagen type Iα1 gene does not predict bone mineral density in postmenopausal women in Sweden. *Calcif. Tissue Int.* 63:293–295.

Mann, V., E. E. Hobson, B. Li et al. 2001. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J. Clin. Invest.* 107:899–907.

Morrison, N. A., J. C. Qi, A. Tokita et al. 1994. Prediction of bone density from vitamin D receptor alleles. *Nature* 367:284–287.

Niu, T. H., C. Z. Chen, H. Cordell et al. 1999. A genome-wide scan for loci linked to forearm bone mineral density. *Hum. Genet.* 104:226–233.

Peacock, M., C. H. Turner, M. J. Econs et al. 2002. Genetics of osteoporosis. *Endocr. Rev.* 23:303–326.

Shimokata, H., F. Ando, and N. Niino. 2000. A new comprehensive study on aging: The National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). *J. Epidemiol.* 10:S1–S9.

Sykes, B. 1990. Bone disease cracks genetics. *Nature* 348:18–20.

Uitterlinden, A. G., H. Burger, Q. Huang et al. 1998. Relation of alleles of the collagen type Iα1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. *New Engl. J. Med.* 338:1,016–1,021.

Yamada, Y., F. Ando, N. Niino et al. 2001. Transforming growth factor-β1 gene polymorphism and bone mineral density. *J. Am. Med. Assoc.* 285:167–168.

Yamada, Y., F. Ando, N. Niino et al. 2003a. Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. *J. Clin. Endocrinol. Metab.* 88:3,372–3,378.

Yamada, Y., F. Ando, N. Niino et al. 2003b. Association of polymorphisms of paraoxonase 1 and 2 genes, alone or in combination, with bone mineral density in community-dwelling Japanese. *J. Hum. Genet.* 48:469–475.

Yamada, Y., H. Izawa, S. Ichihara et al. 2002. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. *New Engl. J. Med.* 347:1,916–1,923.

Chapter 9

高齢者の栄養と食生活

- 日本の社会は現在，世界でも他に例をみないほどの速さで高齢化している．この高齢化の時代に対応する高齢者のための栄養を新たに考えていかなければならない．
- 栄養は高齢者の健康を守るキーポイントである．しかし要介護高齢者などでは栄養の摂取状況はむしろ悪化している．
- 高齢者の健康を考えるとき，肥満よりもやせを防ぐことが重要である．
- 栄養判定には肥満度の測定とともに，血液検査による血清アルブミンの定量などが有用である．
- 高齢になるとエネルギー消費量が減ることが多く，食物摂取量も減り必須栄養素が不足することが多い．高齢者では特に栄養配分に留意した食生活が必要であろう．

高齢社会の進展

平均寿命の延長

日本人の平均寿命は大正の終わりには男性で約 42 歳，女性で 43 歳であったが，昭和期に入ってから急速に伸び始め，1947 年には男女ともに 50 歳を超え，1951 年には 60 歳を超えた．以後，延び率は若干緩やかにはなったが毎年着実に延長し，この傾向は現在も続いている．2001 年度の男性の平均寿命は 78.07 歳，女性では 84.9 歳であり，日本人の平均寿命は男女ともに世界のトップクラスである．

平均寿命
その年に生まれた子どもが，平均して何歳まで生きられるかを推定して求めた寿命．

2001 年度の簡易生命表によると，65 歳まで生存する人は男性が 85.1 %，女性が 92.8 %，80 歳まで生存する人は男性で 53.5 %，女性で 75.3 %となっている．40 歳までの生存率はほぼ頭打ちとなっているが，65 歳，80 歳までの生存率はさらに増加傾向が続いている．

出生率の低下

1 人の女性が一生の間に生むと推定される子どもの数を示す合計特殊出生率は 1949 年ころまで 4 を超えていたが，その後急激に低下し，1957 年には 2.04 となった．その後は 2.0 前後で安定していたが，1974 年以降からは低下傾向が続き，2001 年には 1.33 となった〔図 7-1 (p.161) 参照〕．合計特殊出生率を

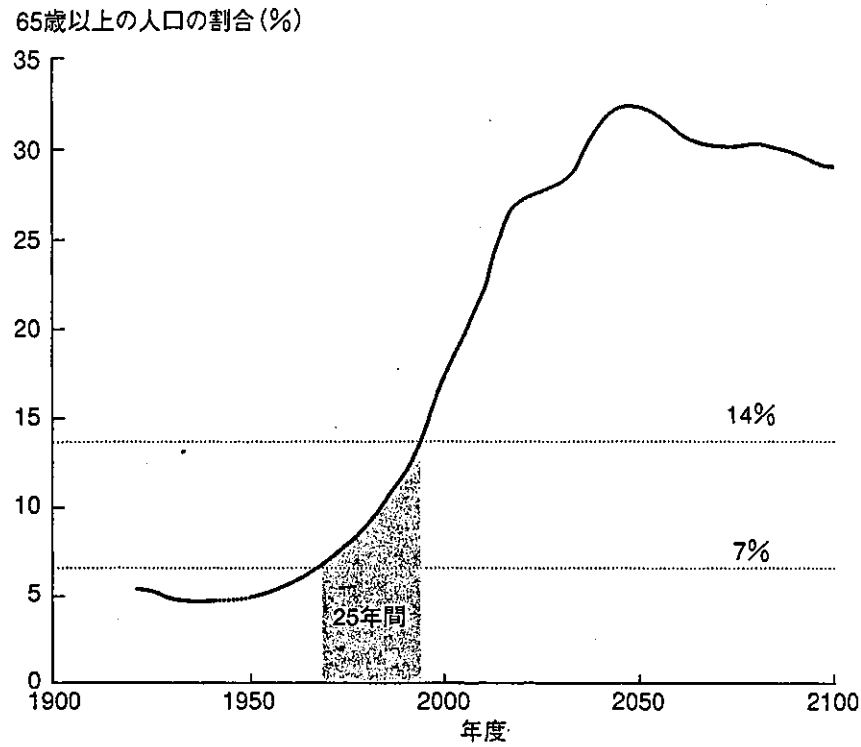

図 9-1 — 日本における老年人口割合の年次別推移および推計
総務庁統計局：国勢調査，人口推計調査
国立社会保障・人口問題研究所：日本の将来推計人口

2.1 以上に維持しなければ人口は徐々に低下し，また人口の高齢化が進むこととなる．

老年人口の増加

65 歳以上の老年人口は大正の終わりには 5.1 %であった．1940 年には政府の多子政策もあり，この比率は 4.7 まで下がった．しかし第二次大戦後は着実に老年人口は多くなってきた（**図 9-1**）．戦後すぐのベビーブーム，そしてそのとき生まれた女性が出生期を迎えた 1973 年前後の第二次ベビーブームに急速に出生数が増加したが，平均寿命の延びによる老年人口の増加のほうがさらに大きかったのである．

2001 年度での 65 歳以上の人口の割合は 18.0 %となった．今後も老年人口の割合は増え続け，高齢化のピークを迎える 2050 年には実に 32.3 %に達すると推計されている．高齢化の速さの指標として国連などの統計で用いられるのは，65 歳以上の人口の割合が 7 %を超える高齢化の第一段階から，その割合が 2 倍の 14 %となるまでの年数である．フランスは，これに実に 130 年を要している．ヨーロッパの他の国々の多くでも 50 年から 100 年近くかかっているが，日本ではわずか 25 年でこれを達成した．現在，スウェーデンをはじめ老年者の人口の割合が日本より高い国はいくつかあるが，将来の老年人口の割合が日本