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Genetic risk score based on the prevalence of vertebral fracture in Japanese women with osteoporosis

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ABSTRACT

A genetic risk score (GRS) was developed for predicting fracture risk based on the prevalence of vertebral fractures in 441 Japanese females with osteoporosis. A total of 979 (858 nonsynonymous and 121 silent) single-nucleotide polymorphisms (SNPs) located in 74 osteoporosis-susceptibility genes were genotyped and evaluated for their association with fracture prevalence. Four SNPs (protein kinase domain containing, cytoplasmic [*PKDCC*; rs4952590], CDK5-regulatory subunit-associated protein 1-like 1 [*CDKAL1*; rs4712556], wingless-type MMTV-integration site family member 16 [*WNT16*; rs2707466], and G-patch domain-containing gene 1 [*GPATCH1*; rs10416265]) showed a significant association ($p < 0.05$) with the fracture, in which the minor allele of the former two SNPs was the protective allele and that of the latter two SNPs was the risk allele. Applying a dominant-genetic model, we allotted −1 point each to the protective-allele carriers and 1 point each to the risk-allele carriers, and GRS values were calculated as the sum of the points. The receiver-operating characteristic curves showed that GRS adequately predicted vertebral fracture. For the model predicted by the GRS with and without the effect of age, areas under the curves were 0.788 (95% confidence interval [CI]: 0.736–0.840) and 0.667 (95% CI: 0.599–0.735), respectively. Multiple logistic regression analysis revealed that the odds ratio for the association between fracture prevalence and GRS was 3.27 (95% CI: 1.36–7.87, $p = 0.008$) for scores of −1 to 0 ($n = 303$) and 12.12 (95% CI: 4.19–35.07, $p < 0.001$) for scores of 1 to 2 ($n = 35$) relative to a score of −2 ($n = 103$). The GRS based on the four SNPs could help identify at-risk individuals and enable implementation of preventive measures for vertebral fracture.

Abbreviations: GRS, genetic risk score; SNP, single-nucleotide polymorphism; *PKDCC*, protein kinase domain containing, cytoplasmic; *CDKAL1*, CDK5-regulatory subunit-associated protein 1-like 1; *WNT16*, wingless-type MMTV-integration site family member 16; *GPATCH1*, G-patch domain-containing gene 1; CI, confidence interval; BMD, bone mineral density; GWAS, genome-wide association studies; OR, odds ratio; AUC, area under the curve; ROC, receiver-operating characteristics.

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1. Introduction

Osteoporosis is among the most common skeletal diseases and affects >200 million individuals worldwide, a figure that continues to increase as populations in developed countries live longer than previous generations (Reginster and Burlet, 2006). Osteoporosis is clinically characterized by reduced bone mass and compromised bone strength, which leads to an increased risk of fracture (Soen et al., 2013).

Fragility fractures, such as vertebral and femoral fractures, are among the most serious complications in elderly patients with osteoporosis. Several important factors, including age, past history of fragility fractures, family history of femoral fracture, bone mineral density (BMD), and history of falls, increase the risk of fracture in a clinical setting. In addition to these factors, genetic variations also determine predisposition to low-trauma fractures as demonstrated by genetic–epidemiological studies (Peacock et al., 2002; Ralston and Uitterlinden, 2010). Recent large-scale meta-analyses of genome-wide association studies (GWAS) identified a number of single-nucleotide polymorphisms (SNPs) associated with low BMD or increased fracture risk (Styrkarsdottir et al., 2008; Rivadeneira et al., 2009; Estrada et al., 2012).

Risk scores have been developed to predict the risk of coronary heart disease (ERICA Research Group, 1991; Tunstall-Pedoe, 1991), diabetes mellitus (Lindström and Tuomilehto, 2003), and dementia (Ngandu et al., 2006), and typically encompass multiple factors that affect disease onset or progression. A risk score can therefore improve the ability to predict common polygenic diseases, such as osteoporosis, by including multiple SNP profiles. Accordingly, we previously performed a study to develop a genetic risk sore (GRS) for predicting lifetime femoral fracture risk using data from consecutive, elderly Japanese autopsy cases at a community-based geriatric hospital (Zhou et al., 2015). The aim of this study was to develop a GRS for predicting vertebral fracture risk in Japanese women with osteoporosis using data registered in Biobank Japan (Nakamura, 2007).

2. Methods

2.1. Subjects

The osteoporosis-case subjects were collected under the support of the BioBank Japan Projects (Nakamura, 2007), and all participants provided written informed consent as approved by the ethics committees of the BioBank Japan Project (Nakamura, 2007) and the University of Tokyo. Osteoporosis was diagnosed based on the Japanese diagnostic criteria for primary osteoporosis (Soen et al., 2013). Patients with malignant neoplasms, liver cirrhosis, nephrotic syndrome, diabetes mellitus, rheumatoid arthritis, cerebral infarction, chronic obstructive pulmonary disease, hyperthyroidism, renal failure, and history of steroid-drug use were excluded from the assessment. Finally, 441 unrelated females with a mean age of 69.6 years were selected for this study. The prevalence of morphological vertebral fracture in all study subjects was determined by examination of lateral thoracolumbar (T_4–L_4) radiographs. The assessment of vertebral fracture was made in accordance with the semi-quantitative method (Genant et al., 1993), and a vertebral fracture was defined as a deformity of more than grade 1 in any of the measured vertebrae. Of the 441 subjects, 72 individuals sustained vertebral fractures, with mean age and age distributions (standard deviation and min–max, respectively) of 74.5 years (7.1 and 53–88) for subjects with fractures and 68.0 years (8.2 and 28–88) for those without.

2.2. SNP selection and genotyping

A large-scale meta-analysis of previous GWASs identified 56 BMD loci and revealed 14 loci associated with fracture risk (Estrada et al., 2012). To select SNPs for this study, those within or close to the 56 BMD loci were evaluated, as well as those on the Illumina HumanExome BeadChip (Grove et al., 2013) (Illumina, Inc., San Diego, CA, USA). A total of 979 (858 nonsynonymous and 121 silent) SNPs in 74 genes were identified (Supplementary Table 1) and evaluated for their association with the incidence of vertebral fracture among the 441 cases. The genotyping data for the 979 SNPs of the study subjects were provided from the Biobank Japan genotyping database generated using Illumina OmniExpressExome BeadChip version 1.2 (Illumina, Inc.) with call rates of >0.99 during the process of genotyping.

2.3. Calculation of GRS

GRS was calculated as reported previously (Zhou et al., 2015). In this study, we applied a dominant-genetic model and allotted −1 point each to the protective-allele carriers and 1 point each to the risk-allele carriers, and unweighted GRS values were calculated as the sum of the points. We also standardized scores using coefficients obtained from the logistic regression analyses (weighted GRS) to ensure that the lowest absolute value of the coefficient was assigned a value of 1 (Zhou et al., 2015). The association of the GRS with vertebral fracture was evaluated by multiple logistic regression analysis.

2.4. Statistical analysis

All statistical analyses were carried out using PLINK 1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007) or SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA). Allelic frequencies of the selected SNPs were calculated using a gene-counting method. Hardy-Weinberg equilibrium for each SNP was assessed by the χ^2 test. The Cochran-Armitage proportion trend test was used to identify changes in fracture incidence with respect to the number of risk or protective alleles. Multiple logistic regression analysis, including age, genotypes of each SNP, or GRS as independent variables, was performed to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association with risk of vertebral fracture. Receiver-operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was calculated to assess the discriminative power of the GRS models (Ngandu et al., 2006). All reported p-values are two-sided, with $p < 0.05$ regarded as statistically significant.

3. Results

After genotyping 979 SNPs located in 74 previously reported osteoporosis-susceptibility genes (Estrada et al., 2012), SNPs that met the following criteria were selected: 1) a statistically significant association ($p < 0.05$) with the prevalence of vertebral fracture according to the Cochran-Armitage trend test, and (2) a minor-allele frequency of >0.01 in the study population. Finally, four SNPs [protein kinase domain containing, cytoplasmic (*PKDCC*; rs4952590), CDK5-regulatory subunit-associated protein 1-like 1 (*CDKAL1*; rs4712556), wingless-type MMTV-integration site family member 16 (*WNT16*; rs2707466), and G-patch domain-containing gene 1 (*GPATCH1*; rs10416265)] were selected for this study (Table 1). Their allele and genotype frequencies were in Hardy-Weinberg equilibrium, and the prevalence of vertebral fracture was calculated for each genotype to identify risk or protective alleles. As shown in Table 1, the T allele of rs4952590 and the A allele of rs4712556 significantly protected vertebral fracture (protective allele), while the T allele of rs2707466 and the A allele of rs10416265 contributed significantly to fracture morbidity (risk allele).

The independent association of each minor allele with vertebral fracture was evaluated by multiple logistic regression analysis. A minor allele-dominant genetic model was used for these four SNPs, because the fracture prevalence was quite similar between heterozygous and homozygous carriers (Table 1). As shown in Table 2, T-allele carriers of rs4952590 and A-allele carriers of rs4712556 showed significantly lower ORs for risk of vertebral fracture, whereas T-allele carriers of rs2707466 and A-allele carriers of rs10416265 showed significantly higher ORs for the risk. In order to calculate GRS values, we allotted