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Single nucleotide polymorphisms in sFRP4 are associated with bone and body composition related parameters in Danish but not in Belgian men

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ABSTRACT

The senescence accelerated mouse P6 (SAMP6) has a low bone mass and has previously shown to be a good model for senile osteoporosis in humans. In addition to a reduced bone mass, SAMP6 mice are obese and have hyperlipidemia. Using positional cloning and expression studies, an increased expression of *sfrp4* was found in these mice. SFRP4 is a modulator of the Wnt signalling pathway. This pathway has been previously shown to be involved in regulating bone mass. Additional evidence that *sFRP4* has an influence on BMD was delivered by linkage and association studies mostly performed in Asian populations. Based on these data we decided to perform an association study between common variants in *sFRP4*, BMD, hip geometry parameters and body composition parameters in a population consisting of 1383 Danish men (783 aged 20–29 years; 600 aged 60–74 years). Afterwards we tried to replicate the significant results in a population of 994 Belgian men. In the Danish population we found 6 SNPs associated with BMD at the hip and/or femoral neck. Furthermore, all 6 SNPs were associated with several hip geometry parameters. The homozygous presence of the minor allele resulted for all SNPs (except rs4720265) in a decrease in bone density and bone strength. Finally, we observed in the Danish population age specific associations with height and fat mass. In the Belgian population we tried to replicate the results of three SNPs with BMD and body composition parameters. Unfortunately, we were not able to replicate the results found in the Danish cohort but we found one SNP (rs2598116) associated with height.

In conclusion, genetic variation in *sFRP4* has an influence on hip fracture risk, percentage body fat and height in a Danish male population. However, we were unable to replicate these results in an independent Belgian population.

1. Introduction

Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone leading to enhanced bone fragility, which results in an increased fracture risk. Worldwide, it is the most common metabolic bone disorder and it affects one in three women and one in eight men over the age of 50 [1]. Consequently, osteoporosis and related fractures have a high impact on the public health care [2]. Bone mass is dependent on the peak bone mass reached in young adulthood and the following age-dependent bone loss [3–5]. It is a complex trait with high heritability (60%–80%) and influenced by many genetic variants with modest effect sizes and the interaction of these variants with environmental factors [1,6–8]. Bone mineral density (BMD) measured by dual X-ray absorptiometry (DXA), is often used as predictor for osteoporosis and related fracture risk [1]. However, BMD shows a linear decline with aging and the prevalence of hip fractures shows an exponential increase with aging [9–11]. This observation indicates that BMD alone is not enough to predict fracture risk [11]. Besides BMD measurements, determinants of bone strength (bone geometry) are important predictors for fractures [11,12]. Considering the importance of geometry in bone quality and strength, deterioration in femoral neck geometry combined with decrease in BMD with aging may explain the exponential rise of hip fractures in the elderly [11]. Based on these results it is important to study BMD as well as bone geometry when investigating susceptibility variants for osteoporosis.

In the past years many genetic and functional studies were performed to identify genes involved in the development of osteoporosis

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and related fractures. One of the studies focused on the senescence-accelerated mouse (SAM) P6 which is a model for senile osteoporosis and is characterized by several features analogous to age-related bone loss in humans [13]. Additionally, SAMP6 mice have a low peak bone mass which is one of the major risk factors of osteoporosis in humans [14]. As a consequence, the SAMP6 mouse is a good model for studying senile osteoporosis in humans. In 2006 Nakanishi and colleagues reported that *sfrp4* is a negative regulator of peak BMD in SAMP6 mice since they observed a 40 times higher expression level of *sfrp4* in SAMP6 mice compared to mice of another SAM strain with normal peak bone mass [14]. SFRP4 is a member of the sFRP family (sFRP1-5) which are extracellular modulators of the Wnt signalling pathway. They can modulate the pathway by binding to either Wnt ligands or Frizzled (Fz) receptors [15,16]. Previous genetic and functional studies demonstrated that the canonical Wnt signalling has an important role in bone formation by regulating osteoblast proliferation and differentiation [16]. Further investigations for the role of sFRP4 and canonical Wnt signalling in the regulation of bone formation and bone mass in mice were performed in *sfrp4* transgenic mice [17,18]. These studies indicated that increased *sfrp4* expression results in decreased bone formation at least partially by attenuating the canonical Wnt signalling [18]. The results of the SAMP6 and transgenic mice studies indicate that *sFRP4* is a strong candidate gene for susceptibility for osteoporosis and related fractures.

The differentiation of mesenchymal stem cells to osteoblasts is regulated by several factors among which canonical Wnt signalling is especially important [19–21]. Therefore, more extensive research was performed in the SAMP6 mice. These studies showed that at 10–22 weeks of age, SAMP6 mice exhibit obesity and hyperlipidemia, besides the low peak bone mass [22]. This observation together with the results of Nakanishi and colleagues suggests that enhanced expression of *sfrp4* in SAMP6 mice may lead to an imbalance in the regulation of mesenchymal stem cells differentiation resulting in osteopenia and enhanced adipogenesis in these mice [14,18,22]. Therefore, the studies in SAMP6 mice indicated that *sfrp4* might be involved in susceptibility to both osteoporosis and obesity. Regarding the latter, to our knowledge no other data are currently available. Meanwhile for osteoporosis, additional evidence that *sFRP4* has an influence on BMD is delivered by linkage and association studies. Linkage or a suggestive linkage signal was achieved at chromosomal region 7p15-14 (containing *sFRP4*) with BMD at different sites in several populations with different ethnical backgrounds [23–27]. Furthermore, a genome wide association study in Korean samples showed that one SNP located close to *sFRP4* is associated with BMD at the radius, tibia and heel [28]. In addition, previous candidate gene studies showed that two SNPs (rs1132553 and rs1802074) are associated with lumbar spine BMD in two different populations containing post-menopausal women from Japan and Korea, respectively [29,30].

Based on the above described literature, we wanted to investigate the effect of common genetic variation in *sFRP4* on several parameters predicting the risk for osteoporosis and related fracture in several cohorts of Caucasian men of different ages. Additionally, we tested whether genetic variation in *sFRP4* has an influence on body composition parameters. In this study, we investigated the effect in two cohorts of a Danish population and we tried to replicate the results of these cohorts in an independent Belgian cohort.

2. Subjects and methods

2.1. Subjects

In this study we analysed 3 cohorts from two independent populations. The first population is part of the Odense Androgen Study (OAS) and consists of 1383 Danish men. The second population is a Belgian population (Siblos) containing data and DNA of 994 men.

2.1.1. OAS population

The Odense Androgen Study (OAS) is a population-based, prospective, observational study on the interrelationship between endocrine status, body composition, muscle function and bone metabolism in men of different age groups. The study consists of two cohorts: a young cohort of 783 non-stratified men aged 20–29 years (OASY) and an older cohort of 600 age-stratified men aged 60–74 years (OASE). Details on the design and inclusion of the participants in the OASY and OASE cohorts were previously reported elsewhere [31,32].

2.1.1.1. Bone mineral density and body composition. Bone mineral density (BMD) at the lumbar spine (LS) and at the femoral neck (FN) was measured by dual energy absorptiometry (DXA) using a Hologic 4500 device (Waltham, MA, USA). Software version 2.3.1 was used to calculate total hip (TH) and whole body (WB) BMD. The coefficient of variance (CV) for BMD ranged 0.7–1.5% at the different skeletal sites.

Body weight and body height were measured (resp. SECA, Hamburg, Germany and Harpenden, Holtain, UK). BMI was calculated as weight divided by height squared (kg/m^2). Lean body mass and fat mass was obtained from whole body DXA scans.

2.1.1.2. Hip geometry. Hip geometry parameters were obtained using Apex 2.3.1 software (Hologic Inc). The software provides data on hip axis length (HAL) and neck-shaft angle (NSA). Similarly, buckling ratio (BR), cross sectional area (CSA) and cross sectional moment of inertia (CSMI, not shown) was assessed. The section modulus (Z) is a strength parameter that is based on and showed instead of the CSMI. The various geometric parameters are calculated at three different regions of interest (ROI): the narrow neck (NN), the intertrochanteric ROI (not shown) and at last the femoral shaft region (FS).

2.1.2. Siblos population

This cohort is part of a study designed to investigate determinants of peak bone mass focusing on genetics and sex steroids. Caucasian men aged between 25 and 45 years were recruited from communities around Ghent, Belgium. The study population contains 994 samples from 529 different families. The population consists of 92 individual samples, 411 brother pairs, 24 brother trios and 2 families with 4 brothers. Details on the design and inclusion of the participants in the Siblos population were previously reported elsewhere [33,34].

2.1.2.1. Bone mineral density and body composition. Body weight and anthropometrics were measured in light indoor clothing without shoes. Whole body soft tissue composition, as well as bone mineral content (BMC) and areal bone mineral density (aBMD) (lumbar spine (LS), proximal femur (total hip region) and whole body (WB)) were measured using DXA with a Hologic QDR-4500A device (software version 11.2.1, Hologic Inc., Bedford, MA).

2.2. SNP selection and genotyping

SNP selection and genotyping of the different tagSNPs were performed in three stages. First we selected tagSNPs which tag most of the common genetic variation in and around *sFRP4* ($\pm$2 kb 5′ and $\pm$ 3.5 kb 3′) and genotyped those SNPs in the OASY cohort. TagSNP selection was based on the results of HapMap (CEU samples, Rel24/phase II Nov 08) and the aggressive tagger method (Haploview, $r^2 > 0.8$) was used for the tagSNP selection. Three SNPs (rs1802073, rs1132552, and rs1132553) were forced included and two additional SNPs at that moment not included in HapMap were selected (rs35937440 and rs1802074). As a result, 12 SNPs (10 tagSNPs and 2 additional SNPs) and 3 multi-marker tests were needed to be analysed to cover most of the common genetic variation. Genotyping of the 10 selected tagSNPs and one additional polymorphism (rs35937440) in the OASY cohort was performed by McGill University and Génome Québec Innovation Centre (Canada) using the Sequenom Iplex Gold