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Glucose-dependent insulinotropic polypeptide (*GIP*) and GIP receptor (*GIPR*) genes: An association analysis of polymorphisms and bone in young and elderly women

Gaurav Garg [a,b,1], Fiona E. McGuigan [a,b,1], Jitender Kumar [c], Holger Luthman [f], Valeriya Lyssenko [d,e], Kristina Akesson [a,b,*]

[a] Clinical and Molecular Osteoporosis Research Unit, Dept. of Clinical Science Malmö, Lund University
[b] Dept of Orthopaedics, Skåne University Hospital Malmö, Sweden
[c] Dept of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[d] Dept of Clinical Sciences, Diabetes and Endocrinology, CRC, Lund University, Lund, Sweden
[e] Steno Diabetes Center, Gentofte, Denmark
[f] Medical Genetics Unit, Dept. of Clinical Sciences Malmö, Lund University, Sweden

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ABSTRACT

Introduction: The gastro-intestinal hormone glucose-dependent insulinotropic polypeptide (GIP) potentiates glucose-induced insulin secretion, with bone anabolic effects through GIP receptor (GIPR) in animal models. We explore its potential in humans by analyzing association between polymorphisms (SNPs) in the *GIP* and *GIPR* genes with bone phenotypes in young and elderly women.
Methods: Association between *GIP* (rs2291725) and *GIPR* (rs10423928) and BMD, bone mineral content (BMC), bone microarchitecture, fracture and body composition was analyzed in the OPRA (75y, n = 1044) and PEAK-25 (25y; n = 1061) cohorts and serum-GIP in OPRA.
Results: The *GIP* receptor AA-genotype was associated with lower ultrasound values in young women (BUA $p = 0.011$; SI $p = 0.030$), with no association to bone phenotypes in the elderly. In the elderly, the *GIP* was associated with lower ultrasound (GG vs. AA; SOS $p_{adj} = 0.021$) and lower femoral neck BMD and BMC after adjusting for fat mass ($p_{adj} = 0.016$ and $p_{adj} = 0.03$). In young women, neither *GIPR* nor *GIP* associated with other bone phenotypes including spine trabecular bone score. In the elderly, neither SNP associated with fracture. *GIP* was associated with body composition only in Peak-25; *GIPR* was not associated with body composition in either cohort. Serum-GIP levels (in elderly) were not associated with bone phenotypes, however lower levels were associated with the *GIPR* A-allele ($\beta = -6.93$; $p_{adj} = 0.03$).
Conclusions: This first exploratory association study between polymorphisms in *GIP* and *GIPR* in relation to bone phenotypes and serum-GIP in women at different ages indicates a possible, albeit complex link between glucose metabolism genes and bone, while recognizing that further studies are warranted.

1. Introduction

Osteoporosis is a silent and progressive systemic skeletal disorder resulting in low bone mineral density (BMD) with fracture as its associated clinical consequence (Consensus Development Conference, 1993). The maintenance of skeletal strength through bone remodeling is regulated through complex interactions between bone cells and endocrine cells (Rosen and Klibanski, 2009). There is evidence for the role of gastro-intestinal hormones secreted in response to food intake in the maintenance of skeletal integrity and altered profiles of bone turnover-markers have been observed in the aftermath of meal ingestion (Elnenaei et al., 2010; Henriksen et al., 2003). Glucose-dependent insulinotropic polypeptide (also known as gastric inhibitory polypeptide (GIP)) is one such gastro-intestinal hormone. Secreted by K cells in the small intestine, GIP potentiates glucose-induced insulin secretion from pancreatic β-cells leading to reduced blood glucose levels (Saxena et al., 2010). *In vitro* studies have shown that GIP inhibits osteoclast differentiation and activity via a direct mechanism which may lead to a net effect of increased bone mass, although the effects of GIP could also be mediated, at least in part, by variation in insulin secretion (Fulzele and Clemens, 2012); in rats, administration of GIP reduces bone loss after ovariectomy (Bollag et al., 2001; Bollag et al., 2000; Zhong et al., 2007).

* Corresponding author at: Department of Orthopaedics, Skåne University Hospital, 205 02 Malmö, Sweden.
E-mail address: kristina.akesson@med.lu.se (K. Akesson).
[1] These authors contributed equally.

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GIP receptor (GIPR) is expressed in osteoblasts, osteocytes and osteoclasts as well as a wide range of tissues including adipocytes, pancreas, lungs, kidney and thyroid (Bollag et al., 2000; Zhong et al., 2007). Studies of transgenic mice overexpressing *GIP* show higher BMD and bone mineral content (BMC) than controls while in addition, they have elevated serum levels of GIP and total osteocalcin (Ding et al., 2008). Furthermore, in these mice, an age dependent decrease in *GIPR* expression has also been observed. Conversely, knockout mice deficient in *GIPR* have deranged cortical microarchitecture of bone leading to reduced bone 'quality' and strength and low fat mass (Mieczkowska et al., 2013). Taken together these observations represent one aspect of the complex shared molecular mechanisms between osteoporosis and diabetes. Type 1 diabetes (T1D) is associated with low BMD and increased fracture risk (Vestergaard et al., 2005) while type 2 diabetes (T2D), with its increased risk of fracture despite normal bone mass (Janghorbani et al., 2007; Nicodemus and Folsom, 2001), is complicated by the complex relationship between body weight, osteoporosis and T2D.

In a meta-analysis of genome-wide association studies, a variant (rs10423928) in the *GIPR* gene has been found to be associated with elevated postprandial glucose and insulin (Saxena et al., 2010) as well as lean body composition including decreased BMI, lean mass and waist circumference (Lyssenko et al., 2011), hence its selection for this study. Only one study however has investigated *GIPR* variation in relation to BMD; reporting that a functional SNP in linkage disequilibrium with rs10423928 was associated with low BMD in early postmenopausal women (Torekov et al., 2014). To date there have been no population-based studies investigating association of variants in the *GIP* gene with bone phenotypes.

The primary aim of our study was to investigate the association of SNPs in the *GIP* and *GIPR* genes with skeletal phenotypes beyond bone density (BMC, bone microarchitecture, fracture), body composition and serum GIP level. Since menopausal (estrogen) status may influence the association, the study was performed in two population-based cohorts consisting of 75 year and 25 year old women.

2. Materials and methods

2.1. Subjects

Two population based cohorts of Swedish women living in Malmö, Sweden were studied; the Osteoporosis Prospective Risk Assessment cohort (OPRA) consisting of 1044 elderly women aged 75 at inclusion and followed-up at 5 years (n = 715) and 10 years (n = 382) and the PEAK-25 cohort consisting of 1061 women all 25 years old at inclusion. Details of the cohorts have been published elsewhere (Gerdhem et al., 2004; McGuigan et al., 2007). All study participants gave written informed consent and the study was approved by the Regional Ethical Review Board in Lund, Sweden.

2.2. DXA — bone phenotypes and body composition

BMD was measured for total body (TB), femoral neck (FN), and lumbar spine (LS) using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy: PEAK-25; Lunar DPX-L: OPRA (Lunar Corporation, Madison, WI, USA). Total body fat mass (FM) and lean mass (LM) were also measured by using DXA. All measurements were performed using the same instrument. At baseline, software versions 1.33 and 1.35 (OPRA) and 2.05, 2.15, 3.60, 5.70 and 7.70 (PEAK-25) were used. Version 4.7e was used for OPRA 10 year follow-up. Calibrations were performed daily using a manufacturer supplied phantom. Precision (coefficient of variation (CV)) for DXA scanning was 0.94% (TB), 1.45% (LS) and 4.01% (FN) in the OPRA cohort (Lenora et al., 2010) and 0.90% (FN) and 0.65% (LS) in PEAK-25 (Callreus et al., 2012).

2.3. Bone microarchitecture at the spine and heel

We also assessed aspects of bone strength as reflected by microarchitecture (or bone 'quality') measured by quantitative ultrasound (QUS): speed of sound (SoS) (m/s), broadband ultrasound attenuation (BUA) (dB/MHz), and stiffness index (SI). Measurements were performed using the Lunar Achilles (R) system (Lunar Corporation Madison, WI, USA) in both cohorts. The CV was 1.5% for derivatives of BUA and SoS (Karlsson et al., 1998). Daily calibrations were performed.

Microarchitecture in the spine was measured using the trabecular bone score (TBS), a novel approach applied to the DXA image. Due to technical limitations TBS could not be calculated from the Lunar DPX-L, therefore spine acquisitions were available only for the PEAK-25 cohort. Posteroanterior spine acquisitions were analyzed using the manufacture's software (Encore 2004; GE Medical-Lunar, Madison, WI) and a standardized protocol (Hans et al., 2011). TBS was calculated as the mean value of the individual measurements for each vertebra (L1 to L4).

2.4. Incident fracture

In the OPRA cohort information on incident fractures was obtained through questionnaires at 1, 3, 5 and 10 years after the baseline investigation. These fractures and all fractures occurring until October 2012 providing a maximum follow-up for fracture of 17.2 years (mean 13.1 years) were verified in files at the Department of Radiology, Skåne University Hospital, Malmö, Sweden. We focused on analyzing "Any Incident Fracture" as a single category. This category included hip, distal radius, vertebra, shoulder, pelvis and proximal tibia fractures. Fractures of the face, hands and feet were excluded. The majority of fractures (>99%) were attributed to low energy trauma. In the PEAK-25 cohort, fracture incidence was not analyzed due to the low numbers of fractures occurring at this age.

2.5. Serum GIP

Serum GIP (s-GIP measurements were available only in the OPRA cohort; at 10 year follow-up. Levels of s-GIP were successfully measured for n = 363/382 participants. s-GIP was measured in fasting samples using a human GIP (Total) ELISA kit (Millipore, R&D Systems, Abingdon, UK) (Ahlqvist et al., 2013). The assay was performed following the manufacturer's instructions. No samples fell below the lower limit of detection (8.2 pg/ml). The inter-assay CV was 2–6% while CV for the study samples was 4.3–5.6.

2.6. Genotyping

Total genomic DNA was isolated from blood using the QIAamp 96 DNA blood kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. In this study, we analyzed rs2291725 (G/A, S103G) located in exon 4 of *GIP* and rs10423928 (T/A) in intron 12 of GIPR (Table 1). The *GIP* SNP rs2291725 was chosen since it is a high frequency missense variation in the *GIP* gene changing amino acid number 103 in the GIP protein (Ser to Gly). The rationale for selection of the *GIPR* SNP lies in the fact that in a combined analysis of several GWAS studies the risk genotype of rs10423928 showed impaired insulin secretion. This *GIPR* SNP is in strong linkage disequilibrium (r2 = 0.99) with the non-synonymous SNP rs1800437 (E354Q) analyzed in the study by Torekov et al. (2014). Consequently the two SNPs reflect the same genetic variation in the gene.

From those who agreed to provide whole blood for DNA analyses, a total of 990 women from OPRA and 992 women from PEAK-25 were genotyped successfully using TaqMan (ABI, Foster City, USA). Approximately 3% of the samples from each cohort were genotyped in duplicate with 100% concordance. Both polymorphisms conformed to Hardy–Weinberg equilibrium and the minor allele frequencies did not