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

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Abstract: 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 (G-G vs. AA; SOS padj = 0.021) and lower femoral neck BMD and BMC after adjusting for fat mass (padj = 0.016 and padj = 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 lower ultrasound (GG vs. AA; SOS padj = 0.021) and lower femoral neck BMD and BMC after adjusting for fat mass (padj = 0.016 and padj = 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.

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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 (Elnein 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).
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., 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 (Lysenko 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 GIPR gene with bone phenotypes.

The primary aim of our study was to investigate the association of SNPs in the GIPR and GIP genes with skeletal phenotypes beyond bone density (BMD, 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.

2.3. 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 manufacturer’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
differ from other European populations. Genotype and allele frequencies did not differ between cohorts.

2.7. Statistical analyses

All statistical analyses were performed using SPSS version 22 (IBM Corp., NY, USA). To analyze association between genotypes and fracture, the \( \chi^2 \) test was used. Logistic regression analysis with adjustment for fat mass and smoking was also used. Unadjusted analyses for continuous variables (BMD, BMC, ultrasound, trabecular bone score and body composition) were performed using the Kruskal-Wallis test for all three genotypes. Genotype association with continuous variables was also performed using regression analysis. Multivariate regression analysis was used to identify confounding factors. Therefore bone phenotypes were adjusted for fat mass (trabecular bone score was additionally adjusted for lumbar spine BMD) and body composition phenotypes adjusted for smoking. The results were not appreciable different when corrected for diabetes, therefore in the presented results women who reported having diabetes (either type I or type II; \( n = 69 \)) were not excluded from the analyses. A priori power analyses, assuming a SD of 0.13 g/cm² in BMD, indicated that our sample size allowed >80% power to detect differences of 0.065 g/cm² between genotypes assuming a minor allele frequency of >0.21. Nominal significance was considered with associations of \( p < 0.05 \). We report the uncorrected \( p \)-values, acknowledging that multiple tests were performed.

3. Results

The characteristics of the women from the two differently aged cohorts have been published previously (Gerdhem et al., 2004; McGuigan et al., 2007). Briefly, elderly women from the Opra cohort, as expected, had higher BMI and fat mass and lower lean mass and bone mineral density than the young women from PEAK-25 cohort (Supplementary Table 1). Both fat and lean mass were strongly positively associated with BMD and QUS (Callleurs et al., 2012; Garg et al., 2014; Gerdhem et al., 2003).

Mean serum GIP level in the Opra cohort at 10 year follow-up was 58.5 pg/ml [SD 33.8]. There was no association between GIP level and any of the bone phenotypes measured at 10 y follow-up, although femoral neck BMD and BMC were lower in the highest GIP tertile compared to the lowest (BMD: 0.693 vs 0.684, \( p = 0.87 \); BMC: 3.564 vs 3.517, \( p = 0.54 \)). Similarly, body composition phenotypes were not associated with this SNP. Neither did bone properties reflecting microarchitecture (i.e. QUS at the calcaneus and TBS at the spine), differ between genotypes (Table 2).

Variation in GIPR was not associated with BMD or BMC in either cohort (Table 3). In PEAK-25, although trabecular bone score did not differ with genotype, women carrying the minor allele had lower calcaneus QUS values, but only BUA and SI reached nominal significance and remained after adjustment for fat mass (BUA \( \beta = -1.63 \) (SE 0.59), \( p_{adj} = 0.006 \); SI \( \beta = -1.86 \) (SE 0.83), \( p_{adj} = 0.026 \)). The results remained similar after further adjustment for femoral neck BMD. Conversely, although non-significant, the trend was towards higher QUS values in the elderly women (Table 3).

In PEAK-25, a general trend for association between weight, BMI, fat and lean mass was observed with the GIP polymorphism (Table 2). Nominal significance was reached for weight and total body fat mass after adjustment for smoking (\( p_{adj} = 0.031 \) and 0.026) (Table 2). There was no association with body composition in Opra. Variation in GIPR was not associated with body composition in either cohort.

Neither GIP nor GIPR SNPs were associated with occurrence of fractures in the Opra cohort (Table 4).

4. Discussion

The basis for this study lies in the role of glucose-insulinotropic peptide (GIP) hormone in the regulation of insulin secretion as well as its anabolic effect on osteoblasts and inhibition of osteoclasts (Tsukiyama et al., 2006; Xie et al., 2005). Transgenic mice over-expressing GIP have increased cortical bone mass while GIP also appears to prevent age related decline in bone mass and bone strength (Ding et al., 2008). Thus GIP and the receptor to GIP (GIPR) genes are attractive biological candidates to understand the genetic relationship between type 2 diabetes and osteoporosis. In this study, comprising two differently aged cohorts of women, we investigated if genetic variants in GIP and GIPR displayed association with phenotypes contributing to bone phenotypes including bone quantity and structure.

In the present study, BMD and BMC were not associated with GIPR; however there was an association, although only in the young women, with lower calcaneal ultrasound values and this was independent of bone density. The absence of an association with bone architecture in the spine however is in line with data from a knock-out mouse model demonstrating that the effects of GIPR deficiency on bone microarchitectural (Mieczkowska et al., 2013) differ between predominantly cortical and trabecular skeletal sites (Gaudin-Audrain et al., 2013).

In the recent study from Denmark, a GIPR SNP (rs1800437), in strong linkage disequilibrium with the rs10423928 SNP used here, was analyzed. They observed an association with lower BMD and increased fracture risk which is in contrast with our findings. Since one would expect Swedish and Danish to be genetically similar, a possible explanation for this divergent finding is that the Danish women were perimenopausal suggesting that GIPR has a more important role in the immediate period

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**Table 1**

| SNP_Gene | Opra | PEAK-25 |
|----------|------|---------|
|          | TT   | TA      | AA    | TT   | TA      | AA    |
| rs10423928 | 610 (61.6%) | 335 (33.8%) | 45 (4.6%) | 0.21 | 606 (61.2%) | 324 (32.7%) | 60 (6.1%) | 0.22 |
| GIP       |      |        |       |      |        |       |
| rs2291725 | 271 (27.4%) | 493 (49.8%) | 226 (22.8%) | 0.47 | 260 (26.2%) | 507 (51.1%) | 225 (22.7%) | 0.48 |
| GIP       |      |        |       |      |        |       |

GIP – gastric inhibitory polypeptide; GIPR – gastric inhibitory polypeptide receptor.
around estrogen withdrawal rather than during bone accrual or in the very elderly. GIPR expression is reduced with age (Ding et al., 2008) which would appear to support this.

In our study, GIPR had an effect on body composition showing small reductions in weight and fat mass. This is largely in line with the findings of Lyssenko et al. (2011), who also demonstrated that the BMI-lowering effect of this SNP neutralized the comitant association with impaired glucose concentration and GIP-stimulated insulin secretion.

Why the GIPR rather than the GIP SNP was associated with serum levels of GIP is unclear although decreased receptor activity has been reported for the GIPR E354Q (rs1800437) variant (Caudin-Audrain et al., 2013) which could influence the cycles of receptor desensitization/resensitization. In cultured adipocytes, the receptor has been shown to be down regulated by GIP stimulation and desensitized to further GIP stimulation for a prolonged period (Ranganath et al., 1998).

### Table 2

Association of GIP rs2291725 with bone phenotypes and body composition.

| OPRA            | GG     | GA     | AA     | \(\beta\)-Value (adj)*  | \(p^\alpha\)  | \(p^\beta\) (adj) |
|-----------------|--------|--------|--------|-------------------------|-------------|-----------------|
| BMD total body (g/cm²) | 1.010 (0.945–1.074) | 1.002 (0.943–1.067) | 0.994 (0.934–1.056) | \(-0.01 \ (-0.01 to 0.001)\) | 0.30 | 0.090 |
| BMD femoral neck (g/cm²) | 0.761 (0.677–0.865) | 0.768 (0.686–0.884) | 0.733 (0.641–0.831) | \(-0.01 \ (-0.03 to -0.003)\) | 0.11 | 0.016 |
| BMC total body (g) | 2.085 (1832–2310) | 2.026 (1812–2260) | 1.977 (1765–2210) | \(-31 \ (-58 to -5)\) | 0.11 | 0.020 |
| BMC femoral neck (g) | 3.8 (3.26–4.6) | 3.8 (3.3–4.4) | 3.7 (3.18–4.4) | \(-0.1 \ (-0.2 to -0.01)\) | 0.21 | 0.030 |
| QUS_BIA (dB/MHz) | 101.4 (94.1–108.0) | 103.0 (96.2–108.7) | 100.7 (94.1–107.7) | \(-0.1 \ (-1.1 to 0.8)\) | 0.11 | 0.76 |
| QUS_SoStiffness | 71.2 (62.1–80.2) | 72.0 (64.0–81.3) | 69.0 (59.4–79.1) | \(-0.76 \ (-1.99 to 0.49)\) | 0.043 | 0.23 |
| Weight (kg) | 67 (59–75) | 67 (60–75) | 67 (61–76) | \(-0.1 \ (-1.03 to 1.01)\) | 0.88 | 0.98 |
| BMI (kg/m²) | 26.1 (23.7–28.6) | 25.9 (23.3–28.7) | 26.0 (23.4–28.7) | \(-0.03 \ (-0.40 to 0.34)\) | 0.83 | 0.86 |
| Total body fat mass (kg) | 25.57 (20.54–31.31) | 25.62 (20.66–31.19) | 26.47 (21.03–31.77) | 173.45 (557.99 to 940.88) | 0.79 | 0.64 |
| Total body lean mass (kg) | 36.98 (34.80–40.21) | 36.82 (34.59–39.74) | 37.20 (34.39–39.14) | \(-108.83 \ (-476.28 to 258.61)\) | 0.86 | 0.56 |

### Table 3

Association between GIPR (rs10423928) and bone composition.

| OPRA            | TT     | TA     | AA     | \(\beta\)-Value (adj)*  | \(p^\alpha\)  | \(p^\beta\) (adj) |
|-----------------|--------|--------|--------|-------------------------|-------------|-----------------|
| BMD total body (g/cm²) | 0.999 (0.943–1.073) | 1.002 (0.934–1.064) | 1.021 (0.967–1.060) | 0.003 \(-0.01 \ (-0.01 to 0.01)\) | 0.56 | 0.49 |
| BMD femoral neck (g/cm²) | 0.758 (0.666–0.851) | 0.739 (0.653–0.835) | 0.763 (0.706–0.867) | \(-0.03 \ (-0.02 to 0.01)\) | 0.44 | 0.70 |
| BMC total body (g) | 2046 (1798–2285) | 2023 (1810–2230) | 2191 (1899–2290) | 12 \(20 \ (-20 to 45)\) | 0.26 | 0.45 |
| BMC femoral neck (g) | 3.8 (3.3–4.5) | 3.67 (3.2–4.4) | 3.9 (3.5–4.5) | \(-0.03 \ (-0.12 to 0.07)\) | 0.21 | 0.58 |
| QUS_BIA (dB/MHz) | 101.4 (96.0–108.0) | 102.5 (96.0–108.0) | 103.0 (95.6–110.4) | \(-0.77 \ (-0.35 to 1.88)\) | 0.62 | 0.18 |
| QUS_SoStiffness | 52.8 (47.2–58.5) | 52.2 (46.9–59.2) | 51.7 (46.1–57.5) | 0.2 \(0.64 \ (-1.04 to 0.74)\) | 0.78 | 0.64 |
| QUS_Stiffness Index | 117.1 (110.4–124.7) | 114.6 (109.1–121.5) | 115.4 (108.8–121.7) | \(-1.63 \ (-2.79 to -0.47)\) | 0.011 | 0.006 |
| Total body lean mass (kg) | 79.8 (78.8–90.9) | 79.6 (86.8–107.0) | 84.5 (87.3–103.9) | \(-1.86 \ (-3.49 to -0.22)\) | 0.03 | 0.026 |

### Notes

- **BMD** (bone mineral density), **BMC** (bone mineral content), **QUS** (quantitative ultrasound), **SoStiffness** (speed of sound), **Stiffness Index** (stiffness index), Values are median (interquartile range).
- **^a** (GG vs. GA vs. AA)
- **^b** Kruskal-Wallis
- **^c** Linear regression — adjusted for fat mass.
- **^d** Additionally adjusted for fat mass & LS BMD.

Why the GIPR rather than the GIP SNP was associated with serum levels of GIP is unclear although decreased receptor activity has been reported for the GIPR E354Q (rs1800437) variant (Caudin-Audrain et al., 2013) which could influence the cycles of receptor desensitization/resensitization. In cultured adipocytes, the receptor has been shown to be down regulated by GIP stimulation and desensitized to further GIP stimulation for a prolonged period (Ranganath et al., 1998).

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Serum GIP levels are reported to be increased in postmenopausal women (Ranganath et al., 1998) and modulated by estrogen replacement therapy (Sztefko et al., 2005), in our study however, GIP does not appear to be exerting a bone anabolic effect, at least in this elderly age group. Whether age related differences in sGIP explain our observed results is uncertain.

The GIP SNP rs2291725 (Gly103Ser) is a functional variant previously reported to be associated with type 2 diabetes (Chia et al., 2009) but not yet explored in relation to altered bone phenotypes. In this study, the GIP polymorphism was adversely associated with components of bone strength including density, mineral content and microarchitecture in the elderly but not the young women, even after adjustment for fat mass. The absence of an association with body composition directly contrasts with the positive association observed specifically with weight and fat mass in the young PEAK-25 cohort.

The strengths of this study include being the first, to our knowledge, evaluating the importance of GIP polymorphisms in relation to bone phenotypes in human cohorts, although a receptor gene polymorphism has been reported once before. In addition, we have not only evaluated BMD but also bone strength and related phenotypes. In the elderly women, we also analyzed serum GIP, although a weakness of the investigation is that this was only available for only 363 individuals, hence the statistical power to detect association was limited. In addition, serum levels could not be determined in the young making it difficult to establish potential interaction between serum GIP levels, bone properties, body composition and genotype at different ages. A limitation of the study is the fact that neither of the SNPs has been identified as major candidate genes in GWAS for osteoporosis related phenotypes. However, since SNP based GWAS do not identify all disease risk variants, studies of variants in related pathways are still warranted. We also acknowledge that the results must be interpreted with caution, since the associations are modest, in the absence of stringent adjustment for multiple testing.

Underlyng this study is the hypothesized importance of insulin and glucose metabolism on bone. In our investigation of GIP and GIPR gene polymorphisms in relation to bone phenotypes in young and elderly women our findings depict a complex relationship between glucose metabolism genes and bone. In the current setting, it is not possible to explore the reason for these observations. Further studies in equivalent populations are merited to explore these associations further.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bonr.2015.12.001.

Conflict of interest

All authors have no conflicts of interest.

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Table 4

Association of GIP and GIPR polymorphisms with incident fracture in OPRA women.

|                | TT             | TA             | AA             | p    |
|----------------|----------------|----------------|----------------|------|
| Fracture       | 312 (62.5%)    | 165 (33.1%)    | 22 (4.4%)      | 0.838|
| No fracture    | 298 (60.7%)    | 170 (34.6%)    | 23 (4.7%)      |      |
| rs2291725_GIP  |                |                |                |      |
| Fracture       | 134 (26.9%)    | 242 (48.6%)    | 122 (24.5%)    | 0.450|
| No fracture    | 137 (27.8%)    | 251 (51.0%)    | 104 (21.1%)    |      |

p-Value calculated by the $\chi^2$ test.

a Incident fracture of any type (includes hip, distal radius, vertebra, shoulder, pelvis and proximal tibia fractures).