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Research

Adaptive selection of an incretin gene in Eurasian populations

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Diversities in human physiology have been partially shaped by adaptation to natural environments and changing cultures. Recent genomic analyses have revealed single nucleotide polymorphisms (SNPs) that are associated with adaptations in immune responses, obvious changes in human body forms, or adaptations to extreme climates in select human populations. Here, we report that the human *GIP* locus was differentially selected among human populations based on the analysis of a nonsynonymous SNP (rs2291725). Comparative and functional analyses showed that the human *GIP* gene encodes a cryptic glucose-dependent insulinotropic polypeptide (GIP) isoform (GIP55S or GIP55G) that encompasses the SNP and is resistant to serum degradation relative to the known mature GIP peptide. Importantly, we found that GIP55G, which is encoded by the derived allele, exhibits a higher bioactivity compared with GIP55S, which is derived from the ancestral allele. Haplotype structure analysis suggests that the derived allele at rs2291725 arose to dominance in East Asians ~8100 yr ago due to positive selection. The combined results suggested that rs2291725 represents a functional mutation and may contribute to the population genetics observation. Given that GIP signaling plays a critical role in homeostasis regulation at both the enteroinsular and enteroadipocyte axes, our study highlights the importance of understanding adaptations in energy-balance regulation in the face of the emerging diabetes and obesity epidemics.

[Supplemental material is available online at <http://www.genome.org>.]

Recent studies have revealed that genetic variation underlies a variety of diversities in human physiology and pathology (Sabeti et al. 2005; Voight et al. 2006, 2010; Sulem et al. 2007; Tishkoff et al. 2007; Genovese et al. 2010; Leslie 2010; Simonson et al. 2010; Yi et al. 2010). Among the sundry forms of genetic variation, single nucleotide polymorphisms (SNPs) with high population differentiation are regarded as candidates of adaptation to recent changes in human environment and culture, and have been shown to play an important role in acquiring distinct physiological traits and susceptibility to different diseases among the populations (Barreiro and Quintana-Murci 2010; Chen et al. 2010; Gibbons 2010; Ingelsson et al. 2010; Laland et al. 2010; Luca et al. 2010; Richerson et al. 2010). Thus, the identification of causal SNPs with signatures of positive selection and underlying functional changes is crucial to a better understanding of the relationship between genomic variation and human health as well as gene–environmental interactions (Nielsen et al. 2007; Laland et al. 2010; Nei et al. 2010). To systematically analyze the contributions of genetic variation in intercellular signaling molecules to physiological diversities in humans, we studied SNPs in the coding region of 839 human polypeptide hormones and their cognate receptors for evidence of

selection using the data from the International HapMap project phases I and II (International HapMap Project 2003; International HapMap Consortium 2007). We focused on these polypeptide ligands and receptors because they represent half of the targets of modern medicine (Drews 2000) and because the genes associated with intercellular communication or the responses to environmental factors (e.g., pathogens and food sources) have been implicated in the evolution of a variety of common traits and pathologies in humans and other vertebrates (Seminara et al. 2003; Sabeti et al. 2005; Hoekstra et al. 2006; Lalueza-Fox et al. 2007; Sulem et al. 2007; Chambers et al. 2008; Prokopenko et al. 2008; Shiao et al. 2008; Shimomura et al. 2008; Anderson et al. 2009; Topaloglu et al. 2009). Here, based on genomic and biochemical analyses, we show that variants in an incretin hormone gene, *GIP*, were differentially selected in human populations, and a nonsynonymous SNP, rs2291725, represents a functional mutation. Because changes in the food source represented one of the most important selection pressures during the transitions of human culture and because incretin hormones play critical roles in homeostasis maintenance at the enteroinsular and enteroadipocyte axes, future studies of potential genotype–phenotype relationships for the selected *GIP* variants could provide a better understanding of which and how these variants contribute to phenotypic variation in energy-balance regulation among individuals or human populations.

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Results

A nonsynonymous SNP (rs2291725) in the human glucose-dependent insulinotropic polypeptide gene exhibits high population differentiation

To investigate whether variation in the polypeptide intercellular signaling molecules contribute to physiological diversities in humans, we curated 457 human G-protein-coupled receptors (GPCRs) and their cognate ligand genes as well as 382 human non-GPCR receptor and ligand genes (Supplemental Table 1; Ben-Shlomo et al. 2003; Semyonov et al. 2008). We started by using the population differentiation statistic F_{ST} to identify the leads for functional characterization (Lewontin and Krakauer 1973; Akey et al. 2002; Li et al. 2008).

We computed the F_{ST} for the coding SNPs of GPCR and ligand genes and compared the result with those for coding SNPs of all other human genes. F_{ST} was computed between all possible pairs of HapMap II populations (YRI [African, Yoruba from Ibadan], CEU [European, United States residents with northern and western European ancestry], and ASN [East Asian, pooled samples of Chinese from Beijing [CHB] and Japanese from Tokyo [JPT]]). Distributions of F_{ST} for either the synonymous or nonsynonymous SNPs between any two HapMap II populations showed no difference (all $P > 0.01$, Kolmogorov–Smirnov test) (Supplemental Fig. 1). Likewise, studies of the F_{ST} of coding SNPs in 382 non-GPCR receptor and ligand genes have shown similar cumulative distribution function (CDF) plots, suggesting that there is no difference

in F_{ST} distribution between the GPCR and the non-GPCR groups ($P > 0.01$, Kolmogorov–Smirnov test) (Supplemental Fig. 2). These results suggest that, when analyzed as a whole set, the coding SNPs of human polypeptide receptor and ligand genes do not have a significantly elevated F_{ST} .

Nevertheless, at the individual-gene level, dozens of coding SNPs in these receptor and ligand genes have a high F_{ST} (>0.5) between select pairs of populations (Supplemental Tables 2, 3). Among GPCRs and their cognate ligands, *DRD5*, *DARC*, *CELSR1*, *CCL23*, *GIP*, *MC1R*, *EMR1*, *GRM1*, *CALCR*, *CXCR6*, *GPR39*, and *DRD3* were found to have nonsynonymous SNPs with a high F_{ST} , which suggests that these SNPs are likely to be targets of selection. On the other hand, nonsynonymous SNPs in *EDAR*, which has been repeatedly shown to be under positive selection (Bryk et al. 2008; Fujimoto et al. 2008), and several immune response-related genes (e.g., *IL4R*, *TNFRSF10A*, *TRAF3*, *IL29*, *IL20RA*, *IL1RL1*, *TNFRSF6B*, and *PTPRA*) in the non-GPCR group were found to have high F_{ST} scores in select pair(s) of the populations.

Importantly, we found that the nonsynonymous SNP (rs2291725) in exon 4 of the glucose-dependent insulinotropic polypeptide gene (or gastric inhibitory peptide, *GIP*; referred to as the *GIP*^{103T/C} mutation in the following text) on chromosome 17 is highly linked with a cluster of neighboring SNPs within a 250-kb region (starting from rs8079874 to rs2291726) and has an F_{ST} value in the top 0.5% of all nonsynonymous SNPs in comparisons between the ASN and YRI populations (Fig. 1A; Supplemental Table 2). In contrast, F_{ST} estimates in comparisons between CEU and YRI or between CEU and ASN were not different from the

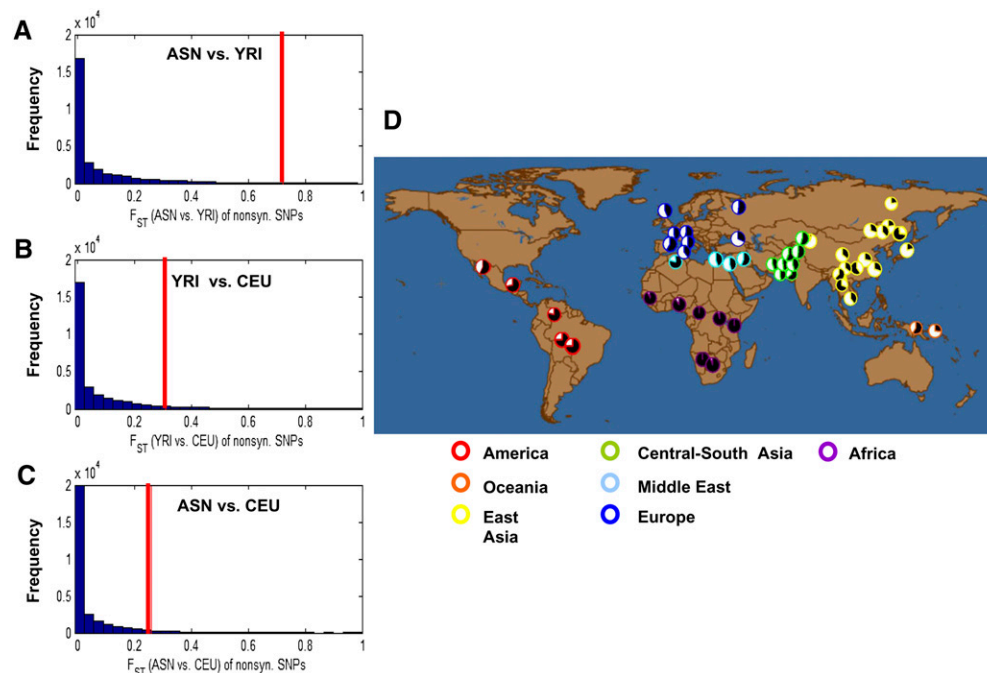


Figure 1. Differential distribution of alleles at rs2291725 in human populations. (A–C) The distribution of the F_{ST} for nonsynonymous SNPs across the human genome and the F_{ST} for rs2291725. F_{ST} estimations between ASN and YRI (A), YRI and CEU (B), and ASN and CEU (C) are shown in the x-axis. The red vertical bar indicates the corresponding values of F_{ST} for rs2291725. The y-axis represents the frequency of SNPs with a given F_{ST} estimate. Statistical significance for comparisons in A, B, and C is indicated with empirical $P = 0.0039$, 0.058, and 0.047, respectively. The number of coding SNPs that were analyzed in the A, B, and C histograms was 48,526, 48,674, and 48,075, respectively. Among these SNPs, the number of nonsynonymous SNPs in the A, B, and C histograms was 29,033, 29,026, and 28,774, respectively. (D) Distribution of rs2291725 in the HGDP–CEPH (944 unrelated samples) populations. Pie charts represent the proportion of each genotype by geographic region. The ancestral *GIP*^{103T} allele (black pie) occurs at a higher frequency in African and American populations, whereas the majority of Eurasian populations have a higher frequency of the derived *GIP*^{103C} allele (white pie).

genome average (Fig. 1B,C). Consistently, data from the HGDP-CEPH project (Center d'Etude du Polymorphisme Humain-Human Genome Diversity Panel; 944 unrelated individuals from 52 populations) (Cann et al. 2002; Rosenberg 2006; Li et al. 2008) and the Human Genome Center at the University of Tokyo (752 Japanese individuals, $GIP^{103C}/GIP^{103T} = 0.732/0.268$) showed that the derived GIP^{103C} allele frequency at rs2291725 is much higher (>60%) in the majority of East Asian populations and varies widely among other populations: ranging from 0.0%–9.5% in sub-Saharan Africans and increasing to >40.0% in European and Middle Eastern populations (Fig. 1D; Table 1; Supplemental Table 5).

GIP encodes one of the two incretin hormones (glucagon-like peptide-1[GLP-1] and GIP) in humans and plays a critical role in normal carbohydrate and lipid metabolism (Kim and Egan 2008). After ingestion of nutrients, GIP secreted from duodenal and jejunal K cells acts on pancreatic β cells to stimulate the release of insulin, which thereby ensures the prompt uptake of glucose and lipids into the tissues. Abnormal regulation of GIP signaling leads to altered carbohydrate metabolism and lipid accumulation at the enteroinsular and enteroadipocyte axis, respectively (Miyawaki et al. 2002; Fulurija et al. 2008; Isken et al. 2008; Kim and Egan 2008). In mice, the deletion of the GIP receptor (*Gipr*) led to impaired first-phase glucose-stimulated insulin release (Miyawaki et al. 2002), whereas exogenous GIP was found to worsen postprandial hyperglycemia in patients with type 2 diabetes (Chia et al. 2009). In addition to effects on glucose homeostasis, GIP has been shown to promote obesity in mice that were fed a high-fat diet (McClean et al. 2007; Gnuli et al. 2010). Because the incretin effect induced by the oral glucose intake leads to a higher insulin response compared with that from a matched intravenous glucose stimulation, the regulation of glucose levels by GIP represents a critical endocrine circuit to monitor exogenous energy intake and regulate subsequent storage (Kim and Egan 2008). In addition, the critical role of GIP signaling in energy-balance regulation has been highlighted by recent studies that showed that variants at the *GIPR* locus are associated with glucose levels 2 h after an oral glucose challenge test used in the diagnosis of type 2 diabetes (Ingelsson et al. 2010; Saxena et al. 2010). On the other hand, epidemiological studies have shown that the prevalence of different forms of diabetes and obesity as well as the regulation of glucose metabolism vary widely among ethnic groups (Buchanan and Xiang 2005; Kim and Egan 2008). Thus, the observed high population differentiation in *GIP* polymorphisms could be associated with the adaptations of incretin physiology to recent changes in diets and cultures in select human populations. To test this hypothesis, we fine mapped the *GIP* locus and tested the functions of GIP variants in vitro and in vivo.

Variants at the *GIP* locus were partially selected in Eurasian populations

Analyses of linkage disequilibrium (LD) in the *GIP* region for the three HapMap II populations showed that extended LD blocks are present in the CEU and ASN chromosomes, and these blocks encompass *GIP* and the neighboring *UBE2Z*, *SNF8*, and *ATP5G1* genes (Supplemental Fig. 3A,B, red square, $D' = 1$, likelihood of odds [LOD] scores >2). In contrast, the majority of SNP pairs in the *GIP* region of the YRI chromosomes exhibited a low LOD and a low r^2 (Supplemental Fig. 3C, blue square, $D' = 1$, LOD scores <2). Consistent with the LD analysis, plots depicting the haplotype map showed that most of the chromosomes with the derived GIP^{103C} allele in the ASN population have haplotypes extending >200 kb and are significantly longer compared with chromosomes with the ancestral GIP^{103T} allele (Fig. 2A, middle panel, $P < 0.001$). In contrast, most chromosomes in YRI did not exhibit an extended haplotype surrounding the GIP^{103} allele (Fig. 2A, bottom panel). These results were reflected in the plots of extended haplotype homozygosity (EHH) decay curves (Fig. 2B). In ASN and CEU, the EHH curve for chromosomes carrying the derived GIP^{103C} allele extends much further than that for the ancestral allele. To test whether the area under the EHH curve is greater for a selected allele than for a neutral allele, we calculated the integrated haplotype score (iHS) for rs2291725 as a core marker (Voight et al. 2006). By using a coalescent model that generated 10,000 data sets from the same length of genome region (750 kb) with the same sample size (180 chromosomes), we found that the observed iHS for the derived allele in ASN (iHS = -0.753)—but not CEU (iHS = -0.131)—deviated significantly from the neutral distribution ($P < 1 \times 10^{-4}$) (Supplemental Fig. 4A). We also performed coalescent simulations that took into account the effects of demography (e.g., population bottleneck) (Supplemental Fig. 4B) and recombination hotspot, respectively (Hellenthal and Stephens 2007; Gutenkunst et al. 2009; Supplemental Methods). Consistently, we found that no iHS for the simulated haplotype sets is more negative than the observed iHS for rs2291725 under the bottleneck scenario (empirical $P < 1 \times 10^{-4}$), and only a few are in the presence of a recombination hotspot (empirical $P < 8 \times 10^{-4}$) (Supplemental Fig. 4A). These results indicated that the observed iHS has deviated significantly from the neutral distribution even after adjusting for the bias that may have been introduced by a bottleneck in demography or heterogeneous recombination events. Thus, it is unlikely that neutral evolution alone explains the observed long haplotypes that carry the derived GIP^{103C} allele in the ASN population.

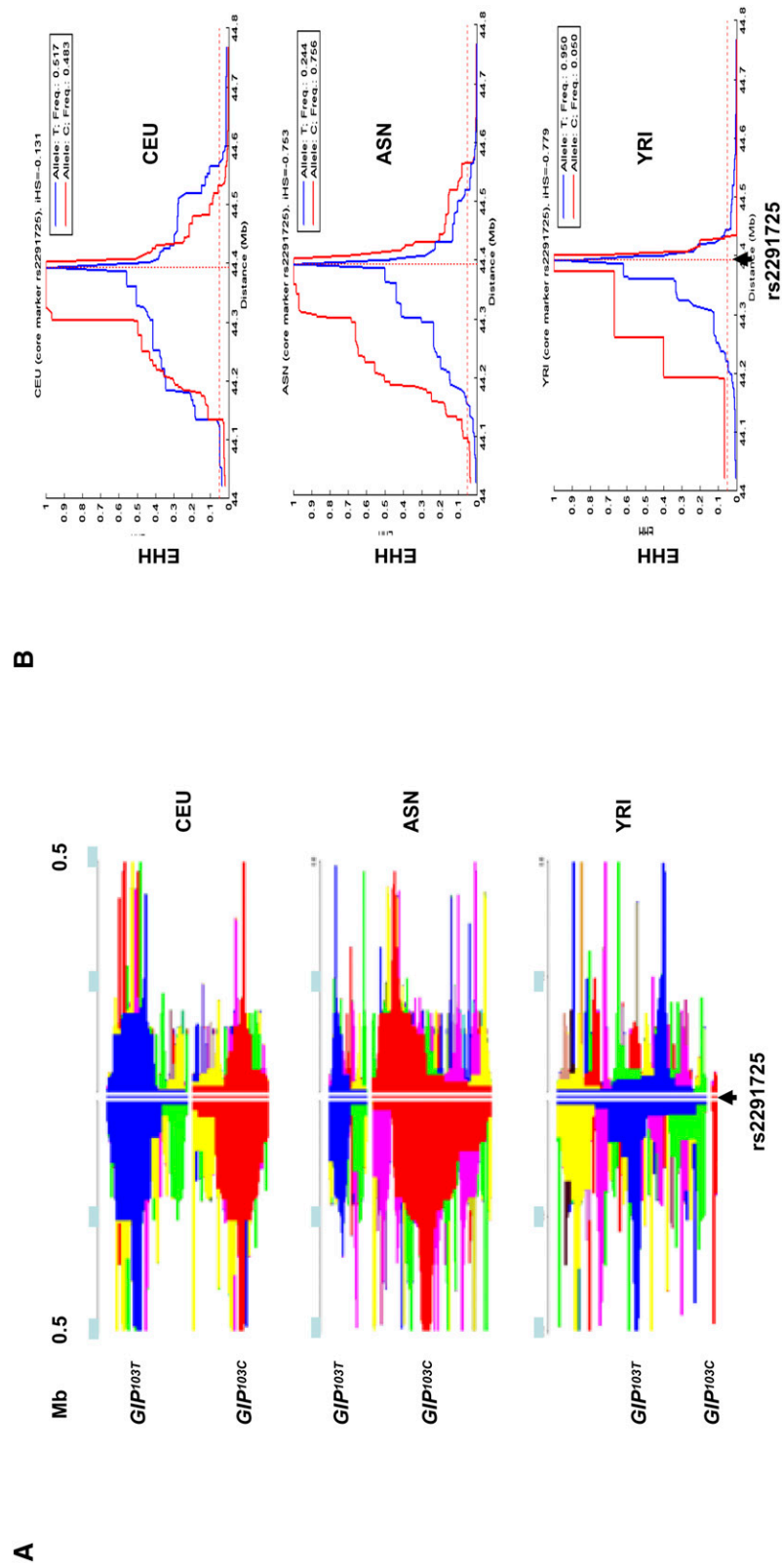
Further examination of the extended haplotype blocks in ASN showed that rs2291725 is highly linked with another 43 SNPs

Table 1. Human *GIP* SNP rs2291725 exhibits a high population differentiation characteristic in the International HapMap II Project data set

Population	Chromosome no.	rs2291725 allele frequency		rs2291725 genotypes			F_{ST} vs.		
		GIP^{103T}	GIP^{103C}	T/T	T/C	C/C	YRI	CEU	ASN
YRI	120	0.950	0.050	0.900	0.100	0.000	—	0.24	0.48 ^a
CEU	120	0.517	0.483	0.267	0.500	0.233	0.24	—	0.08
ASN	178	0.245	0.755	0.090	0.314	0.595	0.48 ^a	0.08	—
All	418	0.524	0.476						

Genotypes were analyzed as described using the SPSmart v3 and Haplotter (Voight et al. 2006; Amigo et al. 2008). The average frequency of two alleles—derived allele GIP^{103C} and ancestral chimpanzee allele GIP^{103T} —at rs2291725 is approximately even in the overall HapMap population: Only 5.0% of YRI chromosomes contain GIP^{103C} compared with 48.3% and 75.5% of CEU and ASN chromosomes that carry the derived allele, respectively.

^a $P < 0.05$.



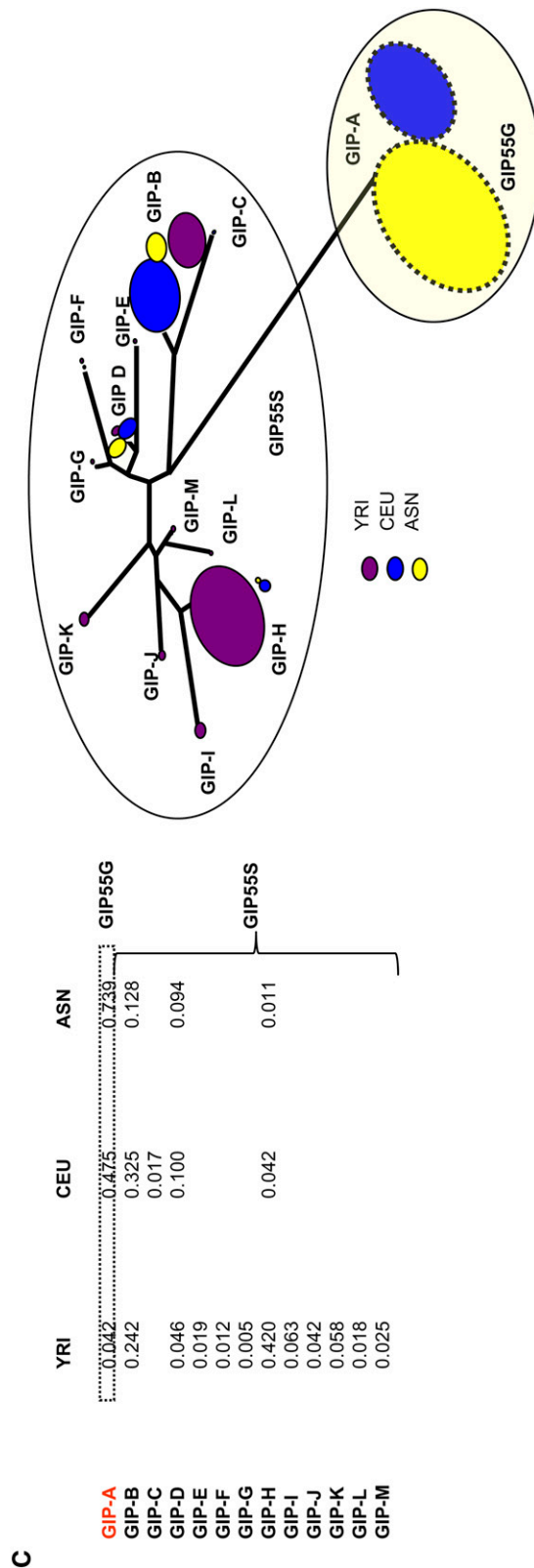


Figure 2. Evolution of haplotypes encompassing the *GIP* locus. (A) Plots of the extent of haplotype homozygosity in the 1.0-Mb region surrounding rs2291725 in CEU, ASN, and YRI as assessed by Haplotype (Voight et al. 2006). These plots are divided into two parts. The upper portion shows haplotypes with the ancestral *GIP*^{103C} allele in blue, and the lower portion shows haplotypes with the derived *GIP*^{103C} allele in red. Adjacent haplotypes with the same color carry identical genotypes spanning the region between a select SNP and rs2291725. (B) Plots of the breakdown of EHH over the distance between rs2291725 and neighboring SNPs at increasing distances. EHH decays much slower at the derived allele (red) compared with the ancestral allele (blue) in ASN and CEU. The position of rs2291725 at the center of the plots is indicated by a vertical dotted line. (C) Evolution of haplotypes within a 70-kb core haplotype in the three HapMap II populations. A total of 13 haplotypes was found in the core region from rs196241 to rs2291726 (37 SNPs at chr17:44,325,258–44,394,253). The frequencies of these haplotypes (GIP-A–M) in YRI, CEU, and ASN are shown in the lower left panel. There are 12 haplotypes in YRI, whereas CEU and ASN are represented by five and four haplotypes, respectively. GIP-A is the only haplotype containing the derived *GIP*^{103C} allele and is indicated by a dotted box. An unrooted tree analysis of these haplotypes showed that the GIP-A haplotype diverged from others early in human evolution (GeneBee). The frequency of each haplotype in a select population is indicated by the size of the pie at the tip of each branch.

investigate this possibility, we analyzed whether the GIP55 peptide is expressed in gut cells of the proximal small intestine. In support of our hypothesis, the immunohistochemical analysis of human duodenum sections with a rabbit GIP55-specific antibody and a mouse anti-GIP antibody showed that immunoreactive GIP55 and GIP are colocalized in select duodenum cells, suggesting that GIP55 is present in gut cells that normally express GIP (Fig. 3B). To study whether GIP55 is secreted into general circulation, we then analyzed the presence of GIP55 in serums of individuals 30 min after a regular breakfast meal using a sandwich ELISA assay that detects the extended C-terminal sequences specific to GIP55. Consistently, we found that GIP55 is present in human serum and constitutes ~1%–3% of the total GIP after a meal (GIP55, 2.91 ± 0.41 pmol/L; total GIP, 95.1 ± 9.41 pmol/L, $N = 6$). Thus, depending on the genotype, humans could contain two (*GIP*^{103T/T}: GIP+GIP55; *GIP*^{103C/C}: GIP+GIP55G) or three GIP isoforms (*GIP*^{103T/C}: GIP+GIP55S+GIP55G) (Fig. 3A).

Because the receptor-activation domain of GIP is located at the N terminus of the peptide, we reasoned that alternative processing at the C terminus of GIP55 peptides is unlikely to decimate their bioactivity. Indeed, functional testing of the synthetic GIP isoforms (GIP, GIP55G, and GIP55S) in vivo showed that similar to conventional GIP, the extended GIP55G and GIP55S suppress hyperglycemia to similar extents in a time-dependent manner in fasting rats (Fig. 3C).

The ancestral GIP55S and the derived GIP55G peptides exhibit distinct bioactivity profiles in vitro

To compare the bioactivity of GIP isoforms and variants, we measured their receptor-activation activities in vitro using HEK293T cells expressing a recombinant human GIP receptor. As expected, treatments of GIP led to dose-dependent increases of cAMP production in transfected cells (Fig. 4A, top left panel). Unlike conventional GIP, which exhibits an EC₅₀ of $\sim 0.9 \pm 0.21$ nM, the extended GIP isoforms have approximately threefold lower potencies (GIP55G, 3.2 ± 0.21 nM; GIP55S, 2.6 ± 0.23 nM) (Supplemental Table 6). Importantly, we found that the derived GIP55G consistently increases cAMP production to significantly higher levels compared with the ancestral GIP55S (Fig. 4A, top left panel). Because GIP is known to be susceptible to serum degradation in vivo, we also studied the stability of GIP isoforms in human serum in vitro. Surprisingly, we found that GIP55G and GIP55S are more resistant to degradation by either pooled normal human serum (Fig. 4A) or pooled complement-preserved human serum (Supplemental Fig. 5). The ranking of potency on receptor activation shifted from GIP > GIP55G > GIP55S at 0 h to GIP = GIP55G = GIP55S and GIP55G > GIP55S \geq GIP, respectively, after a 6-h or a 12-h preincubation with either normal serum (Fig. 4A), or complement-preserved serum (Supplemental Fig. 5). Plots of EC₅₀ data in relation to the length of incubation showed that the slope of changes in the bioactivity for GIP is significantly steeper than that of GIP55G (Fig. 4B, $P = 0.0023$). On the other hand, coincubation with a recombinant dipeptidyl peptidase IV (DPP IV) led to similar extents of degradation of these peptides (Supplemental Table 7). Thus, the resistance to serum degradation by GIP55G cannot be attributed to a resistance of DPP IV, which represents the major processing enzyme that degrades GIP and GLP-1 in vivo by cleaving these peptides at position 2 of the N terminus (Kim and Egan 2008). These data suggested that the rise in the frequency of *GIP*^{103C} in Eurasian populations could be associated with the quantitative increase in the overall potency of GIP55G. Although the nature of the selective

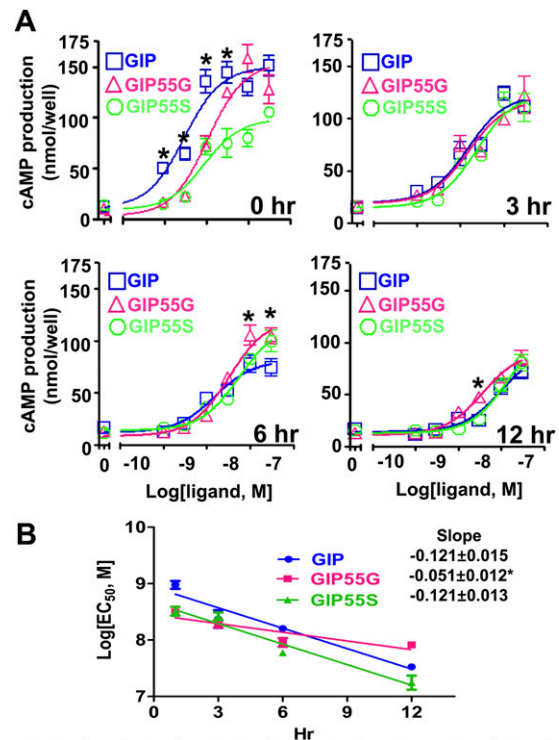


Figure 4. The variation at rs2291725 affects the bioactivity of translated products. (A) GIP55G peptide is resistant to serum degradation. Treatments of GIP receptor-expressing HEK293T cells with GIP, GIP55G or GIP55S led to dose-dependent increases of cAMP production (top left panel). Receptor-activation activities of peptides were also analyzed following incubation with pooled normal human serum for 3, 6, or 12 h. Cells were treated with synthetic peptides for 12 h; the signaling is reported as total cAMP contents in cell lysates. Error bars, SEM of triplicate samples. Significant differences in cAMP production between GIP and GIP55G treatments at a given peptide concentration are indicated by asterisks ($P < 0.01$). In the control group, cells were treated with an aliquot of human serum without a synthetic peptide. Similar results were observed in three separate experiments. (B) Comparison of the slopes of EC₅₀ trend lines for GIP, GIP55G, and GIP55S after treatments with pooled human serum for the indicated time-spans. The slope of the GIP55G group is significantly different from that of the GIP group (* $P = 0.0023$).

advantage conferred by the GIP55 peptides is not clear, we speculate that *GIP*^{103C} could represent a risk allele in ancestors of YRI populations but provide a selective advantage in Eurasians.

The derived *GIP*^{103C} allele arose to dominance in East Asians ~8100 yr ago

Because energy-balance regulation-related loci are subject to selection pressures that fluctuate over time in response to environmental and culture changes, genetic responses to changes in the human diet are more likely associated with incomplete signatures of selection or even signatures of balancing selection (Charlesworth 2006; Pritchard et al. 2010). Based on this understanding, we speculated that the *GIP*^{103C} and *GIP*^{103T} alleles likely confer distinct advantages depending on the history of culture changes (Allison 1956; Turner et al. 1979) and that the selection of *GIP*^{103C} could occur at a time when humans experienced major shifts in subsistence culture. To investigate this possibility, we estimated the age of the *GIP*^{103C}-associated haplotype. Under the neutrality, the average age of a polymorphism with the frequency

p is estimated to be $-4N_e[p(\log p)/(1-p)]$ (Kimura and Ota 1973; Slatkin and Rannala 2000). With the assumption of $N_e = 5000$ for each population, this yielded 77,500, 350,000, and 425,000 yr for the derived allele to arise to its current frequencies in the YRI, CEU, and ASN populations, respectively. These estimates are obviously incompatible with the archaeological evidence showing that modern humans originated ~195 kyr ago in Sub-Saharan Africa and that a first wave of migration to the Arabic peninsula occurred ~60 to 55 kyr ago, which was followed by migration toward Northern Eurasia ~40 kya (McDougall et al. 2005; Klein 2009). Consequently, we estimated the age of the GIP^{103C} -associated haplotype on the basis of the decay of haplotypes (Reich 1998; Stephens et al. 1998). Based on a recombination rate derived from estimates of LD of the HapMap data set (McVean et al. 2004), the analysis showed that ASN contains a dominant ancestral haplotype and that the GIP^{103C} -associated haplotypes arose to dominance ~8100 yr ago in East Asians. Because this dating approach relies on the linkage map derived from estimates of LD, and because discrepancies between LD maps and the pedigree-based recombination maps are significant in many genomic regions (Clark et al. 2010), we also performed the analysis using alternative estimates of recombination rate, which range from 0.5–3.03 cM/Mb in three studies of pedigree-based recombination maps (i.e., deCODE, Marshfield, and Genethon) (Dib et al. 1996; Broman et al. 1998; Kong et al. 2002). Using these recombination rates, we obtained alternative estimates of the age to be between 11,800 and 2000 yr. On the other hand, dating is unattainable for CEU because the ancestral GIP^{103C} haplotype in this population cannot be identified unambiguously, perhaps because that mutation in the region is effectively “saturated” (Supplemental Fig. 6). Given that GIP signaling plays a critical role in the regulation of glucose and lipid metabolism, it is plausible that the increased prevalence of the GIP^{103C} allele in Eurasians is a consequence of changes in foraging skills or in population dynamics associated with the emergence of agricultural societies in the Eurasian continent 12,000 to 7000 yr ago (Balter 2007; Fuller et al. 2009; Jones and Liu 2009; Richards and Trinkaus 2009; Gibbons 2010).

Discussion

Based on the gene age estimation and biochemical analyses, our study revealed a functional mutation that is associated with the selection of the GIP locus in East Asian populations ~8100 yr ago and the presence of a cryptic GIP isoform. Specifically, we showed that the inventory of human GIP peptides has recently diverged and that individuals could express three different combinations of GIP isoforms (GIP, GIP55S, and GIP55G) with distinct bioactivity profiles. Future study of how this phenotypic variation affects glucose and lipid homeostasis in response to different diets and of which physiological variations in humans can be attributed to prior gene–environmental interactions at the GIP locus is crucial to a better understanding of human adaptations in energy-balance regulation.

Taking advantage of the availability of genome information from diverse human populations, recent studies have revealed a number of loci and variants that describe phenotypic variation in appearance, physiological parameters, and pathological responses to diseases (Sabeti et al. 2005; Voight et al. 2006, 2010; Sulem et al. 2007; Tishkoff et al. 2007; Genovese et al. 2010; Gibbons 2010; Leslie 2010; Ng et al. 2010; Simonson et al. 2010). In addition, it has been shown that human genomes contain hundreds of loci that exhibit varying degrees of positive selection (Kelley et al. 2006;

Voight et al. 2006; Sabeti et al. 2007; Barreiro et al. 2008; Akey 2009; Cai et al. 2009; Pickrell et al. 2009). These recent in silico findings on gene selection, perhaps due to differential gene–environmental interactions among populations, have opened the doors to human history and a better understanding of the prevalence of adaptations among human populations. However, few causal variants have been confirmed or linked to a phenotype (Akey 2009; Gibbons 2010; Nei et al. 2010). Thus, our finding that the high-frequency GIP^{103C} -associated haplotypes in Eurasian populations are functionally relevant has provided a rare opportunity to better understand the environmental impact on human physiology at the enteroinsular and enteroadipocyte axes. It is generally accepted that a food source represents a potent force that influences selection and adaptive radiation in nature (Darwin 1859; Freeman and Herron 2003). For example, the external differences in beak morphology and speciation of Darwin’s finches are believed to be results of adaptations that exploit particular types of seeds, insects, and cactus flowers on the Galápagos Islands (Schluter 2000). Likewise, changes in food source represented one of the most important selection pressures during transitions of human culture and posed a wide spectrum of challenges to the digestive and endocrine systems of our ancestors (Piperno et al. 2004; Balter 2007; Fuller et al. 2009; Jones and Liu 2009; Richards and Trinkaus 2009; Gibbons 2010). For instance, the ability to digest lactose in milk (lactase persistence) and the adoption of a pastoral culture has been linked to the selection of a variety of variants at the lactase locus in multiple ethnic groups at various time points during the last 10 millenniums (Tishkoff et al. 2007; Itan et al. 2009). Similarly, individuals from populations with high-starch diets tend to have more copies of amylases than do those from populations with low-starch diets (Perry et al. 2007). It has long been hypothesized that our ancestors ate a low-carbohydrate, high-protein diet and that the adaptive response was manifested as insulin resistance, perhaps for coping with low dietary glucose (Miller and Colagiuri 1994). Because normal GIP signaling is crucial to normal glucose and lipid metabolism, we speculate that the selection of the GIP^{103C} haplotypes could be pertinent to shifts in long-term energy-balance regulation after the emergence of agriculture, which provided a stable supply of high-starch staples and a reduced need for metabolic efficiency as opposed to the traditional hunter-gatherer societies (Miller and Colagiuri 1994; Wang et al. 2006; Balter 2007; Fuller et al. 2009; Jones and Liu 2009; Richards and Trinkaus 2009; Gibbons 2010; Luca et al. 2010; Richerson et al. 2010).

It was hypothesized by Neel almost 50 yr ago that mismatches between prior physiological adaptations and contemporary environments can lead to health risks because the ancestral variants that have been selected for the organism’s fitness or reproductive success may not be optimal for the individual’s health in the new environment (Neel 1962). In support of this thrifty genotype hypothesis, a number of genes in humans and house mice have been implied to have coevolved with the emergence of agricultural societies (Prentice et al. 2005; Vander Molen et al. 2005; Shiao et al. 2008), and a rapid shift in diets is associated with the detrimental effects on human survival in a number of human populations (Anonymous 1989). Conceptually, the serum-resistant GIP55G carried by the GIP^{103C} haplotype may have been beneficial for individuals who have unconstrained access to the food supply in many agricultural societies by preventing severe hyperglycemia. As selection pressure changed in these societies, the ancient GIP^{103T} haplotype could have become a liability and conferred a loss of fitness in the new environment. In addition, we speculate that the selection of GIP in East Asians may contribute to the heterogeneity

in the risk of diabetes among major ethnic groups at the present time (Retnakaran et al. 2006; Nystrom et al. 2008; Ma and Chan 2009). Importantly, regardless of what the selection pressure might have been, our study indicated that the *GIP* locus was susceptible to recent changes in human environment and that the physiological variation stemming from this selection could bear important implications for understanding the phenotypic variation of metabolic syndromes such as diabetes and obesity. Nonetheless, it is important to note that the selection of the derived *GIP*^{103C} haplotypes could be a consequence of the coordinated actions of multiple SNPs at the *GIP* locus. Future analysis of the functionality of rs2291725 and linked SNPs is needed to elucidate the exact nature of the selection of *GIP*^{103C} haplotypes in the last 10 millenniums.

Taken together, our study has highlighted an unexpected complexity in the regulation of sugar and lipid metabolism among human populations; it has also illuminated the importance of understanding adaptations in genes associated with energy-balance regulation in the face of ongoing changes in our diets toward refined and high-energy convenience food as well as the emerging pandemics of diabetes and obesity in modern society.

Methods

Genotype analysis

*F*_{ST} estimation and LD plot

The selected loci that have rapidly increased in frequency due to local positive selection are likely to show high levels of genetic differentiation, which can be quantified with the *F*_{ST} statistic (Lewontin and Krakauer 1973; Weir 1996). The principle of a number of estimators of *F*_{ST} is $F_{ST} = (H_T - H_S)/H_T$, where *H*_T is the heterozygosity of the total population and *H*_S is the average heterozygosity across subpopulations. The empirical distribution of *F*_{ST} or the average of *F*_{ST} over multilocus windows for the genome-wide polymorphism data has been used to detect the genomic regions under positive selection (Akey et al. 2002). We computed the *F*_{ST} for all nonsynonymous SNPs in the HapMap Project phases I and II, and the function *snp_fst.m* in PGEToolbox (Cai 2008) was used to calculate and present an unbiased estimate of *F*_{ST}.

LD plots for SNPs within the *CALCOCO2*, *ATP5G1*, *UBE2Z*, *SNF8*, *GIP*, and *JGF2BP1* gene regions from rs1422645 to rs8069452, spanning 200 kb, were generated using HaploView 4.1 (Barrett et al. 2005). The LD blocks were determined using an *r*² threshold of 0.8.

Analysis of EHH and iHS

The EHH plots were generated as described (Voight et al. 2006). We also computed the EHH statistic for the core SNP (rs2291725) as previously described (Sabeti et al. 2002). The EHH curve measures the decay of identity of haplotypes that carry the core SNP as a function of distance. When an allele rises rapidly in frequency due to strong selection, it tends to exhibit high levels of haplotype homozygosity, extending further than expected under a neutral model. For the analysis of EHH decay, haplotypes in the genomic region that encompasses a 750-kb region centered on the *GIP* gene and contains 388 phased SNPs were downloaded from the HapMap project website.

The iHS statistic detects whether the area under the EHH curve is greater for a selected allele than for a neutral allele (Voight et al. 2006). Large negative values indicate unusually long haplotypes carrying the derived allele. To test whether the observed iHS for rs2291725 is significantly deviated from the expected values derived from neutral evolution, we used a coalescent model and

generated 10,000 replicated haplotype sets using the coalescent simulator *ms* (Hudson 2002). We adopted parameters that are compatible with the sample size (180 chromosomes) of the ASN population and the length of genomic region (750 kb) that was analyzed. The simulation was conditioned based on recombination rate = 9.7×10^{-7} cM/bp, *N*₀ = 10,000, and number of segregating sites = 388. We also required that each replicate contain at least one segregating site in which the derived allele frequency reaches ~0.75 and that the site is located in the center of the haplotype (that is, between a 370- and 380-kb area of the 750-kb region in total). With these conditions, we found no iHS value for a simulated data set to be more negative than the observed iHS (empirical *P* < 1×10^{-4}) (Supplemental Fig. 4A).

Estimation of the age of *GIP*^{103C} haplotype

Under positive selection, a derived, or novel, allele can quickly rise to a high frequency. As the allele ages, recombination causes the breakdown of the LD within the existing linked alleles, and mutation leads to the accumulation of new linked variations. The allele's age can be estimated by the decay of the haplotype carrying the allele (Stephens et al. 1998). The length of the haplotype retained between different copies of the allele shortens over time due to recombination and mutation, which is modeled using a Poisson process by Reich and Goldstein (1999). The probability that a haplotype remains ancestral (i.e., that the haplotype that carries the derived allele retains the status of high frequency right after being selected) is $P = e^{-G(r+u)}$, where *G* is generations, *r* is the recombination rate, and *u* is the mutation rate. For a given allele in the derived haplotype, the haplotype-decay approach estimates the number of generations *G* in terms of *P* (the probability that a given haplotype does not change from its ancestor) (Stephens et al. 1998; Reich and Goldstein 1999).

In ASN, the *GIP* LD block is 91 kb long and contains eight distinct haplotypes (1–8 from top to bottom in Supplemental Fig. 6). Haplotypes 1–4 are *GIP*^{103C} associated and are derived. Among these derived haplotypes, haplotype 1 is apparently the ancestral haplotype from which haplotypes 2–4 were derived. The frequency of haplotype 1 in *GIP*^{103C}-associated haplotypes is *P*' = 0.803. We obtained the recombination rate derived from estimates of LD (McVean et al. 2004) from the website of the HapMap project (<http://hapmap.ncbi.nlm.nih.gov/>). Regression analysis with data points of different physical lengths versus genetic map lengths gave the regression function: $y = 0.93x - 0.018$, where *x* is the physical distance (Mb) and *y* is the genetic distance (cM). The genetic distance of the 91-kb region was estimated to be 0.0666 cM (i.e., 0.0666% chance of crossing over in a single generation), which gives a rate of recombination $r = 6.66 \times 10^{-4}$ per generation. We took the mutation rate of the region $u = 1.0 \times 10^{-5}$, based on an estimation of the haploid mutation rate of $\sim 1.1 \times 10^{-8}$ per base per generation (Roach et al. 2010). We assumed that the most common haplotype is the ancestral haplotype and used *P*' obtained above as an approximation of *P*. Using $G = -\ln(P')/(r+u)$, we obtained *G* = 325 (or 8100 yr).

Phenotype tests

Reagents

Synthetic *GIP* peptides were obtained from Genescript and the American Peptide Company. The extended *GIP*55G and *GIP*55S isoforms were synthesized by the Stanford University PAN facility and GL Biochem. Pooled normal human serum, pooled complement-preserved human serum, and serum from individuals were obtained from Innovative Research and ProteoGenex. Stocks of different hormones were prepared in phosphate-buffered saline

and diluted in a serum-free culture medium. In addition to routine chemistry and mass-spectrometry assessments, we verified the quantity of different GIP isoforms using a human GIP enzyme-linked immunosorbent assay (ELISA; Linco Research).

Immunohistochemical analyses and ELISA

Total GIP levels in human serums were measured using a sandwich human GIP ELISA kit (Linco Research), and assays were performed with a programmable ELISA plate washer. The minimum detection limit of the assay was 8.1 pg/mL. For the measurement of GIP55, human serums were partially purified with C18 chromatography, and the secondary antibody of the human GIP ELISA kit was substituted with a rabbit polyclonal antibody specific for the last 13 amino acids of GIP55 (REARALELASQAN). The GIP55-specific antibodies were generated using a KLH-conjugated CREARALELASQAN peptide (Covance Research Products and Genescript).

For immunohistochemical analyses, human duodenum sections (BioChain Institute) were dewaxed with xylene and maintained for 10 min at 95°C–99°C in 10 mM sodium citrate buffer (pH 6.0), followed by cooling on the bench top. Sections were immunostained with a mouse monoclonal anti-GIP antibody (Abbiotec) and a rabbit GIP55-specific antibody overnight at 4°C, followed by incubation with fluorochrome-conjugated secondary antibodies (Invitrogen) for 1 h at room temperature in the dark. Signals for GIP and GIP55 were detected using an Alexa 488 donkey anti-mouse antibody (488 nm, 1:2500 dilution) and a Texas Red-conjugated goat anti-rabbit antibody (594 nm, 1:500 dilution), respectively, with a Leica SP2 single- and multi-photon confocal microscope.

The *in vivo* glucose suppression assay

Eight-week-old Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were fasted overnight for 20 h. To measure the GIP isoforms' ability to reduce blood glucose levels *in vivo*, fasting rats were injected with a select GIP peptide (100 nmol/kg) dissolved in 0.8 mL of PBS together with glucose (3.8 g/kg body weight). Blood samples were obtained via the tail vein at select time points after the intraperitoneal injection of glucose and the peptide. Glucose levels in the blood were measured with a One-Touch Ultra Blood Glucose Monitoring System and One-Touch Ultra Test Strips (Johnson and Johnson).

Receptor-activation assay

The expression vector for the human GIP receptor (Origene) was transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen). Receptor-activation activities were assayed based on cAMP production in transfected cells and were performed as described (Park et al. 2008; Chang et al. 2010). To quantify resistance to serum degradation by GIP isoforms, aliquots of GIP peptides were incubated in microfuges at 37°C with human serum in a final concentration of 10 μ M for indicated time spans. To analyze the mechanism underlying the serum-degradation-resistant property of GIP55, peptides (10 μ M) were preincubated with a recombinant human DPP IV (1 mU/mL reaction in PBS; Enzo Life Science) for 3, 6, or 12 h and were frozen at –80°C before being tested for receptor-activation activity. The receptor-activation results were analyzed using the GraphPad Prism 5 package (GraphPad Software).

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