Neuronal Genes for Subcutaneous Fat Thickness in Human and Pig Are Identified by Local Genomic Sequencing and Combined SNP Association Study

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Abstract

Obesity represents a major global public health problem that increases the risk for cardiovascular or metabolic disease. The pigs represent an exceptional biomedical model related to energy metabolism and obesity in humans. To pinpoint causal genetic factors for a common form of obesity, we conducted local genomic de novo sequencing, 18.2 Mb, of a porcine QTL region affecting fatness traits, and carried out SNP association studies for backfat thickness and intramuscular fat content in pigs. In order to relate the association studies in pigs to human obesity, we performed a targeted genome wide association study for subcutaneous fat thickness in a cohort population of 8,842 Korean individuals. These combined association studies in human and pig revealed a significant SNP located in a gene family with sequence similarity 73, member A (FAM73A) associated with subscapular skin-fold thickness in humans (rs4121165, GC-corrected p-value = 0.0000175) and with backfat thickness in pigs (ASGA0029495, p-value = 0.000031). Our combined association studies also suggest that eight neuronal genes are responsible for subcutaneous fat thickness: NEGR1, SLC44A5, PDE4B, LPHN2, ELTD1, ST6GALNAC3, ST6GALNAC5, and TTLL7. These results provide strong support for a major involvement of the CNS in the genetic predisposition to a common form of obesity.

Introduction

The pig (Sus scrofa domesticus) was domesticated from Sus scrofa, the wild boar, approximately 9,000 years ago in multiple regions of the world [1,2]. It has become an important animal as one of the major animal protein sources for humans and is also an exceptionally relevant biomedical model related to energy metabolism and obesity in humans since it is devoid of brown fat postnatally and due to its similar metabolic features, cardiovascular systems, and proportional organ sizes [3].

Obesity is increasing in an epidemic manner and represents a major public health problem by increasing risk to cardiovascular disease [4,5] and metabolic disease such as type 2 diabetes [6]. Recently, two genome-wide association (GWA) studies have expanded the number of genetic susceptibility loci for obesity by identifying SNPs associated with body mass index (BMI) and weight, thus, contributing to obesity risk. The loci identified are located in or near ten genes including the neuronal growth regulator 1 (NEGR1) genes [7,8]. Both of the GWA studies hypothesized a role of the central nervous system (CNS) in the predisposition to a common form of obesity as has previously been shown for rare monogenic forms of obesity. Although both, BMI and weight are highly heritable, the variants detected in these large GWA studies explained only a small fraction of the inherited variability in BMI and weight [7,8].

In pigs, high heritability has been estimated for backfat thickness (BFT) and intramuscular fat (IMF) content. Estimates of heritability for BFT are between 50% and 70%, and those for IMF content between 38% and 67% [9]. IMF is necessary to increase meat quality. However, a conflicting relationship exists
between IMF and BFT because extra fat in pigs unnecessarily raises the cost of feed [10]. Various efforts have been made to identify the chromosomal regions influencing BFT and IMF by quantitative trait loci (QTL) analysis on pig chromosome 6 (SSC6) BFT [11,12,13] and IMF [14,15,16,17,18,19,20]. Recent QTL analyses revealed that the region between S0228 and SW1881 might harbor a highly significant QTL affecting fatness and meat quality traits on SSC6 [21,22,23] (Figure 1). Thus, we carried out both a de novo local genomic sequencing and a SNP association study in this region to identify loci associated with BFT and IMF content. Our rationale was that it is important to both identify causal genetic factors in the pig QTL region, and to expand the knowledge of genetic risk factors predisposing to common forms of obesity in humans. To relate and expand the QTL results observed in pigs to human common forms of obesity, we performed a targeted genome wide association study with subcutaneous fat thickness in a cohort population of 8,042 Korean individuals.

Results

Local genomic sequencing of the pig QTL region

To perform de novo local genomic sequencing of the QTL region, a total of 316 markers were developed from pig Bacterial Artificial Chromosome (BAC) end sequences summarized in Table S2. The Korean Native Pig (KNP) BAC library [24] was screened by 4D-PCR [25], and sequenced by a shotgun strategy. Additional BAC clones produced by the International Swine Genome Sequencing Consortium were used for gap filling. Approximately 18.2 Mb sequence was generated after assembling these sequences. Out of 72 protein coding genes on human syntenic regions, 70 orthologous genes were detected in pigs. Three porcine pseudo-genes and four novel genes were annotated in this region. A total of 10 non-coding RNAs were detected (Table S3). Comparative analysis with the human syntenic region exhibited a genomic structure and contents similar to that in the pig QTL region (Figure 2A), which included functional genes, repetitive elements, GC content and CpG islands. A detailed description of the genomic sequencing is provided in the methods. The similarity of the genomic structure and contents with the human syntenic region augments the merit of the pig as a relevant biomedical model to identify and expand causal genetic factors predisposing to common forms of obesity. Visualization of syntenic blocks along the region between pig and four other species, human, mouse, dog and cow, shows closer genomic similarity between pig and human than between pig and mouse (Figure 2B). Rodent models have long been the pillar of obesity and metabolic syndrome research. However, marked differences in metabolism and adipose tissue biology between rodents and humans have been recognized and, thus, the pig is emerging as a more appropriate biomedical model for obesity in humans because of its biological similarities with humans [3].

SNP association studies for backfat thickness and intramuscular fat content in pigs

After filtering SNP genotypes for quality control (Materials and Methods), we conducted a SNP association study of the region using 235 SNPs in 527 pigs for BFT and IMF content traits. We identified 52 SNPs (22.1% of the 235 SNPs) associated with BFT at \( p = 0.000213 \), equivalent to \( p \)-value = 0.05 after Bonferroni correction (Figure 3A and Table S4). Although this genomic region has been suggested to contain QTLs for BFT, more significant SNPs than expected were identified considering that the Bonferroni procedure is a very conservative correction (some of the SNPs are in strong linkage disequilibrium and therefore the tests are not independent). None of the SNPs, however, were
found to be significantly associated with IMF content (Figure 3B). The significant SNPs are located in or near 13 protein coding genes, with 10 genes containing at least one significant SNP each. The SNP (ALGA0122230) showing the strongest association with BFT is located near the neuronal growth regulator 1 (NEGR1) gene. Recently the GIANT consortium [8] reported that the NEGR1 obesity-associated SNPs they detected seemed to be in strong linkage disequilibrium with nearby copy number variations (CNV), although there is currently no functional evidence to support the involvement of the CNV in non-syndromic human obesity. The NEGR1 protein participates in the regulation of neurite outgrowth in the developing brain [26,27]. Interestingly, we found that of the 13 genes associated with BFT, 8 genes including NEGR1 are involved in psychiatric disease, neural development or high expression in CNS. The eight genes include: NEGR1, a member of solute carrier (SLC) superfamily 44 (SLC44A5); phosphodiesterase 4B (PDE4B); latrophilin 2 (LPHN2); epidermal growth factor; latrophilin; seven transmembrane domains containing 1 (ELTD1), ST6 (N-acetylgalactosaminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosamine-α,2,6-sialyltransferase 3 (ST6GALNAC3), ST6GALNAC5; and tubulin tyrosine ligase-like family, member 7 (TTLL7).

The SLC superfamily is a major group of membrane transporter proteins that control cellular uptake and efflux of nutrients, neurotransmitters, metabolites, drugs, and toxins [28]. Although biological and neurological functions for the majority of the SLC genes in the mammalian brain are largely unknown, recently, Dahlin et al. [29] reported that 82% of known SLC genes were expressed in the brain. Among the members of this superfamily, a member of SLC44 was present in oligodendrocytes. To date, the biological function of SLC44A5 is unknown. PDE4B belongs to a family of four PDE4 genes, all coding for phosphodiesterases that hydrolyze the second messenger cyclic adenosine monophosphate (cAMP). Since PDE4B was first suggested as a risk factor for schizophrenia [30], PDE4B has also been suggested as a candidate gene associated with both schizophrenia and bipolar disorder [31]. Variation in the resting electroencephalogram (EEG) is associated with common, complex psychiatric traits including alcoholism, schizophrenia, and anxiety disorders [32]. Recently, genome-wide association identified SNPs with significant association to EEG traits on 1p31.3 of human (Materials and Methods). The genomic control parameter λ value in the SUP-SNP association study was 1.037, indicating no overall inflation of statistical results due to population stratification, while the λ value in the SUB-SNP association study was 1.187 (Figure 4). After genomic control (GC) correction [40], no evidence of inflation remained for either of the association studies. Using a false discovery rate (FDR) q value [41] < 0.05, we identified one SNP located in a gene family with sequence similarity 73, member A (FAM73A) gene associated with SUB (rs4121165, GC-corrected p-value = 0.0000175) (Figure 4A and Table S5). The FAM73A gene was also significantly associated with BFT in pigs. Considering that SUB is measured in the human back, FAM73A is a strong candidate gene responsible for subcutaneous fat thickness in both humans and pigs. The SNP also showed the strongest association with SUP. To our knowledge, no biological function of the gene has been reported. However, based on tissue expression analysis in humans of the GeneCards (www.genecards.org), the gene seems to be expressed prominently in the nervous system. After the FDR correction, there were no significant SNPs associated with the two skin fold thickness measurements except the SNP in the FAM73A gene. However, using a GC-corrected p-value threshold of 0.01, genes containing SNPs with a lower p-value cutoff seem to show enrichment of the 13 genes (Table S4) associated with pig BFT. Using the threshold, out of 14 genes containing significant SNPs in the SUB-SNP association, 7 genes are among 13 functional genes associated with pig BFT. Likewise in the SUP-SNP association, 4 genes out of 9 belong to those 13 functional genes (Figure 4B and Table S6). Considering 72 protein coding genes in the region (probability of success = 13/72), exact binomial probability observing the given number of genes or more in each result, 7 out of 14 genes, is 0.0065 for SUB-SNP association and 0.0621 for SUP-SNP association which is 4 out of 9 genes. Therefore, it is unlikely to observe this number of common genes by chance in both, the human and pig association studies especially for the SUB-SNP association.

**Discussion**

Measurement error in assessing the skin-fold thickness may be considerably larger than the BFT measurement in pigs. Skin-fold thicknesses in human are affected by individual and regional differences in compressibility that vary with age, gender and recent weight loss. In addition, pressure from skin-fold caliper measurement may force some adipose tissue lobules to slide into areas of

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**Figure 2. Comparative genomic analysis of pig QTL region affecting fatness and meat quality on SSC6.** Similar genomic structure and contents was revealed by comparative analysis between the human syntenic region and the pig QTL region (A) and visualization of syntenic blocks along the region between pig (Ss) and the four other species: human(Hs), mouse(Mm), dog(CF) and cow(Bt) (B). In the dot-plot analysis (A), protein coding genes are shown as forward (upper) and reverse (lower) in brown color according to x- and y-axis for pigs and human, respectively. The conserved segments from 70-100% are plotted. The genomic feature of pig appear in order from top to bottom under dot-plot: CpG islands, Tandem elements, Simple repeats and Low complexity. In the synteny maps (B), the rings depict from outside to inside: syntenic regions of each chromosome, SINE (blue) and LINE (red) repeat densities using a sliding window of 100 kbp, Interspersed repeat elements (SINEs, LINEs, LTR elements, Simple repeats and Low complexity). In the synteny maps (B), the rings depict from outside to inside: syntenic regions of each chromosome, SINE (blue) and LINE (red) repeat density using a sliding window of 100 kb and protein-coding genes (purple). Genomic coordinates are shown in 100 kb intervals. Synteny blocks larger than 5 kb are displayed by connecting lines.

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lesser pressure. This sliding may be more marked for thick skin-folds in which the adipose tissue contains little connective tissue [42]. On the contrary, BFT in pigs are accurately measured with a ruler between the 10th and 11th rib on the chilled carcass. Because of these measurement errors, the association study in pigs is likely to provide higher statistical power than that for humans if they are under similar conditions except the measurement errors.

Our combined association studies in human and pig in the predefined fatness related pig QTL region revealed three most likely genes, FAM73A, NEGR1 and TTLL7, as being responsible for genetic predisposition to common forms of obesity, especially subcutaneous fat thickness (Figure 5). The second likely set of genes for genetic predisposition includes five genes, LPHN2, SLC44A5, ELTD1, ST6GALNAC3 and GIPC2 (Figure 5). As mentioned above, two recent GWA studies [7,8] suggested the role of the CNS in the predisposition to the non-syndromic form of obesity. Our results strongly support a major involvement of the CNS in the genetic predisposition, and suggest several neuronal genes as genetic risk factors for the polygenic common form of obesity (Figure 5).

Except for the NEGR1 gene, to our knowledge, the other neuronal genes are newly suggested in our research for the genetic association with obesity related traits. Our findings of candidate causal genes may provide expanded insight into mechanisms underlying obesity biology. Further evaluation of these candidate genes in humans and pig may enable researchers to accelerate gaining knowledge of genetic factors for common forms of obesity.

Materials and Methods

Ethics statement

Approval was granted from relevant review boards in all study sites; all included subjects gave informed written consent. The Korea Centers for Disease Control and Prevention’s review board
reviewed and approved the Korean SNP association studies. For the pigs experiment, the study protocol and standard operating procedures were reviewed and approved by the National Institute of Animal Science’s Institutional Animal Care and Use Committee (No. 2009-077, C-grade).

**Pig BAC sequencing and assembling**

BAC clones consisting of the 18.2 Mb contigs were screened from the Korean Native Pig BAC library [43]. The pig BAC end sequences (BES) corresponding to the syntenic region between 65 Mb and 85 Mb of human chromosome 1 were obtained from Sus scrofa Project site of the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/cgi-bin/Projects/S_scrofa/BESearch.cgi). A total of 316 markers were developed from pig BESs with intervals of 60 kb to screen BAC clones (Table S2). The BAC library was pooled for 4D-PCR screening [25]. The 4D-PCR screening consisted of a two-step screening process: the first PCR was performed on master pools and the second on plate row/column, well row/column pools in a total volume of 15 μL with 10 ng in each pool. PCR amplifications were performed in a PTC 200 thermocycler (MJ Research, USA). Thermal cycling parameters were defined as follows: pre-denaturation at 95°C for 2 min; followed by 32 cycles of 95°C for 30 s, annealing temperature for 30 s, and 72°C for 30 s; and then a final step at 72°C for 5 min. PCR products were separated on a 2% agarose gel containing ethidium bromide and visualized using a UV light source.

The screened BAC clones were sequenced by a shotgun strategy. The BAC DNAs were isolated using the Large Construct Kit (Qiagen, USA). A total of 15 μg BAC DNA was used to obtain random fragments of 2~3 kb. Fragmentation was performed using the HydroShear DNA Shearing Device (Genomic Solution, USA) with the following parameters: 200 μL volume of DNA solution, 11 speed code, and 20 cycles. Small sizes of the fragments were removed using the Sizesep 400 spin column (Amersham Biosciences, USA) and CHROMA SPIN+TE1000 (Clontech, USA) and were subsequently repaired with DNA polymerase and the polynucleotide kinase method (BKL Kit; TaKaRa, Japan). The prepared DNA fragments were cloned into the dephosphorylated Smal site of pUC19 (Qbiogene, USA). Ligates were transformed into DH10B by electroporation (Gene Pulser II, Bio-Rad, USA). Approximately 1000 plasmids in each shotgun DNA library were randomly selected for sequencing. Plasmid DNAs

**Figure 4.** -log10 (Genomic control-corrected p-value) of 2,143 SNPs associated with SUB (A) and SUP (B) in the human syntenic region. Using a false discovery rate (FDR) q value <0.05 [41], a SNP located in FAM73A gene is significantly associated with SUB indicated in red. The genomic control parameter λ value in SUB-SNP association study was 1.037 and the λ value in SUP-SNP association study was 1.187 indicated by the QQ-plots. Genomic control-corrected p-value threshold of 0.01 is indicated by the dotted line.
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were bi-directionally sequenced for each plasmid with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI 3730 automatic sequencer (Applied Biosystems). Sequence data were assembled using the PHRAP program (University of Washington, Seattle, WA, USA). To fill gaps, primer pairs were designed from the high-quality region with a PHRAP score greater than 70 on both ends of each contig. PCR amplifications were performed using appropriate BAC DNA used to construct the shotgun library as a template. PCR products were inserted into the pGEM T Easy vector (Promega, USA) and sequenced. Finishing assembly was performed in Seqman (DNASTAR, USA). The complete sequences of 126 KNP BAC clones and 33 unfinished KNP BAC clones were deposited into EMBL/GenBank (EF488234, FN673706-FN673830, FN674549-FN675238). A total of 29 CHORI242 BAC clones were selected from the FPC clone map of the Wellcome Trust Sanger Institute website to fill the gaps within the KNP BAC clone maps (Table S1). The selected CHORI242 BAC clones were sequenced up to 8-fold depth (FN677038-FN677340).

All BAC clone sequences were assembled with Seqman to construct continuous genomic sequences. The representative genome sequence of approximately 18.2 Mb mainly consisted of KNP BAC clone sequences. The pig genome sequences of Suscrofa9 produced by Swine Genome Sequencing Consortium were used to replace the remaining gaps within the BAC clone contigs (ftp://ftp.ensembl.org/pub/current_fasta/sus_scrofa/dna/).

Sequence annotation and comparative genome analysis

The genomic sequence of 18,261,618 bp was used to predict putative genes using de novo gene prediction programs, that is, GENSCAN [44], AUGUSTUS [45] and GeneMark.hmm [46]. For cis-alignment analysis, the Sus scrofa UniGene build 38 and expressed sequence tag (EST) sequences were downloaded from NCBI. Then, the ESTs were aligned against the sequenced genome using BLAT [47] and filtered by 98% coverage cutoff. For trans-alignment, human and mouse protein sequences from the UniProtKB database (http://www.uniprot.org/downloads) were aligned against the sequenced genome using BLAT [47] and filtered by 95% coverage cutoff. To eliminate false positive gene findings, de novo genes which included EST-aligned or trans-aligned genomic region were selected in the annotation process. Final gene annotation was determined by careful manual inspection.

To perform comparative genome analysis, we downloaded the assembled genome sequences: Human (1:65,592,395-85,484,668); Mouse (4:101,091,882-102,963,468, 6:67,372,926-66,971,344,
Y_{ijk} = \mu + S_i + b_1 D_j + a_k + e_{ijkl} \quad (1)

where $Y_{ijkl}$ is the trait measured in the $k^{th}$ animal of $i^{th}$ sex and $j^{th}$ age at slaughter days; $\mu$ is an overall mean, $S_i$ is the fixed effect of $i^{th}$ sex, $b_1$ is a regression coefficient, $D_j$ is covariate for the age at slaughter days, $a_k$ is additive genetic (polygenic) effect of $k^{th}$ animal, fitted as a random effect and $e_{ijkl}$ is the random residual error. The variance for additive genetic (polygenic) effects of animals is defined as $Var(a) = \sigma^2_a$, based on the pedigree of the offspring and $\sigma_a$ is the additive genetic variance due to polygenes [56]. For the residual random effects, the variance is defined as $Var(e) = \sigma^2_e$, where $I$ is the identity matrix and $\sigma^2_e$ is the residual variance. The residuals from this analysis are given by

$$\hat{e}_i = Y_{ijk} - (\mu + S_i + b_1 D_j + \hat{a}_k)$$

where $\hat{S}_i$ and $\hat{b}_1$ are the estimates of sex and age effects and $\hat{a}_k$ is the estimated contribution from the polygene (breeding value).

In the second step for the whole genome association study, these residuals are used as the phenotype in a simple linear regression for each SNP (i.e.,

$$\hat{e}_i = \mu + b_2 g_i + e_i \quad (2)$$

where $\hat{e}_i$ is the vector of residuals from model (1), $\mu$ is the mean, $g_i$ is the vector of genotypes at the marker $i$, $b_2$ is the marker genotype effect and $e_i$ is the vector of random residuals. This approach is called GRAMMAR [54].

The simple linear regression analysis was performed in the R/ SNPassoc package [57]. Markers with a test statistic exceeding a threshold corresponding to a $p$-value below the region-wise Bonferroni corrected significance threshold (0.05/#SNPs) were selected for the final test using the full animal model in ASREML [55]:

$$Y_{gk} = \mu + S_i + b_1 D_j + b_2 g_k + a_k + e_{gk} \quad (3)$$

where $Y_{gk}$ is the trait measured in the $k^{th}$ animal of $i^{th}$ sex, $j^{th}$ age at slaughter days and $k^{th}$ genotype; $\mu$ is an overall mean, $S_i$ is the fixed effect of $i^{th}$ sex, $b_1$ is a regression coefficient, $D_j$ is covariate for the age at slaughter days, $b_2$ is the marker genotype effect, $g_k$ is the vector of genotypes at the marker $k$, $a_k$ is additive genetic (polygenic) effect of $k^{th}$ animal, fitted as a random effect and $e_{gk}$ is the random residual error. Additive genetic covariances among animals and residual variance are described in the model (1).

**SNP association study of subcutaneous fat thickness in human**

The Korea Association Resource (KARE) project was initiated in 2007 to undertake large-scale GWA analyses. Participants in this project were recruited from two community-based cohorts (i.e., the rural Ansan and urban Ansan cohorts) in the Gyeonggi Province of South Korea. The Ansan and Ansan cohorts consist of 3,018 and 5,020 participants, respectively, ranging in age from 40 to 69 years. Genomic DNAs was isolated from peripheral blood drawn from the participants and genotyped on the Affymetrix Genome-Wide Human SNP array 5.0 containing 500,568 SNPs. Prior to the analysis, we performed genotype calling and quality control as previously described in Cho et al. [39]. After sample and SNP quality controls, a total of 8,942 individuals and 2,143 SNPs in the human syntenic region were included in the association.
studies. Subcutaneous fat in humans was indirectly measured by subscapular and suprailiac skin-fold thickness (SUB and SUP) for the SNP association study. To perform a SNP association study with skin-fold thickness measurement, data transformation of the actual skin-fold thickness measurement is desirable because the frequency distribution of most skin-fold measurements is skewed, and the relationship of body density to skin-folds may not be rectilinear because of a larger proportion of the body fat which is deposited subcutaneously with increasing obesity [58]. Natural logarithmic transformation for SUB measurement and square root transformation for SUP measurement was performed in which the assumption of normal distribution was more reasonable for each transformation for SUP measurement was performed in which the assumption of normal distribution was more reasonable for each trait. Linear regression analysis was performed in an additive model using PLINK [59], including sex, age and geographic region as covariates. The \( p \) values were adjusted by a genomic control method [40] and followed by FDR [41] corrections as implemented in PLINK [59]. As described in the result, we could not find significant SNP associations except one SNP of the traits. Thus, we used GC-corrected \( p \) value threshold of 0.01 to summarize the top highest SNP associations with the traits in human and test enrichment of significant genes in pigs.

Supporting Information

Table S1 References of each QTL region indicated in the Figure 1.

Table S2 List of sequence-tagged sites (STSs) designed used to screen bacterial artificial chromosome (BAC) clones. The STSs were designed from BAC end sequences (BES) mapped on PigMap corresponding to human genomic region between 65 Mb and 85 Mb in chromosome 1.

References

1. Kijas JM, Andersson L (2001) A phylogenetic study of the origin of the domestic pig estimated from the near-complete mtDNA genome. J Mol Evol 52: 392–308.
2. Larson G, Dobney K, Albarrella U, Fang M, Matisoo-Smith E, et al. (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307: 1618–1621.
3. Spurlock ME, Gahle NK (2000) The development of porcine models of obesity and the metabolic syndrome. J Nutr 130: 397–402.
4. Lakka TA, Lakka HM, Salonen R, Kaplan GA, Salonen JT (2001) Abdominal obesity is associated with accelerated progression of carotid atherosclerosis in men. Atherosclerosis 154: 497–504.
5. Cekeniahia S, Evans JC, Levy D, Wilson PW, Benjamin EJ, et al. (2002) Obesity and the risk of heart failure. N Engl J Med 347: 305–313.
6. Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444: 840–846.
7. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, et al. (2009) Genome-wide association study of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat, and serum leptin concentration in Duroc pigs. Journal of Animal Science 87: 2209–2215.
8. Wiiler CJ, Speliotos EK, Loss RJ, Li S, Lindgren CM, et al. (2009) Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41: 18–24.
9. Neuguthn R, Luther H, Reinsch N (2010) Parent-of-origin effects cause associations with body composition on pig chromosome 6. Genetics 185: 57–67.
10. Kollmaris P, Schmid R, Kudoh Y, Wang YM, et al. (1997) Human BAC library: Construction and rapid screening. Gene 191: 69–79.
11. Malek M, Dekker J, Lee H, Baas T, Prusa K, et al. (2001) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. Mammalian Genome 12: 637–643.
12. Okolo C, Oliver A, Noguera J, Clop A, Barragan C, et al. (2002) Test for positional candidate genes for body composition on pig chromosome 6. Genetics Selection Evolution 34: 465–479.
13. Soduja Y, Grindelke E, Liu Z, Lien S (2003) Multivariate mixed inheritance model for QTL detection on porcine chromosome 6. Genetics Research 81: 63–73.
14. de Koning D, Janss L, Rattink A, van Oers P, de Vries B, et al. (1999) Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (Sus scrofa). Genetics 152: 1679.
15. Gerberns F, Van Esp A, Harders F, Verburg F, Meuwissen T, et al. (1999) Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. Journal of Animal Science 77: 846.

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Author Contributions

Conceived and designed the experiments: MJB KSK EWP HSP JTJ BHC GWJ SHC DWK DL HSP MRP. Analyzed the data: SHL WCP DHS SC HYK KWK THH JEP KTL HK JOS W. Wrote the paper: KTL JO LBS HK.
of human membrane transport proteins. Introduction. Pfugers Arch 447: 465–468.

29. Dahlia A, Royall J, Holmann JG, Wang J (2009) Expression Profiling of the Snare Carrier Gene Family in the Mouse Brain. Journal of Pharmacology and Experimental Therapeutics 329: 530–537.

30. Millar JK, Pickard BS, Mackie S, James R, Christie S, et al. (2005) DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science 310: 1187–1191.

31. Kahler AK, Otnaess MK, Wirgenes KV, Hansen T, Jonsson EG, et al. (2010) Association study of PDE4B gene variants in Scandinavian schizophrenia and bipolar disorder multicenter case-control samples. Am J Med Genet B Neuropsychiatr Genet 153B: 86–96.

32. Hodgkinson CA, Enoch MA, Srivastava V, Cummins-Oman JS, Ferrier C, et al. (2010) Genome-wide association identifies candidate genes that influence the human electroencephalogram. Proc Natl Acad Sci U S A 107: 8695–8700.

33. Lang J, Ushkaryov Y, Grasso A, Wollheim CB (1998) Ca2+ independent insulin exocytosis induced by alpha-latrotoxin requires latrophilin, a G protein-coupled receptor. EMBO J 17: 648–657.

34. Okajima T, Fukumoto S, Ito H, Kiso M, Hirabayashi Y, et al. (1999) Molecular cloning of brain-specific GD1alpha synthase (ST6GalNAc V) containing CAG/ Glutamine repeats. J Biol Chem 274: 30557–30562.

35. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, et al. (2009) Genes that mediate breast cancer metastasis to the brain. Nature 459: 1005–1009.

36. Agrawal A, Pergadia ML, Saccone SF, Lynskey MT, Wang JC, et al. (2008) TTLL7 is a mammalian beta-tubulin polyglutamylase required for growth of MAP2-positive neurites. J Biol Chem 283: 30707–30716.

37. Heimann EL, Need AC, Hayden KM, Chiba-Falek O, Roses AD, et al. (2010) Genome-wide scan of copy number variation in late-onset Alzheimer’s disease. J Alzheimers Dis 19: 69–77.

38. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological) 57: 289–300.

39. Scozzafava E, Vicente-Rodriguez G, Manios Y, Beghin L, Iliescu C, et al. (2008) Circos: an information aesthetic for comparative genomics. Genome Res 18: 1639–1645.

40. Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55: 997–1004.

41. Nagy E, Vicente-Rodriguez G, Manios Y, Beghin L, Iliescu C, et al. (2008) A large-scale genome-wide association study of Asia populations uncovers genetic factors influencing rates in human membrane transport proteins.

42. Schneider KL, Pollard KS, Baertsch R, Poli A, Lowe TM (2006) The UCSC Archaeal Genome Browser. Nucleic Acids Res 34: D407–410.

43. Lomsadze A, Ter-Hovhannisyan V, Chernoff YO, Borodovsky M (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. Nucleic Acids Res 33: 6494–6506.

44. Choy Y, Jeon G, Kim T, Choi B, Chung H (2002) Ear type and coat color on growth performances of crossbred pigs. Asian-australasian journal of animal sciences 15: 1178–1181.

45. Choy YH, Jeon GJ, Kim TH, Choi BH, Cheong IC, et al. (2002) Genetic Analyses of Carcass Characteristics in Crossbred Pigs: Cross between Landrace Sows and Korean Wild Boars Asian-Aust J Anim Sci 15: 1080–1084.

46. Lomsadze A, Ter-Hovhannisyan V, Chernoff YO, Borodovsky M (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. Nucleic Acids Res 33: 6494–6506.

47. Kost VJ (2002) BLAT—the BLAST-like alignment tool. Genome Res 12: 656–664.

48. Takai D, Jones PA (2003) The CpG island searcher: a new WWW resource. In Silico Biol 3: 235–240.

49. Kraywinski M, Schein J, Birol I, Conners J, Gascoyne R, et al. (2009) neutron: a new information aesthetic for comparative genomics. Genome Res 19: 1639–1645.

50. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 557–575.

51. Krzywinski M, Schein J, Birol I, Conners J, Gascoyne R, et al. (2009) Circos: an information aesthetic for comparative genomics. Genome Res 19: 1639–1645.

52. Choy Y, Jeon G, Kim T, Choi B, Chung H (2002) Ear type and coat color on growth performances of crossbred pigs. Asian-australasian journal of animal sciences 15: 1178–1181.

53. Choy YH, Jeon GJ, Kim TH, Choi BH, Cheong IC, et al. (2002) Genetic Analyses of Carcass Characteristics in Crossbred Pigs: Cross between Landrace Sows and Korean Wild Boars Asian-Aust J Anim Sci 15: 1080–1084.

54. Aulchenko YS, de Koning DJ, Haley C (2007) Genomewide rapid association using mixed model and regression: a fast and simple method for genomewide pedigree-based quantitative trait loci association analysis. Genetics 177: 577–585.

55. Gilmour A, Gogel B, Nelder J, Thompson R (2006) ASReml user guide release 2.0. UK: VSN International Ltd, Hemel Hempstead.

56. Thompson R, Gilmour A, Botham F, Moggach B, Wray J, et al. (2001) ASReml user guide release 2.0. UK: VSN International Ltd, Hemel Hempstead.

57. Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55: 997–1004.

58. Heimann EL, Need AC, Hayden KM, Chiba-Falek O, Roses AD, et al. (2010) Genome-wide scan of copy number variation in late-onset Alzheimer’s disease. J Alzheimers Dis 19: 69–77.

59. Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, et al. (2009) A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet 41: 527–534.

60. Mant A, Blom J, Milosavljevic D, Krasnik B, Kragh M, et al. (2005) GWAS: a tool for performing genome-wide association studies of complex traits. Bioinformatics 23: 644–645.

61. Durin JV, Womersley J (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32: 77–97.

62. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 557–575.

63. Neuronal Genes for Subcutaneous Fat Thickness