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Published in:
PloS one

DOI:
10.1371/journal.pone.0137356

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Pant, S. D., Karlskov-Mortensen, P., Jacobsen, M. J., Cirera Salicio, S., Kogelman, L., Bruun, C. V. S., ... Fredholm, M. (2015). Comparative analyses of QTLs influencing obesity and metabolic phenotypes in pigs and humans. DOI: 10.1371/journal.pone.0137356
Comparative Analyses of QTLs Influencing Obesity and Metabolic Phenotypes in Pigs and Humans

Sameer D. Pant1*, Peter Karlskov-Mortensen1*, Mette J. Jacobsen1, Susanna Cirera1, Lisette J. A. Kogelman1, Camilla S. Bruun1, Thomas Mark1, Claus B. Jørgensen1, Niels Grarup2, Emil V. R. Appel2, Ehm A. A. Galjatovic2, Torben Hansen2, Oluf Pedersen2, Maryse Guerin3,4,5, Thierry Huby3,4,5, Philippe Lesnik3,4,5, Theo H. E. Meuwissen6, Haja N. Kadarmideen1*, Merete Fredholm1*

1 Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 2 The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 3 INSERM UMR_S 1166, Integrative Biology of Atherosclerosis Team, F-75013, Paris, France, 4 Sorbonne Universités UPMC Univ Paris 06 UMR_S 1166, Integrative Biology of Atherosclerosis Team, F-75013, Paris, France, 5 Institute of Cardiometabolism and Nutrition (ICAN), Pitié-Salpêtrière Hospital, 75013, Paris, France, 6 Institute of Animal and Agricultural Sciences, Norwegian University of Life Sciences, Ås, Norway

* These authors contributed equally to this work.

Abstract

The pig is a well-known animal model used to investigate genetic and mechanistic aspects of human disease biology. They are particularly useful in the context of obesity and metabolic diseases because other widely used models (e.g. mice) do not completely recapitulate key pathophysiological features associated with these diseases in humans. Therefore, we established a F2 pig resource population (n = 564) designed to elucidate the genetics underlying obesity and metabolic phenotypes. Segregation of obesity traits was ensured by using breeds highly divergent with respect to obesity traits in the parental generation. Several obesity and metabolic phenotypes were recorded (n = 35) from birth to slaughter (242 ± 48 days), including body composition determined at about two months of age (63 ± 10 days) via dual-energy x-ray absorptiometry (DXA) scanning. All pigs were genotyped using Illumina Porcine 60k SNP Beadchip and a combined linkage disequilibrium-linkage analysis was used to identify genome-wide significant associations for collected phenotypes. We identified 229 QTLs which associated with adiposity- and metabolic phenotypes at genome-wide significant levels. Subsequently comparative analyses were performed to identify the extent of overlap between previously identified QTLs in both humans and pigs. The combined analysis of a large number of obesity phenotypes has provided insight in the genetic architecture of the molecular mechanisms underlying these traits indicating that QTLs underlying similar phenotypes are clustered in the genome. Our analyses have further confirmed that genetic heterogeneity is an inherent characteristic of obesity traits most likely...
caused by segregation or fixation of different variants of the individual components belonging to cellular pathways in different populations. Several important genes previously associated to obesity in human studies, along with novel genes were identified. Altogether, this study provides novel insight that may further the current understanding of the molecular mechanisms underlying human obesity.

Introduction

Obesity, a condition represented by excessive accumulation of body fat, incurs massive economic costs and predisposes individuals to a number of other diseases including diabetes, cardiovascular disorders and osteoarthritis [1, 2]. Obesity is estimated to increase medical expenses by as much as 2,741 US dollars per person every year [1], and its prevalence is rapidly increasing worldwide. The etiology of obesity is highly complex and influenced by numerous factors including genetics and environmental factors such as diet and exercise. Past studies [3] have demonstrated genetic factors to determine as much as 60–70% of phenotypic variation, though genetic determinants underlying only 10% of the total genetic variance have been identified so far [4]. Genetic heterogeneity, confounding between genetics, epigenetic and environmental factors together with imprecise, costly and difficult measurement systems associated with obesity phenotypes, are some of the factors that are likely to contribute to the discrepancy between the overall genetic contribution to obesity and the identified genetic determinants.

For a complex trait like obesity, animal models can aid and accelerate the identification of underlying genetic determinants. Advantages of animal models include the possibility to design populations with certain genetic characteristics and much better control over environmental factors. Mouse models have been widely used primarily due to their evolutionary proximity to humans, their well characterized genome and the relatively low costs involved in housing, handling and breeding them in controlled environments. However, findings from murine models of obesity have often failed to translate to humans largely due to pathophysiological differences [5]. Given these differences, alternative animal models for human obesity are needed where research findings have a greater probability of being translatable to humans. Pig models are of interest in this regard as the pig genome has been sequenced and they are genetically closer to humans especially in the context of energy metabolism and obesity [6, 7]. Pigs are omnivores like humans, and unlike mice, also exhibit almost all of the pathophysiological features related to obesity and metabolic syndrome in a relatively short time span [7].

Given the potential benefits of using pigs to model human obesity, comprehensively phenotyped and genotyped porcine F2 intercross populations were established as a resource for obesity studies. Genetic determinants (Quantitative Trait Loci–QTLs) underlying a broad range of obesity and metabolic phenotypes were identified via combined linkage disequilibrium linkage analysis (LDLA). Subsequently, human chromosomal regions syntenic to identified QTL regions were investigated for previously reported associations with phenotypes comparable to those in pigs. Brief descriptions of the resource population and statistical methods are presented herein together with an overview of results from analyses.

Results and Discussion

The overall aim of this study was to identify genetic determinants underlying a broad range of obesity phenotypes in a porcine resource population, and also to evaluate the efficacy of using a porcine model of human obesity for genomic investigations. The porcine resource population
was constructed by crossing two sets of Göttingen minipig boars with Duroc and Yorkshire sows separately. Göttingen minipigs are susceptible to diet-induced obesity, and by crossing them to commercial pig breeds that have been genetically selected for leanness over several generations, we aimed to maximize genetic variance for obesity phenotypes in the resultant F2 populations (see Fig 1 for example). The pigs used in this study were raised in highly controlled conditions in order to minimize variation associated with environmental factors, and were subsequently extensively phenotyped. Consequently, these phenotypes (e.g. the body adiposity index, BAI and body mass index, BMI) more accurately represent genetic variation as opposed to corresponding human phenotypes that also include substantial environmental variation.

In order to perform genome-wide association analyses, a strategy based on combined LDLA was used instead of traditional single marker analyses. Given that the resource population was based on an F2 design with a structured pedigree, combined LDLA offered the opportunity to leverage linkage disequilibrium (LD) both within families and across the population, thereby mapping QTLs with narrower confidence intervals [8]. This is in contrast to traditional single marker GWAS which only leverages population-wide LD. On the other hand, since extensive LD has been demonstrated in several livestock genomes, it should be kept in mind that combined LDLA may not offer significant advantage in terms of resolution compared to GWAS in genomic regions of high LD. By mapping QTLs separately in the two crosses derived from
Durocs and Yorkshires, we hoped, to be able to exploit differences in the breed-specific LD structure to map QTLs segregating in both crosses to narrower chromosomal regions. This however, could not be exploited to a great extent since few QTLs underlying obesity and metabolic traits of comparative interest were found to segregate in both crosses. This may be due to limitations associated with statistical power and sample size that did not allow the identification of all QTLs in both crosses, or due to founder effects associated with the limited number of animal used in the founding generation. Although, compared to the human population the individual pig breeds are much more homogeneous genetically, the fact that different QTLs segregate in the two breeds supports the hypothesis that genetic heterogeneity is inherent to obesity traits. Combined LDLA is based on a linkage disequilibrium multi-locus iterative peeling (LDMIP) algorithm [8] that uses marker information surrounding a locus to compute IBD probabilities. Since the pig genome is incompletely annotated and some markers are either misplaced or their position is not currently known, this could potentially influence the analyses and should be considered as a potential limitation.

A total of 229 QTLs for 35 different phenotypes (Table 1) were identified as genome-wide significant (S1 Table). Some of these overlap with QTLs for comparable phenotypes in human syntenic regions. Overlapping QTLs for comparable phenotypes in human syntenic regions are indicative of similar genetic mechanisms driving obesity phenotypes in both pigs and humans. Therefore, it was important for us to assess the extent of overlap to determine the efficacy of using pigs as a model for human obesity. However, a few limitations were associated with our assessment of this overlap. Firstly, the NHGRI GWAS catalog [9] was used as a reference database for identification of comparable QTLs in human syntenic regions and our analysis was confined to results included in this catalog. Secondly, syntenic human chromosomal regions could not be defined for all porcine QTLs. This was primarily due to QTLs spanning synteny breakpoints and to ambiguities in the assembly of the porcine genome.

In addition to overlapping human QTLs, several overlapping pig QTLs for comparable or related phenotypes (e.g. subcutaneous fat, BMI, BAI etc.) were also identified by querying the AnimalQTLdb [10] (S1 Table). Contrary to the NHGRI GWAS catalog, the AnimalQTLdb does not use predefined criteria to determine inclusion of QTLs, but instead exhaustively curates all previously reported QTLs in the literature. Several of these QTLs have confidence intervals that span across the length of entire chromosomes. Consequently, AnimalQTLdb was only queried for previously reported pig QTLs up to 3 Mb in size that overlapped QTLs identified in this study. Thus, information on potential overlap between QTLs spanning larger regions has not been included.

Of the 35 porcine phenotypes analyzed in this study, 11 phenotypes constituted 114 QTLs, of which 20 had QTLs for comparable or related phenotypes in human syntenic regions (Table 2). All porcine autosomes had at least three QTLs each, with the exception of chromosome 17 that harbored a single QTL for total cholesterol measured directly in plasma (ct_s). Porcine autosome 1 (SSC1) harbored the maximum number (n = 44) of QTLs that represented 16 out of the 35 different phenotypes analyzed in this study. However, the highest density of QTL is on SSC13, harboring 0.83 QTLs per Mb (Figs 2 and 3). Overall, clustering of QTLs underlying similar phenotypes can be observed providing support for the notion that genes assigned to the same pathway are clustered in the genome [11].

A substantial proportion of QTLs were identified within the Minipig-Duroc cross compared to the Minipig-Yorkshire cross (S1 Table, second column). We have defined individual significant positions as independent QTLs if located more than 1 cM apart. However, many of the Minipig-Duroc QTLs are located close together and at the same time, extent of LD is greater in the Minipig-Duroc cross (S1 Fig). Hence, our definition of QTLs, while appropriate in the Minipig-Yorkshire cross, may inflate the number of QTLs in the Minipig-Duroc cross by
Also, it should be noted that some of the Minipig-Duroc QTLs are in fact detected in the Minipig-Yorkshire cross, however, at significance levels that are borderline to the level considered to be genome-wide significant in this study (data not shown).

### Table 1. Description of phenotypes measured in pigs at different ages, and covariates used in the statistical model for association analyses.

| Obesity Phenotypes                                                                 | Model Covariates                   |
|------------------------------------------------------------------------------------|------------------------------------|
| Backfat1: Thickness of Subcutaneous Adipose Tissue in Lower Trunk (Measured in mm at Age 3) | Sex, Age 3, (Age 3)^2              |
| Backfat2: Thickness of Subcutaneous Adipose Tissue in Upper Trunk (Measured in mm at Age 3) | Sex, Age 3, (Age 3)^2              |
| Bai: Body Adiposity Index (Measured at Age 2)                                       | Sex, Age 2, (Age 2)^2              |
| Bai_s: Body Adiposity Index (Measured at Age 1)                                     | Sex, Age 1                          |
| Birth_wgt: Birth Weight (Measured in Kgs)                                           | Sex                                |
| Bmi: Body Mass Index (Measured at Age 2)                                            | Sex, Age 2, (Age 2)^2              |
| Bmi_s: Body Mass Index (Measured at Age 1)                                          | Sex, Age 1                          |
| Dg1: Average Daily Weight Gain from Birth to Age 1 (Weight in Kgs)                 | Sex, Age 1                          |
| Dg2: Average Daily Weight Gain From Age 1 to Age 2 (Weight in Kgs)                 | Sex, Age 2, (Age 2)^2              |
| Mesfat: Excision of an 8 cm Diameter Section of Mesenteric Fat in the Triangle Between Ileum and Cecum (Weight in gms) | Sex, Age 3, (Age 3)^2              |
| Leaffat: Blunt Removal of Retroperitoneal Fat (Weight in Kgs)                       | Sex, Age 3, (Age 3)^2, Length (Age 3) |
| OmeFat: Blunt Removal of Greater Omentum (Weight in gms)                            | Sex, Age 3, (Age 3)^2, Length (Age 3) |
| Trpfat: Fat Percentage Trunk Region (DXA scanning)                                  | Sex, Age 1                          |
| Wb_lean: Total Lean Mass in Whole Body (DXA scanning, Weight in Kgs)               | Sex, Age 1, Length (Age 1)          |
| Wb_pfat: Fat Percentage in Whole Body (DXA scanning)                                | Sex, Age 1                          |
| Wb_tf: Total Fat in Whole Body (DXA scanning, Weight in Kgs)                        | Sex, Age 1, Length (Age 1)          |

### Blood Glucose and Lipoprotein Phenotypes Measured in Plasma

| Phenotype                                                                                     | Model Covariates                   |
|------------------------------------------------------------------------------------------------|------------------------------------|
| Cetp_per: Cholesteryl ester transfer protein Activity (CETP activity—Expressed in Percentage at Age 1) | Sex, Age 1                          |
| Ce_s: Esterified Cholesterol (Expressed in mmol/L at Age 1)                                   | Sex, Age 1                          |
| Cl_s: Free Cholesterol (Expressed in mmol/L at Age 1)                                        | Sex, Age 1                          |
| Ct_g: Total Cholesterol (Expressed in mmol/L at Age 3)                                       | Sex, Age 3, (Age 3)^2              |
| Ct_s: Total Cholesterol (Expressed in mmol/L at Age 1)                                       | Sex, Age 1                          |
| Hdl_c_g: High-density-lipoprotein Cholesterol (Expressed in mmol/L at Age 3)                  | Sex, Age 3, (Age 3)^2              |
| Hdl_c_s: High-density-lipoprotein Cholesterol (Expressed in mmol/L at Age 1)                  | Sex, Age 1                          |
| Ldl_c_g: Low-density-lipoprotein Cholesterol (Expressed in mmol/L at Age 3)                   | Sex, Age 3, (Age 3)^2              |
| Ldl_c_s: Low-density-lipoprotein Cholesterol (Expressed in mmol/L at Age 1)                   | Sex, Age 1                          |
| Pl_s: Phospholipids (Expressed in mmol/L at Age 1)                                             | Sex, Age 1                          |
| Tg_s: Triglycerides (Expressed in mmol/L at Age 1)                                             | Sex, Age 1                          |
| Fructosamin: Fructosamin (Expressed in μmol/L at Age 3)                                       | Sex, Age 3, (Age 3)^2              |
| Glucose: Fasting Glucose (Expressed in mmol/L at Age 3)                                       | Sex, Age 3, (Age 3)^2              |
| Lipase: Lipase (Expressed in U/L at Age 3)                                                     | Sex, Age 3, (Age 3)^2              |

### Blood Glucose and Lipoprotein Phenotypes Measured in ApoB depleted Plasma

| Phenotype                                                                                     | Model Covariates                   |
|------------------------------------------------------------------------------------------------|------------------------------------|
| Hdl_ce_s: High-Density-Lipoprotein Esterified Cholesterol (Expressed in mmol/L at Age 1)     | Sex, Age 1                          |
| Hdl_cl_s: High-Density-Lipoprotein Free Cholesterol (Expressed in mmol/L at Age 1)           | Sex, Age 1                          |
| Hdl_ct_s: High-Density-Lipoprotein Cholesterol (Expressed in mmol/L at Age 1)                | Sex, Age 1                          |
| Hdl_pl_s: High-Density-Lipoprotein Phospholipids (Expressed in mmol/L at Age 1)              | Sex, Age 1                          |
| Hdl_tg_s: High-Density-Lipoprotein Triglycerides (Expressed in mmol/L at Age 1)              | Sex, Age 1                          |

Age 1: 63 ± 10 days; Age 2: 218 ± 45 days; Age 3: 242 ± 48 days

doi:10.1371/journal.pone.0137356.001
| Pig Trait | QTL no. | Chr start | end Position | P-value | Chr start | end Position | P-value | Disease/Trait | SNP | Position | Context | P-value | PubMed ID |
|-----------|---------|-----------|--------------|----------|-----------|--------------|----------|--------------|-----|----------|---------|----------|-----------|
| back_fat2 | 1       | 4         | 30776117     | 46626364 | 37649495  | 106785287    | 1.91E-06 | Fat distribution (upper trunk subcutaneous adipose tissue) | rs921231 | 91348168 | intron   | 1.00E-06 | 21897333 |
|           |         |           |              |          |           |              |          | Fat distribution (upper trunk) | rs921231 | 91348168 | intron   | 2.00E-06 | 21897333 |
|           |         |           |              |          |           |              |          | Type 2 diabetes (HIV) | rs10504906 | 91406400 | Intergenic | 8.00E-06 | 21897333 |
|           |         |           |              |          |           |              |          | Type 2 diabetes | rs896854 | 94948283 | intron   | 1.00E-09 | 20581827 |
|           | 2       | 4         | 54865387     | 75907976 | 58665823  | 83944860     | 1.05E-07 | Obesity | rs4735692 | 75703428 | Intergenic | 4.00E-10 | 23563607 |
|           |         |           |              |          |           |              |          | Body mass index | rs2922763 | 75661476 | Intergenic | 6.00E-08 | 20935630 |
|           |         |           |              |          |           |              |          | Waist circumference | rs4471028 | 74382740 | Intergenic | 2.00E-07 | 17903300 |
|           |         |           |              |          |           |              |          | Visceral fat (overall) | rs16909318 | 81532989 | intron   | 7.00E-07 | 22589738 |
|           | 3       | 4         | 90666702     | 91379468 | 91379468  | 16358843     | 7.25E-05 | Response to mTOR inhibitor (rapamycin) | rs2063142 | 16308439 | Intergenic | 4.00E-06 | 24009623 |
|           |         |           |              |          |           |              |          | Visceral fat (men) | rs17744121 | 29341277 | Intergenic | 6.00E-06 | 22589738 |
|           |         |           |              |          |           |              |          | Body mass index | rs933117 | 29728478 | intron   | 6.00E-06 | 22446040 |
|           |         |           |              |          |           |              |          | Response to mTOR inhibitor (everolimus) | rs2832270 | 29222211 | intron   | 8.00E-06 | 24009623 |
|           |         |           |              |          |           |              |          | Response to statin therapy (Triglyceride, sum) | rs9305406 | 30013925 | Intergenic | 8.00E-06 | 20339536 |
|           |         |           |              |          |           |              |          | Visceral adipose tissue/subcutaneous adipose tissue ratio (men) | rs11858577 | 66774225 | intron   | 9.00E-06 | 22589738 |
|           | 6       | 1         | 187060595    | 19532007 | 190184135 | 57582560     | 3.32E-05 | Subcutaneous adipose tissue (overall) | rs7350721 | 55866795 | Intergenic | 6.00E-07 | 23192594 |
|           |         |           |              |          |           |              |          | Visceral adipose tissue/subcutaneous adipose tissue ratio (women) | rs8013477 | 49067707 | Intergenic | 4.00E-06 | 22589738 |
|           |         |           |              |          |           |              |          | Visceral fat (men) | rs1530947 | 49484632 | Intergenic | 5.00E-06 | 22589738 |
|           | 7       | 1         | 196439311    | 215457206 | 214926348 | 27329259     | 3.67E-06 | Body mass index (asthmatics) | rs3780215 | 19579429 | intron   | 7.00E-06 | 23517042 |
|           |         |           |              |          |           |              |          | Obesity-related traits (Fat mass) | rs1340043 | 18458070 | Intergenic | 9.00E-06 | 23251661 |

(Continued)
| Pig Trait QTL Data | Position of Strongest Association | Human Syntenic Region | Association Data from NHGRI GWAS Catalog |
|-------------------|----------------------------------|-----------------------|----------------------------------------|
| Pig Trait no.     | Chr start end                    | Chr start end         | Disease/Trait                          | SNP       | Position | Context    | P-value  | PubMed ID |
| Obesity-related traits (Trunk fat mass) | rs6475216 18444140 Intergenic | 9.00E-06              | Obesity-related traits                  | rs6475216 | 18444140 | Intergenic | 9.00E-06 | 23251661 |
| Visceral adipose tissue/subcutaneous adipose tissue ratio (overall) | rs4978053 26208859 Intergenic | 6.00E-06              | Visceral adipose tissue/subcutaneous adipose tissue ratio (overall) | rs4978053 | 26208859 | Intergenic | 6.00E-06 | 22589738 |
| Fat distribution (HIV) (upper trunk) | rs1944766 18215282 Intergenic | 3.00E-06              | Fat distribution (HIV) (upper trunk)   | rs1944766 | 18215282 | Intergenic | 3.00E-06 | 21897333 |
| Quantitative traits (Waist Circumference) | rs613391 22670716 intron | 5.00E-06              | Quantitative traits (Waist Circumference) | rs613391 | 22670716 | Intergenic | 5.00E-06 | 19197348 |
| Quantitative traits (Weight) | rs2225614 24111282 Intergenic | 3.00E-06              | Quantitative traits (Weight)           | rs2225614 | 24111282 | Intergenic | 3.00E-06 | 19197348 |
| Type 2 diabetes | rs10811661 22134095 Intergenic | 1.00E-27              | Type 2 diabetes                         | rs10811661 | 22134095 | Intergenic | 1.00E-27 | 24509480 |
|                    |                                  | 1.00E-18              |                                        |           |         |           |         | 23945935 |
|                    |                                  | 7.00E-07              |                                        |           |         |           |         | 19056611 |
|                    |                                  | 8.00E-15              |                                        |           |         |           |         | 17463248 |
|                    |                                  | 5.00E-08              |                                        |           |         |           |         | 17463246 |
|                    |                                  | 5.00E-06              |                                        |           |         |           |         | 17463249 |
| Type 2 diabetes    | rs2383208 22132077 Intergenic    | 2.00E-29              | Type 2 diabetes                        | rs2383208 | 22132077 | Intergenic | 2.00E-29 | 19401414 |
|                    |                                  | 3.00E-17              |                                        |           |         |           |         | 22961080 |
|                    |                                  | 3.00E-06              |                                        |           |         |           |         | 23091898 |
| Type 2 diabetes    | rs10965250 22133285 Intergenic   | 1.00E-10              | Type 2 diabetes                        | rs10965250 | 22133285 | Intergenic | 1.00E-10 | 20581827 |
|                    |                                  | 2.00E-07              |                                        |           |         |           |         | 18372903 |
| Type 2 diabetes    | rs7020996 22129580 Intergenic    | 2.00E-08              | Type 2 diabetes                        | rs7020996 | 22129580 | Intergenic | 2.00E-08 | 2293688 |
|                    |                                  | 3.00E-06              |                                        |           |         |           |         | 2383208 |
| Type 2 diabetes    | rs7018475 22137686 Intergenic    | 3.00E-08              | Type 2 diabetes                        | rs7018475 | 22137686 | Intergenic | 3.00E-08 | 2293688 |
|                    |                                  | 6.00E-10              |                                        |           |         |           |         | 2157907 |
| Type 2 diabetes    | rs1333051 22136490 Intergenic    | 6.00E-10              | Type 2 diabetes                        | rs1333051 | 22136490 | Intergenic | 6.00E-10 | 2157907 |
| Type 2 diabetes    | rs564398 22029548 ncRNA          | 1.00E-06              | Type 2 diabetes                        | rs564398 | 22029548 | ncRNA     | 1.00E-06 | 17463249 |
| Obesity-related traits (Dinner intake, adj TEE) | rs294845 10139580 Intergenic | 7.00E-06              | Obesity-related traits                  | rs294845 | 10139580 | Intergenic | 7.00E-06 | 23251661 |
| Type 2 diabetes    | rs649891 10430602 intron         | 6.00E-06              | Type 2 diabetes                        | rs649891 | 10430602 | intron    | 6.00E-06 | 21647700 |

(Continued)
| Pig Trait QTL Data | Position of Strongest Association | Human Syntenic Region | Association Data from NHGRI GWAS Catalog |
|-------------------|-----------------------------------|-----------------------|----------------------------------------|
| Pig Trait         | QTL no. Chr start end             | Position P-value      | Chr start end                          | Disease/ Trait SNP Position Context P-value PubMed ID |
| no.               | Chr start end                     |                      |                                        |                                                        |
| 9                 | 1 227146232 229129499 227628153 6.60E-06 9 2865933 4911598 |                      |                                        | Type 2 diabetes rs7041847 4287466 intron 5.00E-06 24508490 22013104 |
|                   |                                   |                      |                                        |                                                        |
| 10                | 1 246441732 247595657 247234467 1.03E-05 9 34718652 3578536 |                      |                                        | Type 2 diabetes rs10814916 4293150 intron 6.00E-12 22961390 |
|                   |                                   |                      |                                        |                                                        |
| 11                | 1 248776413 251138962 249132600 1.64E-05 9 36738650 38472147 |                      |                                        | Obesity and blood pressure (BMI) rs16933812 36969208 intron 5.00E-06 22013104 |
|                   |                                   |                      |                                        |                                                        |
| ce_s              | 12 3 56636950 57391731 56723081 2.92E-06 2 8109186 81826661 |                      |                                        | Bilirubin levels rs12052359 81645798 intron 7.00E-06 22085899 |
|                   |                                   |                      |                                        |                                                        |
| dg1               | 13 7 60925144 76495215 67628895 1.27E-07 14 29020842 39120983 |                      |                                        | Body mass index rs11847697 30045906 Intergenic 2.00E-06 23669352 |
|                   |                                   |                      |                                        |                                                        |
| 14                | 7 86871288 98757230 90901391 1.37E-06 15 85286426 98423740 |                      |                                        | Response to mTOR inhibitor (rapamycin) rs17664713 94775357 Intergenic 5.00E-06 24009623 |
|                   |                                   |                      |                                        |                                                        |
| ce_s              | 15 1 66931623 67106130 67106130 7.95E-05 6 96545315 96733074 |                      |                                        | Coronary heart disease rs12200560 96632322 Intergenic 6.00E-07 22313920 |
|                   |                                   |                      |                                        |                                                        |
| hdl_ce_s          | 16 4 29131900 31654951 31569807 3.03E-06 8 106022233 108323587 |                      |                                        | Obesity-related traits (HDL) rs7004587 107028124 Intergenic 3.00E-06 23251661 |
|                   |                                   |                      |                                        |                                                        |
| hdl_cl_s          | 17 1 66931623 67106130 67106130 0.000186 6 96545315 96733074 |                      |                                        | Coronary heart disease rs12200560 96632322 Intergenic 6.00E-07 22313920 |
|                   |                                   |                      |                                        |                                                        |
| hdl_ct_s          | 18 2 38562295 39437513 38837764 2.68E-06 11 17003981 17858922 |                      |                                        | Type 2 diabetes rs5215 17387083 missense 3.00E-11 24509480 |
|                   |                                   |                      |                                        |                                                        |
| hdl_tg_s          | 19 1 268191623 268191623 268191623 0.000186 6 268191623 268191623 |                      |                                        | Obesity and blood pressure (Total Fat Mass) rs16933812 36969208 intron 5.00E-06 22013104 |
|                   |                                   |                      |                                        |                                                        |

(Continued)
## Table 2. (Continued)

| Pig Trait | QTL no. | Chr | start | end | Position | P-value | Disease/Trait | SNP | Position | Context | P-value | PubMed ID |
|-----------|--------|-----|-------|-----|----------|---------|---------------|-----|----------|---------|---------|-----------|
| Type 2 diabetes (obese) | rs5219 | 17388025 | missense | 5.00E-07 | 19056611 |
| Type 2 diabetes (non-obese) | rs5219 | 17388025 | missense | 1.00E-09 | 19056611 |
| Type 2 diabetes | rs5219 | 17388025 | missense | 1.00E-07 | 17463246 |
| ld1_c_s | 19 | 3 | 120245799 | 121054453 | 120245799 | 2.78E-05 | 2 | 2043854 | 4034691 |
| Type 2 diabetes | rs11677370 | 3793830 | intron | 3.00E-06 | 21490949 |
| tg_s | 20 | 6 | 43445975 | 43627267 | 43627267 | 0.000331 | 1 | 3084050 | 3542414 |
| Response to statin therapy | rs6658356 | 3363689 | intron | 2.00E-06 | 20339536 |
There were 19 chromosomal regions that were associated with more than one phenotype (Fig 2) (Table 3), of which 2 QTL regions on chromosome 3 (SSC3:56,689,989 and SSC3:56,723,081), and another 2 QTL regions on chromosome 5 (SSC5:58,544,792 and SSC5:60,364,446) are in so close proximity that they may represent two single QTLs influencing multiple phenotypes. Most of these QTLs (n = 14) influenced cholesterol related phenotypes. Two QTLs on SSC1 (15,748,172 bp) and SSC5 (38,544,792 bp) influenced fat percentage of the trunk region as well as of the whole body measured via DXA scanning. QTLs on SSC1
(218,448,574 bp) and SSC7 (132,308,360bp) influenced BMI and the average daily weight gain measured at different ages. Finally, one QTL on SSC10 (19,668,169) influenced both BMI measured at 63 ± 10 days and whole body lean mass measured via DXA scanning at the same age. Hence in most cases, the multiple phenotypes influenced by the same chromosomal position are interrelated phenotypes most likely influenced by similar cellular pathways.

Fig 3. QTLs influencing Metabolic Phenotypes in pigs. (Figure created using Phenogram- http://visualization.ritchielab.psu.edu/phenograms/plot). Vertical columns labeled 1–18 represent porcine autosomes SSC1–18. QTL locations are marked on the chromosomes using a proximity algorithm that minimizes the overlap between individual QTLs for different phenotypes. Different phenotypes are represented by circles filled with different colors and the description of the abbreviated phenotypes is presented in Table 1.

doi:10.1371/journal.pone.0137356.g003
In addition to identifying QTLs for specific phenotypes, some general inferences can also be drawn with respect to the biology driving different phenotypes. For example, storage of fat in intra-abdominal fat compartments (retroperitoneal fat, mesenteric fat, omental fat) appears to be controlled by separate loci except for a locus from 241.4 to 244.7 Mb on SSC1 which is associated with both retroperitoneal and omental fat. None of the loci associated with intra-abdominal fat accumulation are associated with accumulation of subcutaneous fat (back_fat1 and back_fat2). Even accumulation of subcutaneous fat of upper (back_fat2) and lower trunk region (back_fat1) seems to be influenced by different genetic loci i.e. lower trunk subcutaneous fat is associated with loci on SSC15, whereas upper trunk subcutaneous fat is associated with loci on SSC1, 3, 4 and 18. A single locus on SSC1 (15.7 Mb) is associated with both trunk and whole body fat percentage measured via DXA scanning. On the other hand, trunk fat percentage (tr_pfat) as measured by DXA scan does not overlap with upper or lower trunk subcutaneous fat except for a locus on SSC4, 83 Mb. DXA scanning was performed in the young pig whereas trunk subcutaneous fat was measured in adult pigs. The difference in associated QTL may therefore indicate that different molecular mechanisms are involved in fat deposition in the trunk region at different ages, except for the locus on SSC4 which seems to have a role independent of age.

Another locus on SSC1 (213.9–215.0 Mb) is associated with weight of omental fat, BMI and daily weight gain in adolescent pigs (dg2). Four regions on SSC3; around 46.1 Mb, from 51.1 to 58.1 Mb, from 109.5 to 120.2 Mb and from 124.6 to 125.0 Mb, are associated with several blood lipid traits in both early life and during adolescence (Fig 3). Thus, this region seems to harbor a number of different genes affecting different aspects of the phenotypes involved in plasma cholesterol levels indicating that there is genomic clustering of functionally related genes and co-regulatory elements [11]. A locus on SSC4 (69.2 Mb) is associated with total HDL-cholesterol and esterified HDL-cholesterol but not with overall cholesterol level or LDL-cholesterol levels. The same locus is associated with overall phospholipid level as well as HDL-phospholipid level.

### Table 3. Chromosomal positions associated with multiple phenotypes in the pig resource population.

| Position     | Phenotypes                          |
|--------------|-------------------------------------|
| Chr1:11134679| ce_s, ct_s, ld1_c_s                 |
| Chr1:15748172| tr_pfat, wb_pfat                    |
| Chr1:67106130| hdl_ce_s, hdl_ct_s                  |
| Chr1:114718770| hdl_ce_s, hdl_ct_s                  |
| Chr1:218448574| bmi_g, dg2                          |
| Chr3:46117851| hdl_ce_s, hdl_ct_s                  |
| Chr3:56689989| ct_g, ld1_c_g                       |
| Chr3:56723081| ce_s, ct_s, ld1_c_s                 |
| Chr4:45434589| hdl_pl_s, pl_s                      |
| Chr4:69198075| hdl_ce_s, hdl_ct_s, hdl_pl_s, pl_s  |
| Chr5:58544792| tr_pfat, wb_pfat                    |
| Chr5:60364446| ce_s, ct_s                          |
| Chr7:132308360| bmi_s, dg1                          |
| Chr9:2418857| ct_g, hdl_c_g                       |
| Chr9:62416702| cl_s, ct_s, ld1_c_s                 |
| Chr10:19668169| bmi_s, wb Lean                      |
| Chr15:45350470| hdl_ce_s, hdl_ct_s                  |
| Chr18:9431105| ce_s, ct_s, ld1_c_s                 |
| Chr18:32618335| ce_s, ct_s                          |

doi:10.1371/journal.pone.0137356.t003
Several QTL regions contained evidence indicative of biological significance. In some cases, similar QTLs have previously been found in human studies but in other cases, the identified QTLs are novel and to our knowledge not described before in humans, rodents or pigs. A selection of the most attractive and biologically significant results is described below:

**Obesity Phenotypes**

**Body Adiposity Index (BAI).** A total of 11 QTLs were identified for BAI, of which 8 QTLs were identified for BAI measured at around two months of age (64 ± 11 days, bai_s), and 3 other QTLs were identified for BAI measured at slaughter (220 ± 45 days, bai_g). Amongst these, an interesting 2 Mb QTL is located on SSC13 (135,407,662–137,685,786) that includes two genes BACH1 and GRIK1. The corresponding human syntenic region is located on HSA21 that also includes both these genes. Human investigations have identified an intronic SNP (rs17744121) in BACH1 to be associated with visceral fat in men (p = 6.0E-6) [12], and another intronic SNP (rs933117) in GRIK1 to be associated with BMI (p = 6.0E-6) [13]. Functionally, BACH1 is a transcription factor that interacts with MAFK, and can suppress expression of heme-oxidase 1. GRIK1 encodes a glutamate receptor that serves as the predominant excitatory neurotransmitter in the mammalian brain.

**Body Mass Index (BMI).** A total of 23 QTLs were identified for BMI in this study, 6 of which were identified for BMI measured at around two months of age (64 ± 11 days, bmi_s), and another 17 were identified for BMI measured at the time of slaughter (220 ± 45 days, bmi_g). Several of these QTLs, particularly those identified for bmi_g, are located on SSC1, and overlap with QTLs for comparable phenotypes in human syntenic chromosomal regions. For example, QTL5 (Table 2) spanning approximately 5 Mb (168,007,706–172,796,697) contains SMAD6, and is syntenic to a 2.5 Mb chromosomal region on HSA15 containing a SMAD6 intronic variant (rs11858577) associated with subcutaneous fat tissue volume in men and women (p = 9.0E-06) [12].

QTL6 (Table 2) located on SSC1 (187,060,595–195,320,076) is syntenic to a chromosomal region on HSA14 that contains three intergenic SNPs associated with BMI (rs7350721, p = 6.0E-07) [14], visceral adipose tissue to subcutaneous adipose tissue ratio in women (rs8013477, p = 4.0E-06) [12], and visceral fat in men (rs1530947, p = 5.0E-06) [12]. The porcine QTL region along with its corresponding human syntenic region both contains the genes RPS29, encoding a ribosomal protein, and PELI2, encoding a ubiquitin protein ligase family member. None of these have a known biological function that relate directly to obesity.

QTL7 (Table 2) covers approximately 20 Mb (196,439,311–215,457,206) on SSC1. This extended QTL probably represents a series of BMI QTLs on SSC1 which, however, cannot be precisely delimited in the present study. The region corresponds to a human syntenic region extending more than 10 Mb on HSA9 which is also rich in obesity QTLs and contains several variants associated with a range of obesity phenotypes like BMI in asthmatic adults (rs3780215, p = 7.0E-06) [15], fat mass (rs1340043, 9.0E-06), trunk fat mass (rs6475216, p = 9.0E-06) [16], subcutaneous adipose tissue of upper trunk in HIV infected men treated with antiretroviral therapy (rs1944766, p = 3.0E-06) [17], weight (rs2225614, p = 3.0E-06), waist circumference (rs613391, p = 5.0E-06) [18] and overall volume of subcutaneous and visceral fat (rs4978053, p = 6.0E-06) [12]. However, none of these human genetic variants have syntenic porcine positions that are in close proximity to the porcine QTL position with the strongest association (p = 3.7E-06). In fact, the position of strongest association within QTL7 is closer to another variant (rs1927702) that has been associated with BMI (p = 6.0E-06) [19], but is located marginally outside the chromosomal extent of QTL7. Though there are three genes contained in the human syntenic region (ADAMTS1, TUSC1 and BNC2), none of these seems
to have any presently known biological relationship with obesity or any of its related phenotypes.

QTL10 (Table 2) is a narrow QTL (~ 1.15 Mb) extending between 246,441,732–247,595,657 on SSC1, syntenic to a chromosomal regions on HSA9 that includes a variant (rs10972341) associated with ‘weight in males’ (p = 9.0E-06) [19].

QTL11 (Table 2) on SSC1 extending between 248,776,413–251,138,962 includes PAX5, and is syntenic to a narrow human chromosomal region on HSA9 (36,738,650–38,472,147) that contains an intronic variant (rs109723412) of PAX5 associated with BMI (p = 5.0E-06) and total fat mass (p = 9.0E-06) [20]. PAX5 is a member of the PAX transcription factor family with a highly conserved DNA binding motif known as the paired box. PAX5 has been described as a B-cell specific transcription factor and its dysregulation is associated with different types of leukemia. The gene is also expressed in brain and testes. The protein plays a role in cell proliferation and is an important regulator in early development.

**Upper Trunk Subcutaneous Fat.** A total of 13 QTLs for Upper Trunk Subcutaneous Fat were identified in this study, of which 3 QTLs located on SSC4 overlap with QTLs for comparable traits in corresponding human syntenic chromosomal regions.

QTL 1 and 2 (Table 2) are large QTLs and consequently contain several relevant associations reported in human syntenic regions. However, none of them seems to be located close to corresponding porcine chromosomal positions with strongest associations within the QTL regions. For example, QTL1 (SSC4:30,776,117–46,626,364) with its strongest association at SSC4:37,649,495, is syntenic to HSA8 (90,948,835–106,785,287) that contains several variants associated with upper trunk subcutaneous adipose tissue (rs921231, HSA8:91,348,168) [17], arm fat distribution (rs10504906, HSA8:91,406,400) [17], and Type 2 diabetes (rs7845219, HSA8:94,925,274; rs896854, HSA8:94,948,283) [21, 22]. One of these genetic variants (rs921231) is located in the intron of SLC26A7 that belongs to a family of anion transporters (solute carrier family) reported to be primarily involved in renal physiology [23]. Other members of the solute carrier family have been implicated in obesity [24].

Similarly QTL2 (SSC4:54,865,387–75,907,976) with its strongest association at SSC4: 58,665,823, is syntenic to HSA8 (59,931,695–83,944,860) that contains several variants associated with obesity (rs4735692, HSA8:75,703,428) [25], BMI (rs2922763, HSA8:75,661,476) [4], waist circumference (rs4471028, HSA8:74,382,740) [26], and overall visceral fat (rs16909318, HSA8:81,352,989) [12]. One variant (rs16909318) is located in the intron of FABP12 that belongs to a family of fatty acid transport proteins that has been linked to both obesity [27] and metabolic syndrome [28].

**Blood Glucose and Lipoprotein Phenotypes**

**Fasting glucose.** Three distinct QTL regions were identified to be significantly associated with fasting glucose levels measured at 242 ± 48 days of birth, of which one QTL position on SSC9 (5,534,785, p = 3.7E-04) is located within an intron of STIM1. This is a novel QTL and association between fasting glucose and this locus has, to our knowledge, not been reported before neither in rodents nor in humans or pigs. Due to its localization within STIM1 it is however a very interesting QTL. This gene encodes a transmembrane calcium sensor located on the endoplasmic reticulum that regulates store-operated calcium entry (SOCE). In-vitro studies using insulin secreting beta cell lines indicate that non-specific inhibitors of SOCE (e.g. SKF-96365) can inhibit glucose-induced insulin secretion in these cells [29]. Murine studies have demonstrated that high glucose levels can induce Stim1 expression in micro vessel endothelial cells [30] and can restore coronary endothelial function in type 1 diabetic mice [31].
**Free Cholesterol.** A total of 5 QTLs were identified for free cholesterol measured directly in plasma. One QTL on SSC12 (12,081,814, $p = 4.0E-04$) is especially interesting since it has not been identified in rodents or humans before, and because it is located within an intron of STRADA. This gene encodes an adaptor protein that interacts and activates STK11 (Also known as LKB1), which in turn phosphorylates and activates AMPK, a central metabolic sensor that regulates lipid, cholesterol and glucose metabolism in liver, muscles and adipose tissue [32].

Free cholesterol was also measured in apoB depleted plasma and 5 QTLs were identified for this phenotype. One QTL (QTL16, Table 2) had a matching QTL for HDL in its corresponding human syntenic region. QTL16 extends between 29,131,900–31,654,951 on SSC4, and is syntenic to HSA6 (96,545,315–96,733,074) containing an intergenic variant (rs7004587) associated with HDL cholesterol ($p = 3.0E-06$) [16] that is located between three genes ANGPT1, OXRI, and ABRA/STARS. Mechanistically, there is no evidence that indicates a role of either of these genes in regulating plasma cholesterol levels. However, STARS (striated muscle activator of Rho signaling), encodes a membrane bound protein expressed in cardiac and striated muscles that enhances Rho-dependent transcription in muscle cells [33]. Rho-GTPases are small signaling G proteins that can also be activated by HDL proteins (e.g. ApoA1) to influence ‘reverse cholesterol transport’ via HDL carriage from peripheral tissues to liver for eventual elimination from the body [34]. The colocation of human and porcine QTLs in syntenic chromosomal regions together with its known biological function, make STARS a putative candidate for further studies with respect to cholesterol levels in plasma.

**Esterified Cholesterol in Plasma.** A total of 10 QTLs were identified for this phenotype, of which only one QTL (QTL12, Table 2) had a QTL for an indirectly comparable phenotype in the corresponding human syntenic region. QTL15 extends over a narrow chromosomal region on SSC3 (56,636,950–57,391,731) and is syntenic to HSA2:81,091,886–81,826,661 that contains a genetic variant (rs12052359, HSA2:81,645,798) associated with serum bilirubin levels in an African American population ($p = 7.4E-06$) [35]. While both the porcine QTL region and the corresponding human syntenic regions do not contain any known genes, the colocation of these QTLs is interesting because serum bilirubin concentration is known to be inversely correlated to concentration of esterified cholesterol in serum [36].

**Low Density Lipoprotein Cholesterol.** A total of 22 QTLs (the maximum for any phenotype in this study) were identified for low density lipoprotein cholesterol measured directly in plasma at 63 ± 10 days of birth. However, half of these QTLs (n = 11) are located on SSC3 in two separate chromosomal regions extending over approximately 10 Mb. One of these QTL positions on SSC3 (115,804,895, $p = 8.2E-06$) is a novel QTL not previously reported in rodents or human. It is located in an intron of LPIN1 encoding a phosphatatic acid phosphohydrolase that is a member of a broader family of Lipin proteins that play key roles in triglyceride and membrane phospholipid biosynthesis [37]. Murine studies indicate that Lipin expression can influence fat storage capacity of adipocytes and whole-body energy expenditure and fat utilization, both of which can directly influence obesity [38]. Human studies have also found LPIN1 expression in visceral adipose tissue to be correlated with body fat percentage, plasma triglyceride level and plasma leptin level. Additionally, LPIN1 mRNA levels have been found to be positively correlated with PPARG and ADIPOQ mRNA levels in visceral and subcutaneous adipose tissue [39]. PPARG and ADIPOQ are highly expressed in adipose tissue [40, 41]; are involved in cholesterol homeostasis, differentiation of adipocytes and accumulation of lipids (PPARG) [42–44]; and in modulation of glucose levels and fatty acid oxidation (ADIPOQ) [45].

**High Density Lipoprotein Cholesterol.** High density lipoprotein-cholesterol measured directly in plasma at 63 ± 10 and 242 ± 48 days of birth; and indirectly at 63 ± 10 days of birth in apoB depleted (high-density-lipoprotein fraction) plasma. All these measurements were
treated as separate phenotypes in the analyses and a total of 26 QTLs were identified, of which 5 chromosomal regions are associated with two different measures of cholesterol and therefore represent 10 QTLs. Two additional sets of QTLs are within 1.2 Mb of each other that could represent two QTLs instead of four.

In the context of high-density-lipoprotein cholesterol, an interesting novel QTL associated with both total cholesterol ($p = 1.2E-04$) and its esterified fraction ($p = 2.1E-04$) in apoB depleted plasma was identified on SSC1 (114,718,770). This chromosomal position is located in the intron of the $RORA$ gene that encodes a receptor for cholesterol sulphate, 7-dehydroxy-cholesterol and cholesterol [46–48]. Functionally, it is a key regulator of cholesterol levels [49]. We categorize this QTL as a novel QTL even though human studies have identified genetic variants around $RORA$ that are weakly associated with cholesterol (both HDL and LDL fractions) [50].

**Conclusion**

The combined analysis of a large number of obesity phenotypes has provided new and confirmed previous insight in the genetic architecture of the molecular mechanisms underlying these traits. Our analyses have further confirmed that genetic heterogeneity is an inherent characteristic of obesity traits most likely caused by segregation or fixation of different variants of the individual components belonging to cellular pathways in different populations. Overall, several QTLs reported in this study are in good accordance with previously reported QTLs for comparable or related phenotypes in pigs (S1 Table). Several of these QTLs also overlap with previously reported QTLs for comparable human phenotypes which indicate that similar genetic mechanisms drive obesity phenotypes in both pigs and humans. The study provides support for novel QTL regions and candidate genes for obesity and metabolic traits which can be exploited in future whole genome sequencing projects in humans. Several possibilities of further analyses of causative variants and molecular pathways exist since the porcine resource described in this study has not only been extensively phenotyped and genotyped, but also subjected to extensive tissue sampling at slaughter. Results of such future investigations could provide valuable and novel biological insights into obesity that could potentially be translatable to humans.

**Materials and Methods**

**Experimental Design and Genotyping**

The resource population was established in the following way: In the parental generation seven purebred Yorkshire (YY) sows and seven purebred Duroc (DD) sows from a DanBred breeding herd were mated to 14 Göttingen Minipig (MM) boars from Ellegaard A/S (all animals unrelated at the grandparental level). Among the DM F1 animals 28 gilts and 16 boars were mated to produce 285 animals; among the YM F1 animals 26 gilts and 13 boars were mated to produce 279 animals. The animals were produced and slaughtered in three batches with approximately the same number of F2 animals from the Duroc and Yorkshire crosses in each batch. The pigs were kept under normal condition for production pigs in Denmark in pens with 10–15 animals per pen at a temperature around 20±3°C with ad libitum administration of standard pig feed and free access to water. Both Duroc and Yorkshire are production breeds that have undergone extensive selection for leanness and growth traits, while Göttingen minipigs are mainly used for research purposes and are bred primarily for their small size and ease of handling. Unlike the production pigs, Göttingen minipigs are also susceptible to diet induced obesity and share many metabolic dysfunctions associated with human obesity [51] (Fig 1). All 564 pigs were genotyped using Illumina Porcine 60k SNP Beadchip.
The project was approved by the Danish Animal Experimentation Board. Animal care and maintenance have been conducted according to the Danish "Animal Maintenance Act" (Act 432 dated 09/06/2004). The animals were housed at a regular pig farm, and slaughtered at a commercial slaughterhouse by stunning and bleeding under veterinary supervision. Tissue and blood samples were collected at slaughter.

Collection of Phenotypes

Extensive phenotypic collection was performed from birth to slaughter (242 ± 48 days) including obesity, obesity-related, and metabolic phenotypes; and measurements of fat compartments at slaughter. In addition, body composition was determined after weaning using dual-energy x-ray absorptiometry (DXA) scanning at about two months of age (63 ± 10 days). Further details of pedigree and phenotyping of obesity traits are available in Kogelman et al. [52]. Plasma lipid levels were assayed by standardized techniques using a Konelab 20 Clinical Chemistry Analyzer (Thermo Scientific, Sweden) and commercial reagent kits from Roche Diagnostics for Total Cholesterol (CT) and from ThermoElectron for triglycerides (TG) and High Density Lipoprotein Cholesterol (HDL-C) levels (direct method). Free cholesterol (CL) and phospholipid concentrations were measured using reagents from Diasys, Germany. Cholesteryl ester (CE) mass was calculated as CT – CL. Fasting plasma Low Density Lipoprotein-Cholesterol (LDL-C) was calculated using the Friedewald formula [53]. Plasma HDL-C levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins. Plasma CETP activity was assayed by using the method of Guerin et al. [54], which estimates CE transfer from HDL to apoB-containing lipoprotein particles (expressed as percentage). A list of the 35 phenotypes included in the study is provided in Table 1.

Statistical Analyses

Phenotype data were checked for normality and log or square-root transformations were applied when required. Four phenotypes had 1–3 data points that were several standard deviations (5–13) away from the mean, and were consequently considered outliers that were excluded prior to analyses. Statistical analyses were carried out separately within the Duroc and Yorkshire crosses. Preliminary quality control of genotype data was performed by excluding all SNPs that had a minor allele frequency (MAF) < 0.05, Hardy Weinberg equilibrium test p-value < 0.001, and a genotype call rate < 0.95.

Subsequently, identity by descent (IBD) probabilities were estimated chromosome-wise for each sliding marker bracket at its midpoint using the linkage disequilibrium (LD) multi-locus iterative peeling (LDMIP) method as described by Meuwissen and Goddard [8]. Variance component analysis was then performed with ASReml [55] using a mixed linear model. Genome-wide association analysis was performed via a likelihood ratio test, where the test statistic was calculated as follows:

\[ 2\Delta l = 2(l_q - l_a) \approx \chi^2 \text{ with } 1 \text{ d.f.} \]

Where:

- \( 2\Delta l \) is the likelihood ratio test statistic;
- \( l_q \) is the maximum likelihood estimate of a full model that included the fixed effect of gender, a number of covariates depending upon the phenotype (Specified in Table 1), a random QTL effect based on the estimated IBD relationships, as well as a numerator relationship matrix to account for polygenic effects. Batch effect due to production of the animals in three contemporary groups was found to be non-significant, and hence excluded as a
covariate from statistical analyses. Using matrix notation, the full model can be described as follows:

\[
y = \mathbf{1} \mu + \mathbf{X} \mathbf{b} + \mathbf{Z}_1 \mathbf{u} + \mathbf{Z}_2 \mathbf{v} + \mathbf{e}
\]

\( y \) = vector of phenotypes  
\( \mu \) = mean  
\( \mathbf{X}, \mathbf{Z}_1 \) and \( \mathbf{Z}_2 \) are design matrices  
\( \mathbf{b} \) = vector of fixed effects  
\( \mathbf{u} \) = vector of random polygenic effects  
\( \mathbf{v} \) = vector of random QTL effect  
\( \mathbf{e} \) = vector of random residuals.

Assuming the following mutually independent distributions of random variables:

\[
\mathbf{u} \sim N(0, \mathbf{A} \sigma_u^2) \\
\mathbf{v} \sim N(0, \mathbf{G} \sigma_v^2) \\
\mathbf{e} \sim N(0, \mathbf{I} \sigma_e^2)
\]

Where: \( \mathbf{A} \) = Additive genetic relationship matrix  
\( \mathbf{G} \) = Average Identity by Descent matrix  
\( \mathbf{I} \) = Identity matrix

- \( L_0 \) is the maximum likelihood estimate of a null hypothesis that included all effects in the full model except for the QTL effect.

Level of significance (p-values) was computed by assuming \( 2 \Delta l \) to follow a chi-squared distribution with one degree of freedom under the null-hypothesis of no QTL in the tested marker bracket. QTLs with a statistical significance of \( p < 0.0001 \), or those with a point-wise \( p < 0.001 \) and whose \( -\log_{10}(p) \) was >3 times greater than the average \( -\log_{10}(p) \) in the flanking 5 Mb (i.e. 10 Mbs in total) chromosomal window, were considered to be genome-wide significant. Adjacent significant positions were regarded as individual QTLs if located more than 1 Mb apart.

To evaluate extend of LD in each cross, decay of \( r^2 \) over distance was calculated using the method described by Badke et al. (2012) [56].

**Comparative Analyses**

The liftOver tool available via UCSC Genome Browser [57] was used to convert genome coordinates between porcine and human assemblies and to map human chromosomal regions syntenic to porcine chromosomal regions containing QTLs associated with different phenotypes. Since liftovers are currently not available between Sscrofa 10.2 build and the current human genome build, we used Sccrofa 9.2 build for the liftover procedure. Additional information on porcine gene annotation was obtained from Sscrofa 10.2. Successive QTL positions that were genome-wide significant were considered to represent a single QTL whose extent was determined by the genomic positions of the first and the last genome-wide significant QTL position.
Chromosomal extents of QTLs smaller than 100 kilobases were extended to a minimum of 100 kilobases. The National Human Genome Research Institute (NHGRI) catalog [9] was used to identify human SNP-trait associations in chromosomal regions syntenic to porcine QTLs. These associations were manually curated to identify SNP associations with human phenotypes that were comparable to the porcine phenotypes. Data on 12,618 pig QTLs from 461 publications representing 656 different traits were also downloaded from the Animal QTL database (Animal QTLdb) [10] and subsequently used to identify previously identified porcine QTLs up to 3 Mb in size that overlapped QTL regions identified in this study. These overlapping QTLs were manually curated to identify phenotypes comparable or related to those identified in the present study. Results of these comparisons are presented in S1 Table. All porcine chromosomal locations described in this study are based on the Sscrofa 9.2 assembly of the pig genome.

Supporting Information

S1 Fig. Decay of average $r^2$ over distance. Decay of average $r^2$ over distance calculated by the method describe by Badke et al. (2012) [56]. Average LD over short distances corresponds well to within-population LD observed previously [56]. Over longer distances, significantly stronger LD is found in the present cross which is in accordance with the LD generated by crossing different breeds.

(PDF)

S1 Table. Complete list of QTLs. Description of the number of QTLs identified for each phenotype, the cross in which they were identified, their genomic position, size and significance levels; along with previously reported QTLs derived from the AnimalQTLdb for comparable phenotypes that overlap QTLs identified in the current study. Name (No. of QTLs) = abbreviations for each phenotype as described in Table 1, along with the number of QTLs identified for this phenotype; Cross = cross in which QTL was identified; Chr = Chromosome; Pos = Chromosomal positions with peak significance (Sscrofa 9.2); start, end = QTL start and end chromosomal positions (Sscrofa 9.2); Pvalue = peak significance of the QTL; Animal_QTL_DB_qtls (QTL id) = Previously reported QTLs for comparable phenotypes derived from the AnimalQTLdb along with their QTL ids.

(XLSX)

Acknowledgments

The authors wish to thank Minna Jakobsen, Tina Neergaard Mahler and Christel A. Halberg for excellent technical assistance.

Author Contributions

Conceived and designed the experiments: MF. Performed the experiments: PKM MJJ SC CSB TM CBJ MF. Analyzed the data: SDP PKM HNK TM THEM NG EVRA EAAG T. Hansen OP LJAK. Contributed reagents/materials/analysis tools: MG T. Huby PL. Wrote the paper: SDP PKM MF.

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