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The FAGenomicH project: towards a whole candidate gene approach to identify markers associated with fatness and production traits in pigs and investigate the pig as a model for human obesity

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ABSTRACT - Fatness in pigs is a complex trait for which a large number of genes are expected to be involved. Genetics of human obesity could take advantages from genetic information coming from the pig and *vice versa*. To these aims, a comprehensive candidate gene approach could be helpful. We catalogued all genes affecting fatness on both species, and identified *in silico* and by resequencing porcine SNPs on a large number of candidate genes. In addition, we applied a selective genotyping approach to identify markers associated with fat deposition in pigs. This approach was tested genotyping the *IGF2* intron3-g.3072G>A mutation and novel markers in the *PCSK1* and *TBC1D1* genes. Polymorphisms in these genes resulted associated with back fat thickness in Italian Large White pigs.

Key words: Pig, Fatness, Obesity, Candidate genes.

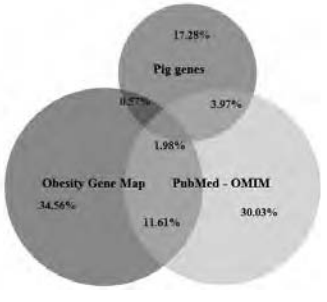
Introduction - Fatness is an important pig breeding objective due to its impact on carcass quality, performance traits, meat quality and, in turn, consumer acceptance. The pig is also an important biomedical model, and for its physiological similarities to humans, it is more relevant than the mouse to human health research priorities, such as obesity and diabetes. Fat deposition in pig is a complex trait for which several studies in experimental populations have identified a large number of QTL. More than 450 QTLs for fatness traits have been positioned in the Pig QTL database map (Hu *et al*., 2007); some of which have been shown to segregate in outbred commercial populations. In human, more than 700 genes or chromosome regions have been associated with obesity (Rankinen *et al*., 2006). Combining the genetic information on fat deposition in pig and human could help to clarify the genetic factors affecting this trait in both species. Pig breeding stocks represent a valuable resource for deciphering the genetic determinants involved in fat deposition, because they are carefully phenotyped for several carcass traits, usually including direct or indirect measures of fatness. Here we investigated fat deposition in pigs, designing an integrated approach

having as first objective the identification of DNA markers associated with fat deposition in Italian heavy pigs. We report parts of the results obtained including i) literature and database surveys and mining, that created a core of candidate genes, ii) *in silico* mining of porcine DNA sequences and resequencing or *de-novo* sequencing, that identified single nucleotide polymorphisms (SNPs) in these genes, and iii) genotyping five of these markers using a selective genotyping approach in two groups of Italian heavy pigs, that allowed to evaluate their association with fat deposition traits.

Material and methods - Literature and database surveys for genes involved in human and mouse obesity, fat deposition in pig, and fat metabolism were carried out on PubMed database Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/), Obesity Gene Map Database (http://obesitygene.pbrc.edu/), and through a systematic inspection of pig genetics publications. Results of these surveys were summarized using BioVenn web application (http://www.cmbi.ru.nl/cdd/biovenn/). Mining porcine *expressed* sequence tags (ESTs) for *in silico* SNPs identification was obtained as described by Fronza *et al*. (2009). Resequencing or *de-novo* sequencing of fragments of 54 obesity candidate genes in pig (a partial list is given in Table 1) was carried out on fragments amplified from a panel of 10-12 pigs belonging to different breeds and including four pools of DNA constituted each by 5 equimolar genomic DNA samples obtained from Italian Large White pigs with extreme divergent values for back fat thickness (BFT) estimated breeding values (EBV; 2 pools with low EBV and 2 pools with high EBV) (see below). PCR primers were designed on porcine sequences identified through BLAST analysis using homologous human cDNA sequences on nr/nt, HTGS, gss, est_others, dbsts GenBank sections or trace archive repositories (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The selective genotyping approach was based on: 1) 100 Italian Large White pigs with extreme EBV for BFT (50 with the most negative and 50 with the most positive values), selected among 3,591 sib-tested animals of this breed; 2) 100 Italian Duroc pigs with extreme EBV for visible intermuscular fat (VIF; 50 with the most negative and 50 with the most positive values), selected among 1,225 sib-tested Italian pigs of this breed. Genotyping of these two groups of pigs was obtained by PCR-RFLP or by high resolution melting analysis for the *IGF2* intron3-g.3072G>A polymorphism (Van Laere *et al.*, 2003), and for 3 SNPs in the *PCSK1* gene and one SNP in the *TBC1D1* gene, identified by resequencing. These latter genes were selected because some mutations in the corresponding human genes are associated with obesity (Benzinou *et al*., 2008; Meyre *et al.*, 2008).

Results and conclusions - As human obesity is becoming a common plague, a large number of studies, having as main objective the identification and analysis of genes affecting this phenotype, have been exponentially issued since the last few years. In addition, polymorphisms in about 90 different genes have shown association with fat deposition traits in pigs. Partial overlapping among different sources of "obesity related" genes clearly supports the usefulness of a combined approach between human and pig and the use of more databases to identify potential candidate genes (Figure 1).

Figure 1. BioVenn diagram showing overlapping of different groups of "obesity related" genes.



Mining about 3.3 million of porcine ESTs using as probes a core of 249 human obesity associated genes, we constructed 598 contigs and identified 621 SNPs. These SNPs, together with SNPs identified by resequencing or *de-novo* sequencing (see Table 1 for a partial list), will be used for genotyping the pigs in the tails of the EBV distributions for fat deposition traits.

The *IGF2* intron3-g.3072G>A polymorphism, underlying an imprinted QTL with strong effects on muscle mass and fat deposition in pig, was used to test the feasibility of the selective genotyping approach based on BFT and VIF EBV. Difference of al-

lele frequencies between the two extreme BFT groups of Italian Large White pigs was highly significant (two tailed Fisher exact test, $P<0.000001$) confirming, on one hand the effect of this marker on fat deposition and, on the other hand, indicating that the selection criteria used in the genotyping is useful in reducing the cost of the experiment, maintaining a high power in detecting associations between DNA markers and quantitative traits. The Italian Duroc pigs selected for VIF were fixed for allele *IGF2* intron3-g.3072A. Differences of allele frequencies for one *PCSK1* SNP and one *TBC1D1* polymorphism were significant (two tailed Fisher exact test, $P<0.05$) between the two BFT and the two VIF selected groups. These results indicated that a human-pig comparative approach can be successful in choosing candidate genes, as also reported for a common obesity associated gene (*FTO*), for which we already showed its association with fat deposition traits in Italian heavy pigs (Fontanesi *et al*., 2009). From the results presented in pig, it could be expected that an inverse comparative approach (pig-human), can identify new genes affecting human obesity.

Table 1. Partial list of SNPs identified by sequencing of porcine gene fragments.

Gene symbol	Gene name	N. of SNPs
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	2
ACVR2B	activin receptor IIB	3
ADRB2	adrenergic receptor, beta 2	1
ADRB3	adrenergic receptor, beta 3	2
CNR1	endocannabinoid receptor 1	4
CTSK	cathepsin K	1
FTO	fat mass and obesity associated	7
MKKS	McKusick-Kaufman syndrome protein	2
PCSK1[1]	proprotein convertase subtilisin/kexin type 1	10
TBC1D1[1]	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1	5

[1]*Markers in these genes were genotyped in the extreme divergent groups of pigs.*

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