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SNP Haplotype Map Defining Growth QTL On SSC1

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Abstract

Haplotype based methods utilizing SNPs offer a powerful approach to map polygenic traits based on the association between causal mutations and the ancestral haplotypes on which they arose. Previously we have identified a QTL on the telomeric region of SSC1q contributing to growth in pigs from birth to slaughter (Paszak *et al.,* 1999; 2001). To maximally fine map genetic intervals containing QTL, we generated a pooled shotgun sub-library targeting the growth QTL defined by *SW1301* and *SW373* with an interval of 21 cM. Nine BAC clones mapped by utilizing a radiation hybrid panel and evenly spaced in this region, were used to generate a pooled shotgun sub-library. Skim sequencing of the pooled BAC sub-library was performed and the shotgun sequences were masked for repetitive elements and subjected to BLAST analysis for similarity to human genome sequences of HSA9. Sequences with significant BLAST hits were then used for SNP discovery. We were able to identify 145 SNP markers and 7 insertions/deletions using a panel of DNA from eight diversified pig breeds (Yorkshire, Chinese Meishan, Berkshire, Duroc, Hampshire, Landrace, Large White and Pietrain). Those SNPs that were heterozygous for the majority of the UIUC Resource Family F1 individuals are being used to genotype using a commercial population by a high-throughput SNP genotyping platform for use in linkage/linkage disequilibrium analyses. A positional candidate gene transforming growth factor receptor type 1 *(TGFBR1)* within the growth QTL region on SSC1q was isolated and characterized. Twenty SNP markers, 5 insertions/deletions and 10 microsatellites were identified in the porcine *TGFBR1* gene.

Introduction

In an attempt to map QTL, the University of Illinois created a divergent cross by three Chinese Meishan boars and seven domestic Yorkshire sows. Using that resource population and performing a genomic scan with 119 microsatellite markers, a putative QTL for average daily gain for all stages between birth and slaughter was detected (Paszek *et al.,* 1999; 2001). This QTL mapped between *SW373* and *SW1301* on SSC1q and accounted for 25% of the F2 phenotypic variance. A number of independent studies have confirmed the presence of a QTL on SSC1 that contributes to growth (average daily gain), development (number of vertebrates) and caracass traits (backfat thickness).

Within the growth QTL region on SSC1q, *TGFBR1* a gene physiologically essential for normal growth and development of different organ systems, was chosen as a candidate gene affecting growth and carcass traits in pigs. The TGF-β superfamily comprises a large and diverse group of polypeptide morphogens, that include the prototype of the family-the TGF-β themselves, bone morphogenic proteins, and myostatin which is an inhibitor of skeletal muscle growth. Mutations in human *TGFBR1* and *TGFBR2* genes are linked to inherited disorders in cardiovascular, craniofacial, neurocogitive and skeletal development (Loeys *et al.,* 2005).

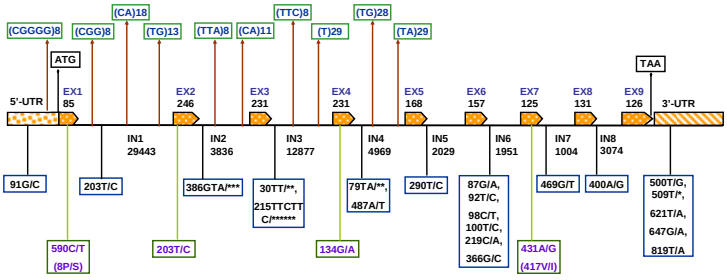
Objectives

The objectives of this project are (I) creating high resolution physical maps of the QTL region; (II) creating SNP haplotype maps for making linkage disequilibrium analysis of the QTL region; (III) determining LD for SNPs defining QTL region in commercial populations; (IV) evaluating positional candidate genes within the growth QTL region on SSC1q.

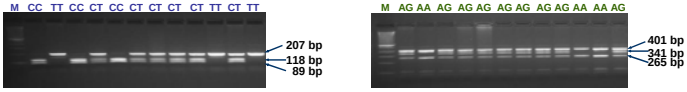
Materials and Methods

Nine BAC clones were selected from the RPCI-44 porcine BAC library (http://bacpac.chori.org/mporcine44.htm) to generate a pooled shotgun sub-library (Invitrogen). Skim sequencing of the pooled BAC sub-library was performed using ABI 3730 automated DNA sequencer. Shotgun sequences were assembled by phred/phrap/consed (http://www.phrap.org/phredphrapconsed.html). All sequences were trimmed for vectors and *E.coli* DNA sequences. After quality assessment (Q>20), sequences were masked for repetitive elements (http://www.repeatmasker.org/), and subjected to BLAST analysis for similarity to human genome sequences of HSA9 using NCBI-BLASTn. An expectation value (E) of e^{-5} was used as the threshold. Primers were designed using primer3 (http://frodo.wi.mit.edu /cgi-bin/primer3/primer3 _www.cgi). SNP discovery was performed by directly sequencing approach, and sequence comparison was done by Phrap and Gap4 integration (http://staden.sourceforge.net/phrap.html).

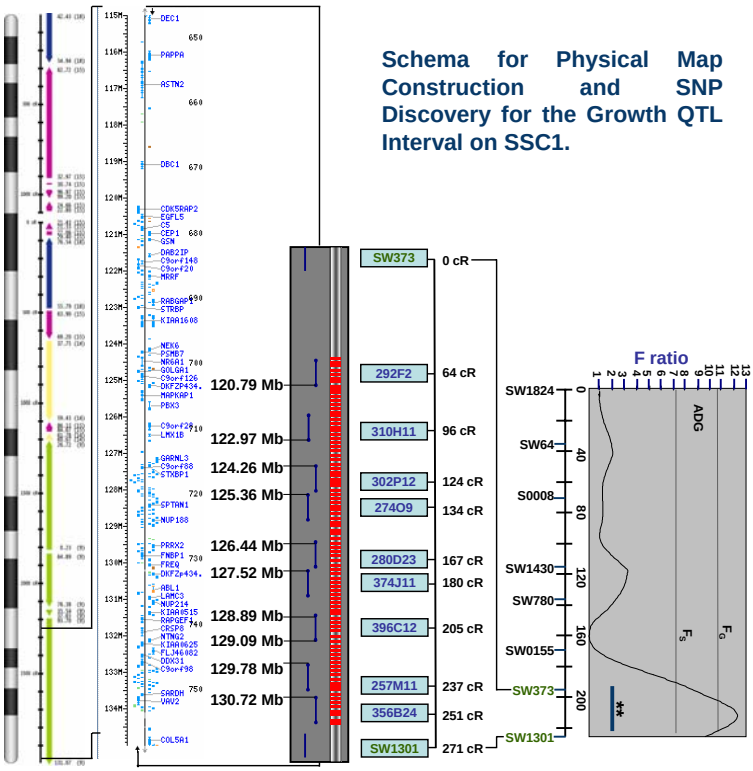
Positional Candidate Gene *TGFBR1*



Genomic Structure and SNPs/Microsatellites Identified in the Porcine *TGFBR1* Gene.



PCR-RFLP Assays for *TGFBR1* SNPs [Left: PCR-*BSP1286I*-RFLP genotyping of SNP at exon1 (590C/T), C=89 +118 bp, T=207 bp; Right: PCR-*HinfI*-RFLP genotyping of SNP at intron 6 (87G/A), A=401 + 265 bp, G=341 + 265 bp; M is 100 bp ladder.].



Schema for Physical Map Construction and SNP Discovery for the Growth QTL Interval on SSC1.

A. Represents porcine SSC1q cytogenetic map.
B. Comparative human-pig map showing HSA9 homologue (olive green) for telomeric region of SSC1q (Meyers *et al.,* 2005).
C. HSA9 physical map (115 Mb to 134 Mb) and annotated genes corresponding to QTL interval.
D. Depicts RPCI-44 BAC clones selected for SNP discovery. Distances in human Mb as defined by BAC end-sequence blasts against Human Draft 34 (http://www.sanger.ac.uk/Projects/S_scrofa/mapping.shtml).
E. Red bars indicate location of selected SNPs for use in the initial low resolution probe set.
F. RH map location (cR) of selected BACs as defined using IMpRH7000 panel.
G. Location of microsatellites (cM) used to define the original QTL interval (Paszek *et al.,* 1999).
H. QTL map showing average daily gain QTL at genome-wide significance.

Results and Disscusion

145 SNP markers and 7 insertions/deletions using a panel of DNA from eight diversified pig breeds (Yorkshire, Chinese Meishan, Berkshire, Duroc, Hampshire, Landrace, Large White and Pietrain) were developed for fine mapping the growth QTL on SSC1. Those SNPs heterozygous for the majority of the UIUC Resource Family F1 individuals are being used to genotype a commercial population by a high-throughput SNP genotyping platform for use in linkage/linkage disequilibrium analyses. The porcine *TGFBR1* gene was isolated and genomic structure was characterized. Twenty SNP markers, 5 insertion/deletions and 10 microsatellites were identified in the porcine *TGFBR1* gene.

References

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