Allelic to Genome Wide Perspectives of Swine Genetic Variation to Litter Size and Its Component Traits

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Abstract

Litter size is a complex and sex limited trait that depends on various biological, managemental and environmental factors. Owing to its low heritability it is inefficaciously selected by traditional methods. However, due to higher heritability of ovulation rate and embryo survival, selection based on component traits of litter size is advocated. QTL analysis and candidate gene approach are among the various supplementary/alternate strategies for selection of litter size. QTL analysis is aimed at identifying genomic regions affecting trait of interest significantly. Candidate gene approach necessitates identification of genes potentially affecting the trait. There are various genes that significantly affect litter size and its component traits viz. ESR, LEP, BF, IGFBP, RBP4, PRLR, CTNNAL1, WNT10B, TCF12, DAZ, and RNF4. These genes affect litter size in a complex interacting manner. Lately, genome wide association study (GWAS) have been utilized to unveil the genetic and biological background of litter traits, and elucidate the genes governing litter size. Favorable SNPs in these genes have been identified and offers a scope for inclusion in selection programs thereby increasing breeding efficiency and profit in pigs.

1. Introduction

Livestock sector has been an integral part of human civilization, fulfilling the cultural, economical and nutritional needs of man. The demand for livestock products are ever expanding being driven by population growth, urbanization and increasing incomes. The intensively raised livestock in the developed countries are the main source of the world's poultry and pig meat production, and such systems are being established in developing countries, particularly in Asia, to meet increasing demand. In particular factory farm based swine operations have huge scope for fulfilling the global meat demand. Profit gained from the swine industry is immediately reflected via improvement of reproductive traits enhancing efficiency of production. However, reproductive traits are complex traits exhibiting low heritability and strong heterosis thereby limiting the efficacy of selection [1]. Among various reproductive traits, litter size is of prime interest to pig producers and breeders and is the most important economic trait. Realizing its extreme importance, selection of replacement gilts having potential for larger litters has proven beneficial to the swine industry [2].

Litter size- a complex and sex limited metric trait is measured as the number of alive piglets born or total piglets born. Prior knowledge regarding determinants of litter size and their interaction is required to facilitate litter size improvement. Litter size depends on various factors such as rate of ovulation, rate of fetal survival, uterine capacity, management and environmental conditions as well as genetics [3]. However primary factors influencing litter size, also known to be the main components of the trait include rate of ovulation, fertilization, survival of embryo and fetus [4]. The average degree of embryonic losses has been documented as 20–30% whereas fetal losses as 10–20% [5]. Though the chance of occurrence of these losses is at any stage, the principal factor limiting porcine litter size is pre-implantation embryonic losses. Owing to its low heritability and being influenced by various biological factors that are difficult to intensely estimate, litter size is inefficaciously selected by conventional schemes and hence genetic improvement by marker assisted selection and genomic selection are proposed as a

complimentary tools in order to amplify the economic performance of piggery. Furthermore, advancement in pig industry through the use of improved feeding and housing conditions has reached a limit therefore genetic improvement of economic traits via selection is of prime importance [6].

Marker assisted selection that allows division of litter size into its component traits can be utilized for greater progress as it optimizes different physiological mechanisms affecting litter size [7]. This has been further proven by selection experiments which produced insignificant response for increased litter size. A study on selection for ten generations found absence of response [8]. The reasons for failure of these experiments are multi causal such as maternal effect, inbreeding, management issues, small population size and within family selection. However, owing to comparatively higher heritability of ovulation rate and embryo survival, selection based on components of litter size is encouraged. Also index selection for viable embryo and litter size is more efficacious in improvement of litter size than single trait selection. Alternatively, litter size may be measured as minimum number of embryos allowed by uterine space or minimum viable embryos. Basis of this method was laid by measurement of uterine space via surgical procedures.

Genetic variability which exists for various reproductive traits is prerequisite for selection. However, choice of selection program should also involve keen observation of the predicted outcome. For instance, if selection is undertaken for increased ovulation rate then it would detrimentally affect early embryonic survivability. Likewise, selection for improved litter size would affect the birth weight. Additionally, uterine overcrowding 44 days post fertilization affects development of placenta along with fetal and post natal development [9]. Presence of genetic variability within breed is indicative of possible genetic improvement of a particular trait. Rate of improvement can be increased if genes that are responsible for physiology and biochemistry of the trait are manipulated directly. Since most of the genetic control is evident at the level of gene therefore gross chromosomal abnormalities severely affect reproduction. However, incidence of such abnormalities is relatively rare. In this review, studies in the domain of swine genetic variation to litter size and its component traits at allelic and genome wide level have been thoroughly reviewed. Further comparison of strategies for genetic improvement has been surveyed along with understanding allelic to genome wide perspectives. However, for wider application various strategies need to be merged across varied breeds and lines. In addition, advanced tools should be incorporated for profound understanding of genetics of reproductive traits in swine.

2. Association Of Litter Size With Sow And Piglet Characteristics

Litter size is positively associated with postnatal survival, however probability of stillbirth increases with abnormally large or small litters [10]. Significant association also occurs between farrowing duration and number of stillbirth. Longer farrowing for over three hours leads to asphyxia due to rupture of umbilical cord or placental detachment. Risk of hypoxia and farrowing time also increases with large litters whereas in small litters, the oversized piglets are subjected to greater difficulties in gestation or farrowing and their birth is blocked leading to hypoxia and death. The most crucial factors involved in survival of piglet from birth to weaning are individual and relative birth weight. It has been reported that male piglets

have higher incidence of stillbirth and lower birth weight [11]. In order to decrease stillbirth, increase in individual birth weight was recommended. Additionally, selecting individuals with higher birth weight did not increased pre weaning survival rate indicating the need for balanced selection.

3. Strategies For Improvement Of Porcine Reproductive Traits

Improvement of reproductive traits has gained much importance in view of the fact that moderate increase results in large profits especially in swine. This improvement is made through selection which is routinely based on performance of the animal but the procedure is tedious and expensive. These lacunae can be filled with the cohesive use of marker assisted selection, resulting in a faster rate of change and hence improvement. Association of genes with trait of interest has been unveiled with advent of DNA technologies thereby necessitating designing of selection methodologies based on information obtained from DNA. Genes influencing these traits are determined by employing following two different strategies:

1. Linkage analysis wherein genomic regions accommodating the concerned genes are scanned to detect quantitative trait loci.

2. Candidate gene approach to identify polymorphism in genes causing phenotypic variation through their physiological and biochemical role or their location in genome, linked with the trait or with differentially expressed genes for the trait. It is pursued as direct evaluation.

3.1 QTL ANALYSIS

Mapping of genomic regions in linkage analysis is based on co-segregation of marker alleles with phenotypic traits. These genomic regions which are linked to polygenic phenotypic traits are known quantitative trait loci (QTL). Microsatellites owing to their high polymorphism are usually employed in QTL analysis as an indirect strategy. QTL analysis is costly and time taking, since it requires at least three generations. Also they are comparatively difficult to use in MAS as they often span more than 10-20 cM hence fine mapping of QTL is necessary **[6].** However, increase in number of alleles and uniform distribution in the population, enhances information content of markers. QTL analysis is pursued with the aim of identifying genomic region significantly affecting a desirable trait. Crosses between Landrace, White composite, Large White Yorkshire and Chinese Meishan have been frequently utilized as resource population in order to undertake genomic scan. However the number of individual animals measured in the analysis was low than that of mice or in few candidate gene analyses primarily because of longer generation turnover in pigs and because genotyping for numerous markers has to be performed unlike in candidate gene approach wherein few genes are studied.

Owing to the fact that several genes contribute for only limited magnitude of phenotypic variance in fecundity parameters and the QTL regions are wide enough to pose difficulty in defining the gene effect, no gene with a causative mutation has been detected in pigs through linkage analysis. Moreover, standardization of environmental influences for longer duration is difficult. Therefore fine mapping at early stage of QTLs should be advocated for economic traits and must be extended in future.

3.2 CANDIDATE GENE APPROACH

Characterization and genotyping of candidate gene is done by using intragenic DNA variants. These variants differ in single base pair and referred as SNPs which are usually biallelic resulting in three distinct genotypes. The effect of candidate gene is evident by significant linkage disequilibrium studies in large population. To validate the significant role of candidate gene, results are tested in various populations of a breed or even on several breeds. Sometimes, there may be inconsistency of results which does not essentially indicate that gene is not potential candidate for the respective trait. Rather discrepancy may arise due to different allelic and genotypic frequency, epistatic effects or different linkage phases between marker and causal mutation.

Prior knowledge of gene having probable role in target trait is necessary in candidate gene analysis. This is an attractive approach for animal breeders as positive results can be readily applied. Inconsistency of genetic effects across varied population and breeds poses limitation to its use. Before application of the approach, various key points have to be taken into account such as polymorphisms in candidate genes are intragenic markers rather than causative mutation therefore the recombination and mutation events may not strictly be as with causal mutations. Furthermore, low allelic frequency of polymorphism results in insignificant result. With these approaches, successful identification of genes influencing economic traits has been carried out with ESR gene being earliest proof of association between gene and litter size.

Mapped porcine genes are proportionately small, limiting the total positional candidate genes. Principally every animal can be studied from any population and hence feasible genotyping of candidate genes for particular trait by various research groups is possible. Testing of gene variants in various populations is pre-requisite in candidate gene strategy to detect its general effects. However, variation in resource population and number of individuals in different studies has been witnessed, some studies using reference families while some using commercial pig population. Owing to differences in housing, feeding and other management conditions, results are difficult to compare. The approach can be directly employed when gene has appreciable effect on physiological trait and is known in advance (physiological candidate), or the gene is distinctly located in QTL region (positional candidate) or influences the trait in other species (comparative candidate gene). Since then various QTL and candidate gene analysis have been performed to find regions or genes affecting reproductive traits.

Economic traits are influenced by genes via two possible pathways **[6]:**

1. Type of encoded protein may be changed due to mutation in the coding region of particular gene. 2. Amount of gene transcripts may alter in the cell, changing the quantity of functional protein due to mutation in regulatory region of particular gene.

These two gene effects cannot be differentiated with linkage and association studies because both pathways lead to appreciable phenotypic changes. Nevertheless, major phenotypic changes are often caused by change in structure of primary protein which results in absence of functional protein whereas

minor changes are expected due to mutation in regulatory regions and neutral substitution of amino acids.

4. Candidate Genes For Litter Size In Pigs:

4.1 ESTROGEN RECEPTORS (ESR)

Steroid hormones such as estrogen play a central role in the postnatal female physiology and their effects are exerted through its receptors. The function of the estrogen hormone is mediated by binding with its receptors namely ESR1 and ESR2, which have cooperative action as heterodimers. Knockout mouse model technique has been utilized to infer the functional similarity of both receptors [12]. The estrogen receptor gene (ESR) located on the pig chromosome 1 [13] is one of the most extensively investigated candidate genes for litter size traits in pigs. Embryotrophic role of the gene is considered crucial for growth and maturation of ovarian follicles and embryo as well as peri-implantation development [14]. Loci coding for ESR gene affects the litter size of sows and their specific genotypes show additive effect with the same hence it is considered as candidate gene for prolificacy traits.

Different studies have been conducted in purebred, crossbred and hybrid sows concerning litter size traits, especially total number born (TNB) and number born alive (NBA). In a study conducted on Meishan breed, a PvuII polymorphism of the gene was significantly associated with litter size [15]. Contrarily, in Chinese-European pigs non- significant association of PvuII polymorphism with litter size but significant association with TNB was observed [16]. Also, in Italian Large White population, genetic variation of the gene was not observed and hence was not associated with litter size in the target breed [17]. In another study, a polymorphism of ESR2 at telomere of q arm of SCC1 was identified and no significant association with litter size of Iberian pig population was found [13]. In a study on population of autochthonous pigs, no significant difference was observed for ESR2 loci. Nevertheless, BB genotype showing an increment of 2 piglets per litter was noticeably superior ($p < 0.001$) to AA genotype [18]. Despite the variable results obtained in different findings due to difference in sample size and target population, the gene was found to be associated with litter size traits and therefore is a potential candidate gene for litter size in pigs.

4.2 LEPTIN (LEP)

Leptin is a 16 KDa polypeptide hormone encoded by LEP gene. It is secreted by white adipocytes and implicated in the regulation of reproductive functions in conjunction with feed consumption and energy homeostasis [19]. During puberty, the concentration of the circulating leptin hormone increases reaching a threshold that activates reproductive axis. Hence, it acts as a metabolic gateway for puberty [20]. The porcine leptin gene has been localized on chromosome 18 [21] and consists of three exons and seven single base polymorphism [22]. It interacts with its receptor LEPR mapped on chromosome 6 to mediate its effect at hypothalamic level [23]. High degree sequence homology of porcine LEPR genes with that of human and mice have been utilized in primer designing [24].

Gene polymorphism has been studied in different population to study its effect on litter size and its component traits. In a study conducted on cross of Polish Large White and Landrace sows, two distinct alleles of the LEP gene were recognized. However, non-significant differences for litter size traits were observed between animals of different genotypes [25]. Weak association between leptin SNP and mummified fetus in four line composite pig population was also reported [26]. In Large White population, LEP polymorphism was significantly associated with total litter size at birth and total litter size born alive [27]. Similar significant effect was also observed in Luchuan pigs [28]. Polymorphism in LEPR gene was also studied in Landrace, Yorkshire and Duroc breeds. However, association was evident between polymorphism in intron 2, exon 2 and exon 18 with litter size in two breeds namely Yorkshire and Duroc [24]. Another study in Suzhong sows revealed the beneficial effect of T allele of LEPR locus on litter size and litter weight as genotype TT had greater TNB and NBA as compared to sows with genotype CC [29]. These studies indicated the potential use of leptin gene and it's receptor in marker assisted selection.

4.3 PROPERDIN (BF)

Properdin – a single chain glycoprotein of 93 kDa is encoded by complement factor B (BF) gene. Porcine BF gene has been mapped within dense gene cluster (MHC) harboring genes regulating reproductive physiology and is located on centromeric region of chromosome 7 [30]. The gene plays vital role in growth of uterine epithelium which is crucial in establishment and maintenance of pregnancy [31]. The first RFLP of the porcine BF gene was demonstrated using SmaI restriction enzyme [32].

Various investigations have been undertaken which showed significant association of the gene with components of litter size i.e. total number born and number born alive. However, noticeable association was evident in multiparous sows [33]. In crossbred pig population (Large White X Landrace X Leicoma), allele B of the gene was found favorable as sows with BB genotype had higher TNB and NBA as compared to other genotypes [34]. Similar results were observed in commercial pigs in Greece where TNB and NBA showed significant statistical difference ($p < 0.05$) from 2nd to 5th birth (high productive period) and BB sows had higher TNB and NBA per parity than AB sows [35]. However, when all births were examined, significance level was insufficient even though BB sows had higher litter size. Furthermore, the concept of removal of sows from the breeding herd once they reach 5th parity or attain 30 months age was supported by the finding that litter size decreased 5th parity onwards. In another population of autochthonous Greek breed higher beneficial effect of the BB genotype was observed for TNB and NBA traits compared to the other two genotypes [18]. Owing to its significant effect, the gene may be considered as a candidate gene for litter traits in pigs.

4.4 INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN (IGFBP)

Fertility is regulated by interaction of various reproductive organs, among which uterus plays primary role in implantation and fetal growth by expressing numerous proteins. One such protein is IGFBP which participates in reproductive physiology by regulating ovulation, implantation, fetal development and pregnancy maintenance [36]. IGFBP is a family of six binding proteins that are potent modulators of IGF1

and IGF2. They are produced in ovarian follicles as signaling molecules and regulate growth and differentiation of endometrial cells [37]. During follicular growth, IGFs play key role in sensitizing the granulosa cells to FSH in addition to regulating proliferation, survival and migration of trophoectoderm during early gestation [38]. The mRNA level of IGFBP2 is linked with implantation and varies during different stages of endometrium secretion. During development of placenta, mRNA expression of the gene is significantly increased and the action of IGF is modulated thereby controlling embryonic development. Gene up-regulation was witnessed in endometrium on 14th day of gestation in pregnant mares and sows indicating its vital role in endometrium. Another most abundant member of IGFBP family i.e. IGFBP3 regulates the biological activity of IGF-1 in mammalian reproduction.

Considering crucial role of the genes of IGFBP family in reproductive physiology, various studies were undertaken. In a study on Berkshire pigs, SNP was identified in intronic regions of IGFBP2 and IGFBP3. The SNP in IGFBP2 was found to affect its own mRNA expression. Further it was witnessed that animals with AT genotype had largest TNB and NBA while TT genotype had highest transcript level, additionally suggesting nil correlation between the two traits. For IGFBP3 gene, GG genotype exhibited highest litter size traits [39]. Similar study on Finnish Landrace sows suggested that animals with minor allele for IGFBP1 and IGFBP2 gene had positive effects on litter size traits [40]. In a composite population of Landrace-Duroc-Yorkshire, the IGFBP3 gene significantly affected estrus interval and stillborn births [26] thereby regulating the litter size. These findings proposed that SNPs in IGFBP gene family could be utilized as biomarkers for litter size in pigs.

4.5 RETINOL BINDING PROTEIN 4 (RBP4)

RBP4 is a major component of uterine histotroph and secretory product of pig conceptus that plays key role in transportation of vitamin A to the developing embryo [41]. Vitamin A in turn have numerous effects with respect to establishment and maintenance of pregnancy as well as embryonic development. Porcine RBP4 gene has been mapped on chromosome 14 [42] and is suggested to be a strong candidate gene for litter size in pigs as supplementing pregnant sows with vitamin A significantly increases litter size [43].

Various studies have been undertaken to investigate the association of gene with litter size owing to its increased production during the critical stage of blastocyst elongation [44] as well as its role in reproductive physiology. In an investigation pertaining to Polish sows, the gene showed polymorphism and was found to be significantly associated with component traits of litter size such as TNB, NBA and number of piglets weaned. Sows with BB genotype showed large litter size as compared to AA and AB genotype. The difference was statistically significant in first parity [45]. However in Tibet pigs, sows with AA genotype had significantly larger TNB and NBA as compared to BB and AB genotype [46]. Similar results were obtained in commercial crossbred from Large White X Landrace sows [47] and in hyper prolific Landrace X Large White sows where animals with AA genotypes had higher TNB [48]. In Autochthonous pig population, sows with genotype AB showed largest TNB and NBA as compared to other genotypes [18]. As the gene was significantly associated with litter size in all these findings, therefore it can be used for selecting sows with large litter size.

4.6 PROLACTIN RECEPTOR (PRL)

PRLR was first cloned from rat liver comprising 310 amino acids and later from the ovary of same species comprising 610 amino acids. Multiple isoforms of the gene in the findings are indicative of alternate splicing [49]. Porcine PRLR has been located on SSC16 and encodes receptor for prolactin hormone [50]. The hormone plays vital role in reproduction and lactation as attested by large body of literature.

Various studies have been undertaken to study the association of gene with litter size traits in pigs. The gene was significantly associated with reproductive attributes in various breeds such as Large White, Duroc, Landrace, Chinese Meishan and Pietrain [51]; Landrace, Meishan and Large White synthetics [50]; and crossbred gilts of Meishan and Large White [52]. A study on crossbred pigs (Large White and Landrace) demonstrated that in first parity, AA genotype sows had significantly largest and BB genotype smallest litter size. However in later parities, though the results were same but the difference between genotype was not significant. This may be due to large residual variance in later parities [25]. Nevertheless, difference can be proven to be significant by increasing the sample size. Similar studies were conducted in Large White [53], crossbred (Large White and Meishan) population [52] and Beijing Black pig population [54]. In these findings, frequency for favorable allele A was similar. However, in a study lower frequency in Landrace and a higher frequency in Duroc was observed [55]. Study on synthetic Large White line showed that sows with AA genotype had 0.66 more NBA than others while in Landrace synthetics AA sows had one more TNB and NBA than BB sows. However, in Meishan synthetics, these parameters were highest for sows belonging to AB genotype [50]. These studies suggested evident effect of PRL gene on improved litter size.

4.7 CTNNAL1, WNT10B AND TCF12

WNT signaling is complex interactive pathway comprising of proteins, receptors and other regulatory elements. The pathway has clear and crucial role in embryonic development, implantation and various other reproductive processes such as formation of ovarian follicles, ovulation, maintenance of normal pregnancy and lactation [56, 57]. Catenin alpha-like protein 1 (abbreviated as CTNNAL1), wingless-type MMTV integration site family member 10B (abbreviated as WNT10B) and transcription factor 12 (abbreviated as TCF12) are among the various pathway genes. Down regulation of CTNNAL1 gene in placental tissues of women suffering from pre-eclampsia, hemolysis, elevated liver enzymes and low platelets or HELLP syndrome indicated the role of gene in maintaining normal pregnancy [58]. Up regulation of WNT10B was evident in endometrium of horse in late diestrus period [59], indicating its effect on early embryonic development along with maintenance of pregnancy. The gene was also expressed in murine blastocyst [60]. TCF12 played vital role in oogenesis and sex determination in drosophila [61]. Differential expression of these pathway genes was witnessed in Chinese Taihu and Large White sows [62].

Owing to the observed expression pattern and close proximity of these genes with QTLs for TNB and NBA, they are regarded candidates for litter size. Association analysis of TCF12 gene was conducted on

Large White pigs. Animals with GG genotype had more NBA in first parity than other genotypes. In Large White pigs, for CTNNAL1 at locus c.1878 G > C, CC homozygotes had 1.14 more alive piglets per litter than GG. However, in Chinese DIV difference between CC and GG sows were 2.07 and 2.62 pigs per litter for NBA and TNB respectively. For WNT10B gene, TC gilts had lower NBA than other genotypes of Large White [63]. These findings suggest that these genes are potential markers for pig selection and breeding.

4.8 DELETED IN AZOOSPERMIA LIKE (DAZL)

DAZL is one of the members of DAZ gene family that comprises of three genes namely DAZ, DAZL and BOULE. The genes of the family encode proteins that show germ cell specific expression pattern regulating development and differentiation of germ cells [64]. This has been validated via disruption of DZAL gene in adult mice that resulted in complete loss of germ cells and follicles in ovary [65]. Additionally the gene has been found to be expressed throughout human peri-implantation period suggesting its crucial role in implantation as well as embryo survival [64]. Porcine DAZL gene has been mapped on SSC13 and is located near QTL for ovulation rate [66] and stillborn birth [67].

Owing to its role in germ cell development, embryo survival and other reproductive processes, role of the gene as potential marker for litter traits was realized and association studies were conducted. In one such study on Italian Large White pigs, the gene was non- segregating [68]. However in another finding, A/G mutation in intron 7 and a C/A mutation in intron 9 of the gene was observed. This study reported that in DIV pig line during first parity, the BB pigs varied from AB with dominance effect for NBA as 0.06 pigs per litter. However, for SNP C/A (intron 9) in Large White CC genotype had 0.716 more pigs per litter than CD for NBA in all parities. Additionally, in DIV pig line animals with CC genotype had 1.940 and 2.017 more NBA than DD and CD respectively during first parity whereas CC differed from genotype CD in DIV line in all parities [69]. Linkage disequilibrium of the C/A mutation with QTL affecting reproductive processes indicated the role of gene as potential marker for improved litter size.

4.9 RING FINGER PROTEIN 4 (RNF4)

RNF4 gene encodes for ring finger protein that was originally termed as SNURF (small nuclear ring finger protein) in rats. It co-regulates the transcription via steroid receptor dependent and independent promoters [70]. Overexpression of this protein enhances the transcription of various other steroid receptors such as estrogen and progesterone [71]. Additionally, the protein coordinates activities of numerous transcriptional signals and acts as a bridging factor. Spatial and temporal expression analysis of SNURF mRNA revealed that the gene was expressed significantly in oocytes in stage-dependent manner and participated in folliculogenesis. Additionally it regulated the development of fetal germ cells along with maturation of oocytes and granulosa cells [72]. The RNF4 gene is located on SSC8 and QTL influencing ovulation rate has also been identified within p arm of SSC8 [73].

Owing to its role in reproductive physiology, association analysis of the gene with litter size traits was conducted in pigs. One such study was undertaken in three distinct population and it was observed that TT sows in Minpigs had more TNB as compared to CC sows. Similar results were observed in Qingping and Line DIV population, however the results were not significant [74]. In another study the gene showed

non-significant association with reproductive traits in Qingping pigs and Line DIV sows during first parity. However, in subsequent parities in Qingping sows, CC genotype had more TNB and NBA than sows with TT genotype [75]. These studies indicate significant yet inconsistent relationship between genotypes and litter size traits indicating that upon further validation the polymorphism in the gene can be considered a potential marker for litter size traits in pigs.

5. Genome Wide Association Study

Genetic architecture of economic traits has remained a mystifying matter for researchers since many decades. However with advent of GWAS, new hopes have been pinned. GWAS is a technical approach for mining functional genes, SNPs, QTLs and other relevant genetic information regarding traits that are controlled by many genes and gene interactions [76]. It enhances our understanding of biological relevance and genetic background of economic traits thereby facilitating efficient MAS or genomic selection for genetic improvement. Currently, genome wide studies utilize SNP Bead Chip technology rather than NGS owing to its affordability. SNP Bead chips face several disadvantages such as rigid structure and uneven marker density across genome [77]. However they are being widely used in livestock species particularly in pigs for exploration of the genetic architecture of polygenic traits [78]. In piggery sector, GWAS has been invariably applied to unveil the genetics of various economic traits such as carcass quality [79], genetic disorders [80], coat color [81] etc. Several studies based on GWAS has also been conducted for reproductive traits especially litter size that plays crucial role for economic success. However due to low heritability, polygenic inheritance, maternal and environmental effects, the conventional breeding program is challenging.

Various findings have suggested that 35,384 QTLs are associated with 716 different traits (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). Among these, 1274 QTLs are associated with litter traits [82]. In a study on two Duroc population i.e. U.S and Canadian, GWAS was conducted across two parities based on GeneSeek Porcine 50K Chip data. Total 76 SNPs related to litter traits were identified in both population. However 10 significant SNPs were determined in Canadian population. Intriguingly, 13 pleiotropic SNPs for litter traits were found SSC 7, 9, 14 and 15 [82]. GWAS on Large White and Landrace breeds have also yielded notable results. In a study, samples were genotyped using Illumina Porcine SNP60 Bead Chip and quality control of SNPs was undertaken. Further GWAS analysis revealed that in Large White, significant markers were found on SSC5 and SSC10 [83]. PPARα gene was mapped near the marker on SCC5. Expression of the gene was significantly higher in endometrial tissues during early gestation and was lower during maternal recognition of pregnancy and after implantation in Polish LR and Pietrain breed [84]. This indicated the vital role of gene during pregnancy and thereby its close proximity with the marker associated with litter trait. Another gene integrin β 1 (ITGβ1) was closely associated with marker in SSC10. The gene is reported to significantly affect litter size in Large White and Landrace [85]. However in Landrace, significant SNPs were associated on SSC7, SSC9, SSC11 and SSC16 [83]. Another finding in Landrace and Large White identified 80, 227 and 187 SNPs affecting TNB, NBA and LWB (litter weight born alive) respectively. Of these 22 loci were shared by the three component traits of litter size. In addition four candidate genes affecting litter size across six parities were also

suggested [86]. Twenty potential SNPs and several candidate genes underlying litter traits have also been identified in Duroc pigs through genome wide association study [87]. Another GWAS study in Duroc population identified 10 putative regions associated with litter traits such as NBA, number of stillborn (NS) and mummified piglets (NM). Within these regions seven candidate genes were identified. In addition it was inferred that the genome wide significant SNPs in the candidate genes identified were parity specific and therefore the effect of these genes might be temporal [88]. In Large White population, GWAS was undertaken using Porcine SNP80 bead chip and the results identified 29 significant SNPs within regions known to be associated with reproductive traits including litter size [89]. In Yorkshire pigs, five significant genes i.e. MSX1, spindlin 1 (SPIN1), VEGFA, FOXQ1, and LHFPL3 regulating TNB and NBA through different physiological pathways were identified [90]. In Bama Xiang pigs, 29 significant SNPs and 12 genes for litter size traits were identified [91].

These findings suggest that GWAS analysis advances our understanding regarding genetic mechanism of litter size traits and thereby aid in efficient selection for genetic improvement of pigs. However there are still few limitations which can be overcome by single traits meta-analysis for identifying SNPs across multiple population. This will improve the power of identification of genetic information and will provide thorough insight of litter traits to undertake selection and enhance genetic improvement.

6. Conclusion

Genetic improvement of litter size is of immense interest for pig producers owing to the fact that improvement in feeding regime and housing system is limited. Initial improvements were made in growth and carcass traits which led to constant or even decreased litter size in pigs. However, improvement in litter size is not only economically vital but also indicates breeding efficiency of pigs. Therefore, selection for litter size is greatly emphasized and carried out in most selection programs.

Litter size is sex limited trait with low heritability and phenotyping of the trait is time consuming hampering efficient improvement using conventional selection. In order to maximize profit from piggery various efforts have been made using different strategies. Marker assisted selection along with conventional selection is one such approach that is considered most effective. Notable success has been observed using candidate gene approach specifically in reproduction related markers. Several genes play biological role in improving litter size and polymorphisms in these genes serve as crucial markers for litter size selection. These genes can be identified with recent technical approach such as genome wide association studies (GWAS). Further using GWAS, genomic selection can be applied for accurate and efficient selection response.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with animals or humans performed by any of the authors.

References

1. Noguera JL, Rodriguez C, Varona L, Tomas A, Munoz G, Ramirez O, Barragan C, Arque M, Bidanel JP, Amills M, Ovilo C (2009) A bi-dimensional genome scan for prolificacy traits in pigs shows the existence of multiple epistatic QTL. BMC Genomics 10:1–12. https://doi.org/10.1186/1471-2164-10-636
2. Mills KM, Schinckel AP, Stevens JG, Casey TM, Stewart KR (2020) Evaluation of on farm indicators of gilt reproductive performance potential at 21 days of age. Translational Anim Sci 4:210. https://doi.org/10.1093/tas/txaa210
3. Sanchez-Davila F, Bernal-Barragan H, Padilla-Rivas G, Del Bosque-González AS, Vázquez-Armijo JF, Ledezma-Torres RA (2015) Environmental factors and ram influence litter size, birth, and weaning weight in Saint Croix hair sheep under semi-arid conditions in Mexico. Trop Anim Health Prod 47:825–831. https://doi.org/10.1007/s11250-015-0795-6
4. Argente MJ (2016) Major components in limiting litter size. In: Payan Carreira R (ed) Insights from Animal Reproduction, London, UK, pp 87–114. http://dx.doi.org/10.5772/62280
5. Pope WF (1994) Embryonic mortality in swine. Embryonic Mortal Domest species 53:77
6. Buske B, Sternstein I, Brockmann G (2006) QTL and candidate genes for fecundity in sows. Anim Reprod Sci 95:167–183. https://doi.org/10.1016/j.anireprosci.2005.12.015
7. Distl O (2007) Mechanisms of regulation of litter size in pigs on the genome level. Reprod Domest Anim 42:10–16. https://doi.org/10.1111/j.1439-0531.2007.00887.x
8. Bolet G, Ollivier L, Dando P (1989) Selection for prolificacy in the pig. I. Results of an eleven-generation selection experiment. Genet Selection Evol 21:93–106. https://doi.org/10.1186/1297-9686-21-1-93
9. Town SC, Putman CT, Turchinsky NJ, Dixon WT, Foxcroft GR (2004) Number of conceptuses in utero affects porcine fetal muscle development. Reproduction 128:443–454. https://doi.org/10.1530/rep.1.00069
10. Vanderhaeghe C, Dewulf J, de Kruif A, Maes D (2013) Non-infectious factors associated with stillbirth in pigs: a review. Anim Reprod Sci 139:76–88. https://doi.org/10.1016/j.anireprosci.2013.03.007
11. Canario L, Cantoni ELBE, Le Bihan E, Caritez JC, Billon Y, Bidanel JP, Foulley JL (2006) Between-breed variability of stillbirth and its relationship with sow and piglet characteristics. J Anim Sci 84:3185–3196. https://doi.org/10.2527/jas.2005-775
12. Couse JF, Korach K (1999) Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 20:358–417. https://doi.org/10.1210/edrv.20.3.0370

13. Munoz G, Ovilo C, Amills M, Rodriguez C (2004) Mapping of the porcine oestrogen receptor 2 gene and association study with litter size in Iberian pigs. Anim Genet 35:242–244. https://doi.org/10.1111/j.1365-2052.2004.01141.x
14. Kowalski AA, Graddy LG, Vale-Cruz DS, Choi I, Katzenellenbogen BS, Simmen FA, Simmen RC (2002) Molecular cloning of porcine estrogen receptor-β complementary DNAs and developmental expression in peri implantation embryos. Biol Reprod 66:760–769. https://doi.org/10.1095/biolreprod66.3.760
15. Rothschild F (1996) Genetics and reproduction in the pig. Reprod Livest Sci 42:143–151. https://doi.org/10.1016/0378-4320(96)01486-8
16. Muñoz G, Ovilo C, Estellé J, Silió L, Fernández A, Rodriguez C (2007) Association with litter size of new polymorphisms on ESR1 and ESR2 genes in a Chinese-European pig line. Genet Selection Evol 39:1–12. https://doi.org/10.1186/1297-9686-39-2-195
17. Dall'Olio S, Fontanesi L, Tognazzi L, Buttazzoni L, Gallo M, Russo V (2011) ESR1 and ESR2 gene markers are not associated with number of piglets born alive in Italian Large White sows. Italian J Anim Sci 10:35. https://doi.org/10.4081/ijas.2011.e35
18. Laliotis GP, Marantidis A, Avdi M (2017) Association of BF, RBP4, and ESR2 genotypes with litter size in an autochthonous pig population. Animal Biotechnol 28:138–143. https://doi.org/10.1080/10495398.2016.1242490
19. Magni P, Motta M, Martini L (2000) Leptin: a possible link between food intake, energy expenditure, and reproductive function. Regul Pept 92:51–56. https://doi.org/10.1016/S0167-0115(00)00149-X
20. Al-Hussaniy HA, Alburghaif AH, Naji MA (2021) Leptin hormone and its effectiveness in reproduction, metabolism, immunity, diabetes, hopes and ambitions. J Med Life 14:600–605. https://doi.org/10.25122%2Fjml-2021-0153
21. Neuenschwander S, Rettenberger G, Meijerink H, Jorg H, Stranzinger G (1996) Partial characterization of porcine obesity gene (OBS) and its localization to chromosome 18 by somatic cell hybrids. Anim Genet 27:275–278. https://doi.org/10.1111/j.1365-2052.1996.tb00489.x
22. Stratil A, Peelman L, Van Poucke M, C epica S (1997) A HinfI PCR-RFLP at the porcine leptin (LEP) gene. Anim Genet 28:371–372. https://doi.org/10.1111/j.1365-2052.1997.tb03272.x
23. Perez-Montarelo D, Fernandez A, Barragan C, Noguera JL, Folch JM, Rodríguez MC, Ovilo C, Silio L, Fernandez AI (2013) Transcriptional characterization of porcine leptin and leptin receptor genes. PLoS ONE 8:66398. https://doi.org/10.1371/journal.pone.0066398
24. Chen CC, Chang T, Su HY (2004) Characterization of porcine leptin receptor polymorphisms and their association with reproduction and production traits. Animal Biotechnol 15:89–102. https://doi.org/10.1081/ABIO-120037903
25. Terman A (2005) Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. J Anim Breed Genet 122:400–404. https://doi.org/10.1111/j.1439-0388.2005.00547.x

26. Rempel LA, Nonneman DJ, Wise TH, Erkens T, Peelman LJ, Rohrer GA (2010) Association analyses of candidate single nucleotide polymorphisms on reproductive traits in swine. J Anim Sci 88:1–15. https://doi.org/10.2527/jas.2009-1985
27. Vega RS, Castillo RMC, Barrientos NNB, Llanes-Autriz MM, Cho BW, Celia B, Villa NO (2018) Leptin (T3469C) and estrogen receptor (T1665G) gene polymorphisms and their associations to backfat thickness and reproductive traits of large white pigs (Sus scrofa L.). Philippine J Sci 147:293–300
28. Bing Z, Gongshe Y, Chao S, Minrui H (2010) Effects of leptin gene on litter size in Luchuan and largewhite pig
29. Fu Y, Li L, Li B, Fang X, Ren S (2016) Long form leptin receptor and SNP effect on reproductive traits during embryo attachment in Suzhong sows. Anim Reprod Sci 168:57–65. https://doi.org/10.1016/j.anireprosci.2016.02.026
30. Ponsuksili S, Wimmers K, Yerle M, Schellander K (2001) Mapping of 93 porcine ESTs preferentially expressed in liver. Mamm Genome 12:869–872. https://doi.org/10.1007/s00335-001-3024-8
31. Schillo KK (2009) Reproductive physiology of mammals: from farm to field and beyond. Delmar Publishers
32. Jiang ZH, Gibson JP (1998) A PCR-RFLP marker at the porcine complement factor B gene locus shows between-population frequency variation. J Anim Sci 76:1716–1717. https://doi.org/10.2527/1998.7661716x
33. Chen LH, Wang LX, Ji YG, Zhang LC, Yan H (2009) Association of polymorphism for porcine BF gene with reproductive traits and placental efficiency in Large White. Yi Chuan 31:615–619. https://doi.org/10.3724/sp.j.1005.2009.00615
34. Buske B, Brunsch C, Zeller K, Reinecke P, Brockmann G (2005) Analysis of properdin (BF) genotypes associated with litter size in a commercial pig cross population. J Anim Breed Genet 122:259–263. https://doi.org/10.1111/j.1439-0388.2005.00528.x
35. Marantidis A, Papadopoulos AI, Michailidis G, Avdi M (2013) Association of BF gene polymorphism with litter size in a commercial pig crosses population. Anim Reprod Sci 141:75–79. https://doi.org/10.1016/j.anireprosci.2013.06.011
36. Giudice LC (2006) Endometrium in PCOS: Implantation and predisposition to endocrine CA. Best practice & research. Clin Endocrinol metabolism 20:235–244. https://doi.org/10.1016/j.beem.2006.03.005
37. Ghazal S, McKinnon B, Zhou J, Mueller M, Men Y, Yang L, Mueller M, Flannery C, Huang Y, Taylor HS (2015) H19 lncRNA alters stromal cell growth via IGF signaling in the endometrium of women with endometriosis. EMBO Mol Med 7:996–1003. https://doi.org/10.15252/emmm.201505245
38. Mazerbourg S, Monget P (2018) Insulin-like growth factor binding proteins and IGFBP proteases: a dynamic system regulating the ovarian folliculogenesis. Front Endocrinol 9:134. https://doi.org/10.3389/fendo.2018.00134
39. An SM, Hwang JH, Kwon S, Yu GE, Park DH, Kang DG, Kim TW, Park HC, Ha J, Kim CW (2018) Effect of single nucleotide polymorphisms in IGFBP2 and IGFBP3 genes on litter size traits in Berkshire

pigs. Animal Biotechnol 29:301–308. https://doi.org/10.1080/10495398.2017.1395345

40. Sironen AI, Uimari P, Serenius T, Mote B, Rothschild M, Vilkki J (2010) Effect of polymorphisms in candidate genes on reproduction traits in Finnish pig populations. J Anim Sci 88:821–827. https://doi.org/10.2527/jas.2009-2426
41. Harney JP, Ott TL, Geisert RD, Bazer FW (1993) Retinol-binding protein gene expression in cyclic and pregnant endometrium of pigs, sheep, and cattle. Biol Reprod 49:1066–1073. https://doi.org/10.1095/biolreprod49.5.1066
42. Messer LA, Wang L, Yelich J, Pomp D, Geisert RD, Rothschild MF (1996) Linkage mapping of the retinol-binding protein 4 (RBP4) gene to porcine chromosome 14. Mamm Genome 7:396–396. https://doi.org/10.1007/s003359900117
43. Brief S, Chew BP (1985) Effects of vitamin A and beta-carotene on reproductive performance in gilts. J Anim Sci 60:998–1004. https://doi.org/10.2527/jas1985.604998x
44. Ollivier L, Messer LA, Rothschild MF, Legault C (1997) The use of selection experiments for detecting quantitative trait loci. Genet Res 69:227–232. https://doi.org/10.1017/s0016672397002802
45. Terman A, Kmiec M, Polasik D, Rybarczyk A (2011) Association between RBP4 gene polymorphism and reproductive traits in Polish sows. J Anim Veterinary Adv 10:2639–2641. http://dx.doi.org/10.3923/javaa.2011.2639.2641
46. Niu SY, Wang XP, Hao FG, Zhao RX (2008) Effect of the polymorphism of RBP4 and OPN genes on litter size in Tibet pigs. Acta Agriculturae Scand Section A 58:10–13. https://doi.org/10.1080/09064700802054170
47. Marantidis A, Laliotis GP, Avdi M (2016) Association of RBP4 genotype with phenotypic reproductive traits of sows. Genetics research international, 2016. https://doi.org/10.1155/2016/4940532
48. Mencik S, Vukovic V, Spehar M, Modric M, Ostovic M, Kabalin AE (2019) Association between ESR1 and RBP4 genes and litter size traits in a hyperprolific line of Landrace× Large White cross sows. Veterinární medicína 64:109–117. https://doi.org/10.17221/87/2018-VETMED
49. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA (1998) Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 19:225–268. https://doi.org/10.1210/edrv.19.3.0334
50. Vincent AL, Tuggle CK, Rothschild MF, Evans G, Short TH, Southwood OI, Plastow GS (1998) The prolactin receptor gene is associated with increased litter size in pigs.Iowa State University Animal Industry Report1(1)
51. Rothschild MF, Vincent AL, Tuggle CK, Evans G, Short TH, Southwood OI, Wales R, Plastow GS (1998) A mutation in the prolactin receptor gene is associated with increased litter size in pigs. Anim Genet 29:60–74
52. Rens BV, Lende TV (2002) Litter size and piglet traits of gilts with different prolactin receptor genotypes. Theriogenology 57:883–893. https://doi.org/10.1016/S0093-691X%2801%2900693-8
53. Putnova L, Knoll A, Dvorak J, Cepica S (2002) A new HpaII PCR-RFLP within the porcine prolactin receptor (PRLR) gene and study of its effect on litter size and number of teats. J Anim Breed Genet

119:57–63. https://doi.org/10.1046/j.1439-0388.2002.00316.x

54. Xing-ping WANG, Li-xian WANG, Zhuo-ma LR, Shi-duo SUN (2008) Analysis of PRLR and BF genotypes associated with litter size in Beijing black pig population. Agricultural Sci China 7:1374–1378. https://doi.org/10.1016/S1671-2927(08)60187-X
55. Drogemuller C, Hamann H, Distl O (2001) Candidate gene markers for litter size in different German pig lines. J Anim Sci 79:2565–2570. https://doi.org/10.2527/2001.79102565x
56. Ricken A, Lochhead P, Kontogiannea M, Farookhi R (2002) Wnt signaling in the ovary: identification and compartmentalized expression of wnt-2, wnt-2b, and frizzled-4 mRNAs. Endocrinology 143:2741–2749. https://doi.org/10.1210/endo.143.7.8908
57. Tepekoy F, Akkoyunlu G, Demir R (2015) The role of Wnt signaling members in the uterus and embryo during pre-implantation and implantation. J Assist Reprod Genet 32:337–346. https://doi.org/10.1007/s10815-014-0409-7
58. Buimer M, Keijser R, Jebbink JM, Wehkamp D, van Kampen AH, Boer K, van der Post JA, Ris-Stalpers C (2008) Seven placental transcripts characterize HELLP-syndrome. Placenta 29:444–453. https://doi.org/10.1016/j.placenta.2008.02.007
59. Atli MO, Guzeloglu A, Dinc DA (2011) Expression of wingless type (WNT) genes and their antagonists at mRNA levels in equine endometrium during the estrous cycle and early pregnancy. Anim Reprod Sci 125:94–102. https://doi.org/10.1016/j.anireprosci.2011.04.001
60. Kemp C, Willems E, Abdo S, Lambiv L, Leyns L (2005) Expression of all Wnt genes and their secreted antagonists during mouse blastocyst and post implantation development. Dev Dyn 233:1064–1075. https://doi.org/10.1002/dvdy.20408
61. Brown NL, Paddock SW, Sattler CA, Cronmiller C, Thomas BJ, Carroll SB (1996) Daughterless is required for Drosophila photoreceptor cell determination, eye morphogenesis, and cell cycle progression. Dev Biol 179:65–78. https://doi.org/10.1006/dbio.1996.0241
62. Sun X, Mei S, Tao H, Wang G, Su L, Jiang S, Deng C, Xiong Y, Li F (2011) Microarray profiling for differential gene expression in PMSG-hCG stimulated preovulatory ovarian follicles of Chinese Taihu and Large White sows. BMC Genomics 12:1–10. https://doi.org/10.1186/1471-2164-12-111
63. Tao H, Mei S, Sun X, Peng X, Zhang X, Ma C, Wang L, Hua L, Li F (2013) Associations of TCF12, CTNNAL1 and WNT10B gene polymorphisms with litter size in pigs. Anim Reprod Sci 140:189–194. https://doi.org/10.1016/j.anireprosci.2013.05.013
64. Cauffman G, Van de Velde H, Liebaers I, Van Steirteghem A (2005) DAZL expression in human oocytes, preimplantation embryos and embryonic stem cells. Mol Hum Reprod 11:405–411. https://doi.org/10.1093/molehr/gah167
65. Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, Dorin J, Cooke HJ (1997) The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature 389:73–77. https://doi.org/10.1038/37987
66. Rathje TA, Rohrer GA, Johnson RK (1997) Evidence for quantitative trait loci affecting ovulation rate in pigs. J Anim Sci 75:1486–1494. https://doi.org/10.2527/1997.7561486x

67. Cassady JP, Johnson RK, Pomp D, Rohrer GA, Van Vleck LD, Spiegel EK, Gilson KM (2001) Identification of quantitative trait loci affecting reproduction in pigs. J Anim Sci 79:623–633. https://doi.org/10.2527/2001.793623x
68. Dall'Olio S, Fontanesi L, Tognazzi L, Russo V (2010) Genetic structure of candidate genes for litter size in Italian Large White pigs. Vet Res Commun 34:203–206. https://doi.org/10.1007/s11259-010-9380-7
69. Zhang YH, Mei SQ, Peng XW, Niu BY, Ren ZQ, Zuo B, Xu DQ, Lei MG, Zheng R, Jiang SW, Deng CY (2009) Molecular characterization and SNPs analysis of the porcine Deleted in AZoospermia Like (pDAZL) gene. Anim Reprod Sci 112:415–422. https://doi.org/10.1016/j.anireprosci.2008.05.069
70. Curtin D, Ferris HA, Häkli M, Gibson M, Janne OA, Palvimo JJ, Shupnik MA (2004) Small nuclear RING finger protein stimulates the rat luteinizing hormone-β promoter by interacting with Sp1 and steroidogenic factor-1 and protects from androgen suppression. Mol Endocrinol 18:1263–1276. https://doi.org/10.1210/me.2003-0221
71. Saville B, Poukka H, Wormke M, Jänne OA, Palvimo JJ, Stoner M, Samudio I, Safe S (2002) Cooperative coactivation of estrogen receptor α in ZR-75 human breast cancer cells by SNURF and TATA-binding protein. J Biol Chem 277:2485–2497. https://doi.org/10.1074/jbc.M109021200
72. Hirvonen-Santti SJ, Sriraman V, Anttonen M, Savolainen S, Palvimo JJ, Heikinheimo M, Richards JS, Jänne OA (2004) Small nuclear RING finger protein expression during gonad development: regulation by gonadotropins and estrogen in the postnatal ovary. Endocrinology 145:2433–2444. https://doi.org/10.1210/en.2003-1328
73. King AH, Jiang Z, Gibson JP, Haley CS, Archibald AL (2003) Mapping quantitative trait loci affecting female reproductive traits on porcine chromosome 8. Biol Reprod 68:2172–2179. https://doi.org/10.1095/biolreprod.102.012955
74. Bu-yue N, Xiao-ming L, Yuan-zhu X, Xi-biao W (2016) Identification of Novel Polymorphisms in Porcine Ring Finger Protein 4 and Matrix Metalloproteinase 9 Genes and Association Analysis with Litter Size Traits. J Northeast Agricultural Univ (English Edition) 23:31–38. https://doi.org/10.1016/S1006-8104(16)30056-3
75. Niu BY, Ye LZ, Li FE, Deng CY, Jiang SW, Lei MG, Xiong YZ (2009) Identification of polymorphism and association analysis with reproductive traits in the porcine RNF4 gene. Anim Reprod Sci 110:283–292. https://doi.org/10.1016/j.anireprosci.2008.01.020
76. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, Yang J (2017) 10 years of GWAS discovery: biology, function, and translation. Am J Hum Genet 101:5–22. https://doi.org/10.1016/j.ajhg.2017.06.005
77. Jiang Z, Wang H, Michal JJ, Zhou X, Liu B, Woods LCS, Fuchs RA (2016) Genome wide sampling sequencing for SNP genotyping: methods, challenges and future development. Int J Biol Sci 12:100. doi: 10.7150/ijbs.13498
78. Guo Y, Huang Y, Hou L, Ma J, Chen C, Ai H, Huang L, Ren J (2017) Genome-wide detection of genetic markers associated with growth and fatness in four pig populations using four approaches. Genet

Selection Evol 49:1–11. https://doi.org/10.1186/s12711-017-0295-4

79. Cho IC, Park HB, Ahn JS, Han SH, Lee JB, Lim HT, Yoo CK, Jung EJ, Kim DH, Sun WS, Ramayo-Caldas Y (2019) A functional regulatory variant of MYH3 influences muscle fiber-type composition and intramuscular fat content in pigs. PLoS Genet 15:1008279. https://doi.org/10.1371/journal.pgen.1008279
80. Nosková A, Hiltpold M, Janett F, Echtermann T, Fang ZH, Sidler X, Selige C, Hofer A, Neuenschwander S, Pausch H (2021) Infertility due to defective sperm flagella caused by an intronic deletion in DNAH17 that perturbs splicing. Genetic 217:33. https://doi.org/10.1093/genetics/iyaa033
81. Ren J, Mao H, Zhang Z, Xiao S, Ding N, Huang L (2011) A 6-bp deletion in the TYRP1 gene causes the brown colouration phenotype in Chinese indigenous pigs. Heredity 106:862–868. https://doi.org/10.1038/hdy.2010.129
82. Ding R, Qiu Y, Zhuang Z, Ruan D, Wu J, Zhou S, Ye J, Cao L, Hong L, Xu Z, Zheng E (2021) Genome-wide association studies reveals polygenic genetic architecture of litter traits in Duroc pigs. Theriogenology 173:269–278. https://doi.org/10.1016/j.theriogenology.2021.08.012
83. Bergfelder-Drüing S, Grosse-Brinkhaus C, Lind B, Erbe M, Schellander K, Simianer H, Tholen E (2015) A genome-wide association study in large white and landrace pig populations for number piglets born alive. PLoS ONE 10:0117468. https://doi.org/10.1371/journal.pone.0117468
84. Bogacka I, Bogacki M (2011) The quantitative expression of peroxisome proliferator activated receptor (PPAR) genes in porcine endometrium through the estrous cycle and early pregnancy. J Physiol Pharmacol 62:559. https://doi.org/10.1016/S1642-431X(12)60083-7
85. Guo LY, Fu JL, Wang AG (2012) Analysis of association between CRS-PCR polymorphisms of integrin β1 gene and litter size in pigs. Yi Chuan 34:879–886. https://doi.org/10.3724/sp.j.1005.2012.00879
86. Wu P, Wang K, Yang Q, Zhou J, Chen D, Ma J, Tang Q, Jin L, Xiao W, Jiang A, Jiang Y (2018) Identifying SNPs and candidate genes for three litter traits using single-step GWAS across six parities in Landrace and Large White pigs. Physiol Genom 50:1026–1035. https://doi.org/10.1152/physiolgenomics.00071.2018
87. Zhang Z, Chen Z, Ye S, He Y, Huang S, Yuan X, Chen Z, Zhang Ha, Li J (2019) Genome-wide association study for reproductive traits in a Duroc pig population. Animals 9:732. https://doi.org/10.3390/ani9100732
88. Chen Z, Ye S, Teng J, Diao S, Yuan X, Chen Z, Zhang H, Li J, Zhang Z (2019) Genome-wide association studies for the number of animals born alive and dead in duroc pigs. Theriogenology 139:36–42. https://doi.org/10.1016/j.theriogenology.2019.07.013
89. Wang Y, Ding X, Tan Z, Xing K, Yang T, Pan Y, Wang Y, Mi S, Sun D, Wang C (2018) Genome-wide association study for reproductive traits in a Large White pig population. Anim Genet 49:127–131. https://doi.org/10.1111/age.12638
90. Jiang Y, Tang S, Xiao W, Yun P, Ding X (2020) A genome-wide association study of reproduction traits in four pig populations with different genetic backgrounds. Asian-Australasian J Anim Sci 33:1400. https://doi.org/10.5713/ajas.19.0411

91. Mo J, Lu Y, Zhu S, Feng L, Qi W, Chen X, Xie B, Chen B, Lan G, Liang J (2022) Genome-Wide Association Studies, Runs of Homozygosity Analysis, and Copy Number Variation Detection to Identify Reproduction-Related Genes in Bama Xiang Pigs. 9. Frontiers in veterinary sciencehttps://doi.org/10.3389/fvets.2022.892815

Table

Table 1. Comparison between QTL analysis and Candidate gene approach

PROPERTY	QTL ANALYSIS	CANDIDATE GENE APPROACH
Utility	Preliminary step	Ultimate goal
Principle	Indirect gene assay	Direct gene assay
Economy	Expensive	Comparatively less expensive
Accuracy	Moderate	Highly accurate
Expressiveness	No information regarding the number of genes, favorable allele or causative gene as QTL region can contain hundreds of genes	High due to direct gene assay
Constraints	Pleiotropy; requirement of at least three generations; decision regarding which QTL to be considered for further analysis	Pleiotropy, unbalanced genotypic frequencies

Figures

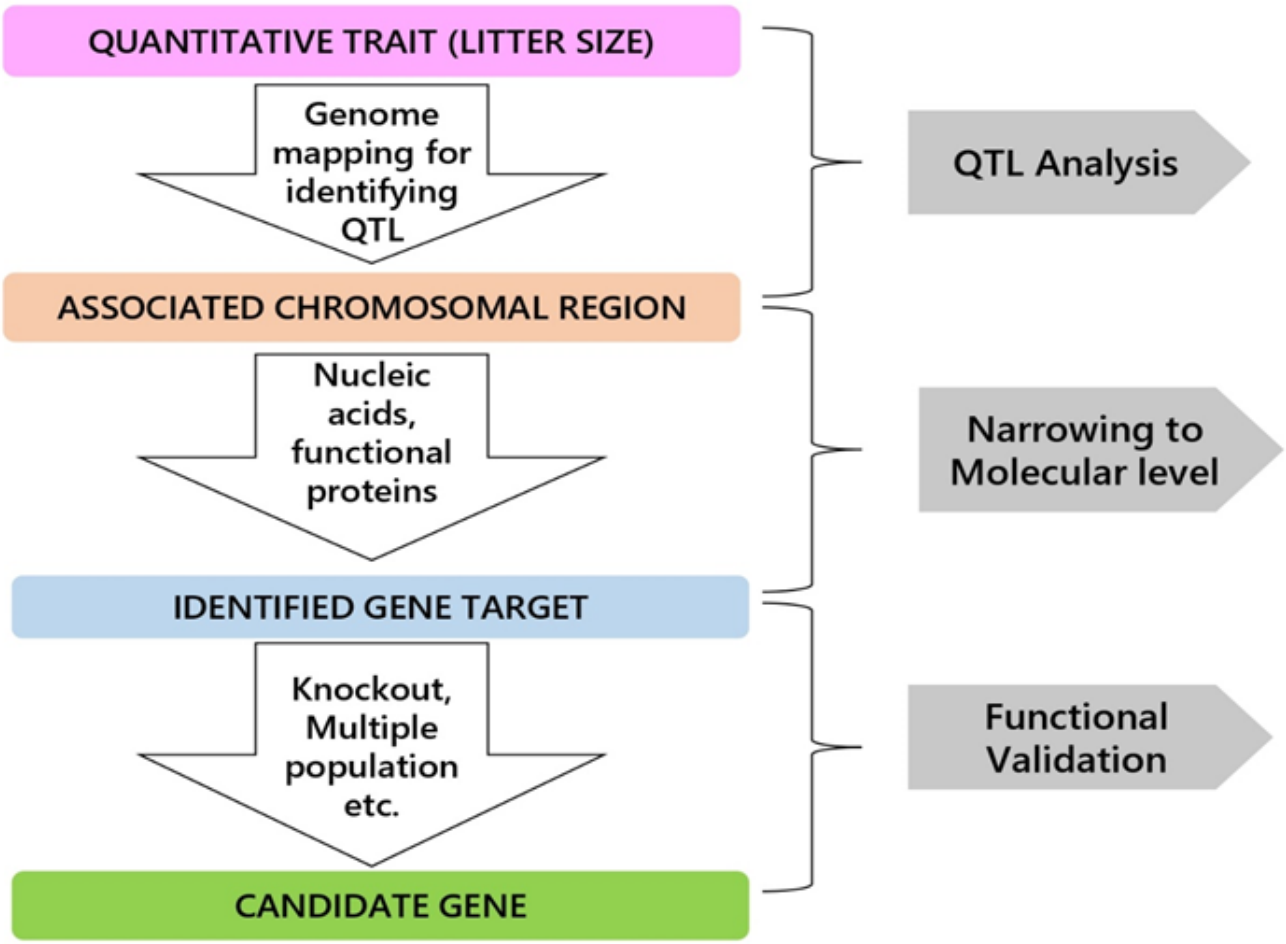

Figure 1

Steps for identification of candidate genes for a quantitative trait

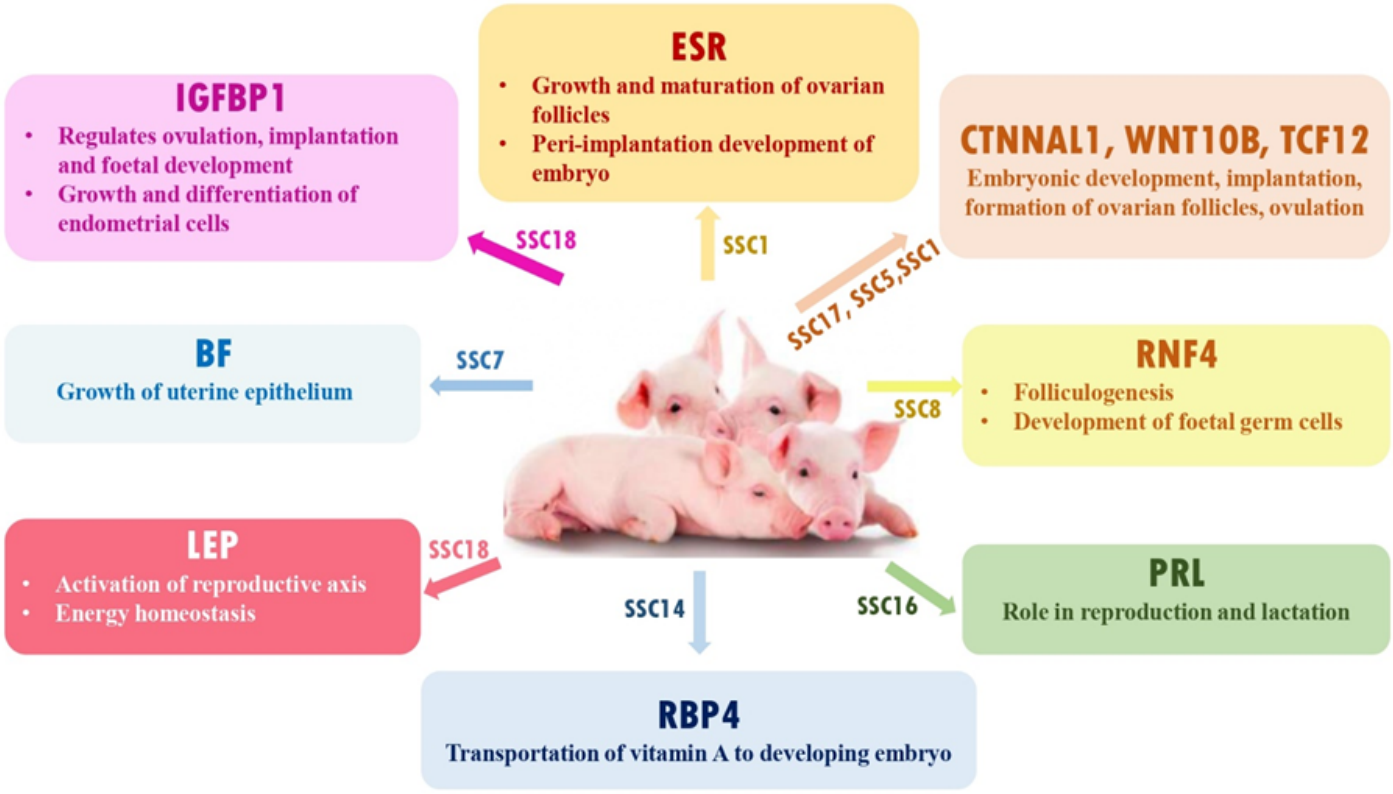

Figure 2

Localisation and physiological role of candidate genes affecting litter traits in pigs

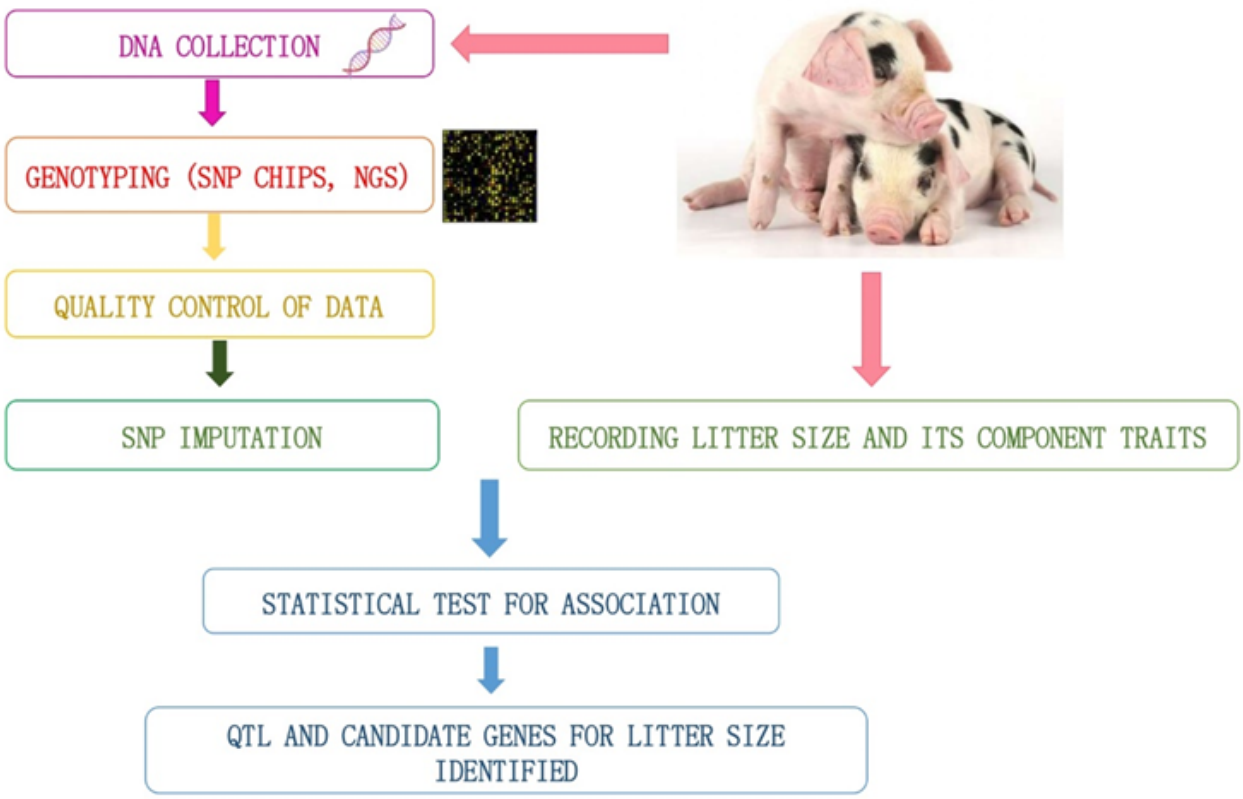

Figure 3

Genome Wide Association Study pipeline

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