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UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE

**DETECTION OF QTL INFLUENCING BODY
CONFORMATION TRAITS IN DAIRY SHEEP**

MASTER'S THESIS

Valentina Blatančić

Zagreb, September, 2023

UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE

Master study:

Animal Genetics and Breeding

**DETECTION OF QTL INFLUENCING BODY
CONFORMATION TRAITS IN DAIRY SHEEP**

MASTER'S THESIS

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Zagreb, September, 2023

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Contents

1. Introduction	1
2. Literature review	5
2.1. Importance of dairy sheep and traits of interest in dairy sheep breeding programs	5
2.2. Evolution of gene mapping studies in sheep	8
2.3. Dairy sheep in the Spanish region of Castilla y Leon and the Churra milk selection program	13
2.4. The use of molecular genetic information in Churra sheep.....	14
2.5. Importance of body morphology traits in Churra sheep program	16
3. Materials and methods.....	18
3.1. Population, Phenotypes and Genotypes	18
3.2. Phenotypes used for QTL mapping.....	18
3.3. QTL mapping analysis	19
3.4. Overlapping with previously reported QTL and gene annotation	20
4. Results	21
4.1. QTL regions detected	21
4.2. Results of the LA analysis performed for body conformation traits	21
4.3. Results of the GWAS analysis performed for body conformation traits.....	24
4.4. Correspondence with previous studies and potential candidate genes	28
5. Discussion.....	32
6. Conclusion	39
7. References	40
Biography	54

Sažetak

Diplomskog rada studenta/ice **Valentina Blatančić**, naslova

DETECTION OF QTL INFLUENCING BODY CONFORMATION TRAITS IN DAIRY SHEEP

Svojstva tjelesne konformacije igraju značajnu ulogu u kontekstu uzgoja mliječnih ovaca, s obzirom da su povezana s ukupnim zdravljem, dugovječnošću i proizvodnim sposobnostima životinja. Osnovni cilj ovog istraživanja bio je identificirati kvantitativne lokuse povezane sa svojstvima (eng. *Quantitative trait loci*, QTL) tjelesne konformacije kod mliječnih ovaca pasmine Churra koristeći podatke generirane pomoću Illumina OvineSNP50 BeadChip (50K-čipa). Za mapiranje QTL-ova primijenjene su dvije različite metode, tradicionalna analiza povezanosti (eng. *Linkage analysis*, LA) i cjelogenomska asocijacijska studija (eng. *Genome-wide association study*, GWAS). Ispitivana populacija sastojala se od 1.680 ovaca iz Seleksijskog nukleusa za mliječne ovce Churra (eng. *National Association of Churra Breeders*, ANCHE), raspoređenih u 16 polubrat/polusestra obitelji. Predmet istraživanja bila su četiri svojstva (stas, širina kukova, stražnje noge (pogled straga) i kut papaka) te je identificirano sedam QTL-ova značajnih na razini kromosoma putem LA i 27 regija značajnih na razini kromosoma putem GWAS-a. Kako bi se poboljšalo razumijevanje identificiranih QTL-ova, uspostavljeni su intervali pouzdanosti za svaki značajan QTL, nakon čega su slijedili anotacija gena i anotacija QTL-ova, kao i analiza obogaćivanja (eng. *Enrichment analysis*). Usporedbom s prethodno identificiranim QTL-ovima kod ovaca za svojstva tjelesne konformacije, otkrivene su sličnosti, što podržava autentičnost identificiranih QTL-ova. Regije koje pokazuju najveći stupanj podudaranja s prethodno identificiranim QTL-ovima ovaca za svojstva od interesa detaljno su analizirane kako bi se identificirali najizgledniji funkcionalni kandidat geni. Međutim, potrebna su daljnja istraživanja kako bi se potvrdila izravna povezanost kandidat gena s odgovarajućim svojstvima tjelesne konformacije od interesa. Ova saznanja imaju potencijal za identifikaciju genetskih markera koji bi se mogli koristiti za poboljšanje uzgojnih programa pasmine Churra, posebice s ciljem poboljšanja proučavanih svojstava tjelesne konformacije.

Ključne riječi: QTL, svojstva tjelesne konformacije, ovce, GWAS, analiza povezanosti, kandidat geni

Summary

Of the master's thesis – student **Valentina Blatančić**, entitled

DETECTION OF QTL INFLUENCING BODY CONFORMATION TRAITS IN DAIRY SHEEP

Body conformation traits hold significant importance in the context of dairy sheep, as they are linked with the animals' overall health, longevity, and production capabilities. The primary objective of this study was to identify quantitative trait loci (QTLs) associated with these traits in Churra dairy sheep using data generated by the Illumina OvineSNP50 BeadChip (50K-chip). Two different approaches, traditional linkage analysis (LA) and a genome-wide association study (GWAS), were employed for QTL mapping. The study population comprises 1,680 ewes from the Churra Dairy Selection Nucleus (ANCHE), distributed across 16 half-sibling families. Four specific traits (Stature, Rump Width, Rear Legs (Rear View), and Feet Angle) were examined, resulting in the detection of eight chromosome-wise significant QTLs through LA and 27 chromosome-wise significant regions through GWAS. To enhance the understanding of the identified QTLs, confidence intervals were established for each significant signal, followed by gene and QTL annotation, as well as QTL enrichment analysis. Comparisons with previously reported QTLs in sheep for body conformation traits revealed intriguing similarities, supporting the authenticity of the identified QTLs. The regions showing the highest level of correspondence with previously reported sheep QTL for traits of interest were analyzed in detail for the identification of the most promising functional candidate genes. However, further research is needed to confirm the direct association of these candidate genes with the corresponding body morphology traits of interest. These findings have the potential to serve for the identification of genetic markers that could be used ultimately to enhance the efficiency of Churra sheep breeding program, particularly for the improvement of body morphology traits.

Keywords: QTL, body conformation traits, sheep, GWAS, Linkage Analysis (LA), candidate genes

1. Introduction

In recent decades, gene technology has undergone significant advancements, primarily due to the emergence of genomics. This has enabled a global-scale exploration of genome structure and function. Animal genomics has witnessed remarkable progress, with significant implications for livestock breeding programs (Meuwissen et al., 2011). These advancements have been driven by developments in sequencing technologies and have paved the way for more efficient and targeted breeding strategies in livestock species like sheep (Clarke et al., 2016). Dairy sheep play a vital role in Mediterranean countries, where agriculture and traditional livestock farming have deep-rooted significance. In the broader context of Europe, with approximately 59 million sheep according to Eurostat (2022), these Mediterranean regions significantly contribute to the continent's diverse sheep population. The importance of dairy sheep in these areas extends beyond mere numbers, as they are valued for their unique ability to thrive in Mediterranean climates and rugged terrains while providing high-quality milk. In dairy sheep, milk production traits have held paramount importance, with Mediterranean countries cherishing sheep's milk for its exceptional nutritional value and suitability for high-quality cheese production. However, the significance of dairy sheep goes beyond milk yield alone. Functional traits, including mammary morphology traits and body morphology traits, are equally crucial. The efficient extraction of milk, influenced by mammary morphology traits, directly impacts milk production. Additionally, body morphology traits, such as stature, rump width, feet angle and rear legs are indicators of mobility, productivity, longevity and overall dairy sheep health. Therefore, the genetic improvement of these functional traits is integral to enhancing the sustainability and competitiveness of dairy sheep farming in Mediterranean countries and across Europe.

During the mid-1990s, numerous projects were initiated to identify regions influencing quantitative traits (QTL) in domestic animals, with a focus on swine and cattle. Although fewer projects were conducted in sheep, several noteworthy genomic sweeps were carried out across different sheep breeds (Barillet et al., 2006; Gutiérrez-Gil et al., 2009; Raadsma et al., 2009a; Mateescu and Thonney, 2010a). These studies often utilized linkage analysis techniques and microsatellites as genetic markers. The genome coverage of these mapping studies was limited as it was based on the analysis of about 200 microsatellites distributed across the sheep genome.

From the early 21st century, the development of Next Generation Sequencing (NGS) technologies, have significantly improved the speed and cost-effectiveness of DNA sequencing, allowing for the sequencing of entire genomes (Meuwissen and Goddard, 2017). NGS has facilitated the discovery of novel genetic variants, improved genome assembly, and enhanced our understanding of the genetic basis of complex traits (Meuwissen and Goddard, 2017). This led to the advancement of genome knowledge in various domestic species. Moreover, the extensive information derived from genome sequencing projects spurred the development of molecular tools, with SNP-chips being the most prominent. For sheep, this international effort was undertaken by various research groups

within the International Sheep Genomics Consortium (ISGC). Their initial objective was to create the OvineSNP50 BeadChip, a medium-density SNP-chip that included more than 50,000 markers for analyzing the genetic architecture of heritable traits in sheep. Notably, the development of this chip, which will be referred here from now on as 50K-SNP-chip, occurred at an early stage of the genome sequencing project when only a preliminary draft of the sheep genome was available. The draft was obtained by aligning the ovine genomic sequence with bovine, canine, and human genomes and is referred to as the Virtual Sheep Genome (Dalrymple et al., 2007). This version of the sheep reference genome served as a foundational resource for understanding the genetic makeup of sheep and played a significant role in the development of genomic tools and research in the field of sheep genomics.

The limitations of early genome scans were overcome with the development of high-throughput SNP genotyping arrays, such as the ovine 50K-SNP-chip (Clarke and Jopson, 2017). This powerful tool enabled the identification of causal mutations of inherited diseases (Becker et al., 2010; Suarez-Vega et al., 2013) and allowed the identification of many QTLs associated with economically important traits with an increased marker density, which significantly enhanced the power to detect regions of interest and improved gene mapping accuracy.

This genomic progress transitioned genomic scans from microsatellite markers in segregating families to high-density genomic scans using affordable SNP-chips. These chips enabled the analysis of genetically unrelated animals through genome-wide association studies (GWAS), which rely on linkage disequilibrium within domestic animal populations. This eliminated the need for costly experimental populations involving crosses between divergent breeds or the need of specific family-structured populations required by linkage analyses. GWAS yielded more precise detection of chromosomal regions associated with productive traits, although results varied across species, there were substantial advances in understanding the genetic basis of both simple and complex productive traits.

In addition, genomic selection (GS) has significantly transformed the field of animal genomics. GS involves using medium to high-density SNP-chips to predict the genomic values of individuals across a range of traits (Savić and Milenković, 2017). By combining genomic data with phenotypic information available in a reference population, breeders can identify animals with superior genetic potential at an early age only based on their genotypes, accelerating genetic gain and enabling selection for complex traits that are challenging to measure directly (Savić and Milenković, 2017).

These advancements in animal genomics hold great promise for improving livestock breeding strategies and enhancing the productivity and resilience of livestock populations (Hayes et al., 2013). Given the current challenges in animal production, genomics can play a crucial role in developing more efficient and sustainable production systems, benefiting both producers and consumers (Hayes et al., 2013). The identification of genetic markers associated to traits of economic interest could be further exploited through GS as it has been shown that the introduction

of causal mutations in SNP-chips used for GS can improve the accuracy of the genomic predictions of genetic merit (Bhat et al., 2016).

The group of animal breeding of the University of León (MEGA-ULE group), in Spain, where this Master's Thesis has been performed, has developed for many years research projects in the field of genetics applied to dairy sheep populations. Since 1964, this group technically supervises the breeding program of the dairy Churra sheep breed managed by the Spanish Association of Sheep Breeders (ANCHE) (Notter, 2016). The program uses artificial insemination and classical genetics selection of sires and dams to improve milk production and breed competitiveness. Initially, the focus of the program was on boosting milk yield and milk quality, vital for cheese production (Notter, 2016). Later, in 2003, secondary objectives included traits related to mammary and body morphology, along with scrapie resistance, to align with European regulations (Notter, 2016). These efforts have been instrumental in preserving and enhancing the Churra breed's genetic merit. Currently, the Churra breeding program is initiating a slow transition towards the implementation of GS, whose use is limited due to the progressive decrease of the Churra breed census associated to social and economic reasons (the retirement of traditional breeders and the replacement of the breed by other foreign breeds with higher production potential such as Spanish Assaf).

Through the collaboration with ANCHE, the MEGA-ULE group has developed gene mapping projects to identify genetic markers associated with different traits of economic interest in dairy sheep. Whereas for milk production traits, mammary morphology and disease resistance traits, published genome scans were performed firstly based on microsatellite markers (Gutierrez-Gil et al., 2007, 2008; 2009a,b; 2011) and later based on the 50K-SNP-chip (García-Gómez et al., 2012a; Gutierrez-Gil et al., 2018; Atlija et al., 2016; Vrčan, 2022), the analysis for body morphology traits has not been performed with the medium density SNP-chip genotypes.

Considering all the above mentioned, this Master's Thesis was defined with the aim of identifying and annotating QTL for body conformation traits in a commercial population of Spanish Churra sheep utilizing a medium-density SNP-chip to discover potential candidate genes that could be employed to enhance classical or genomic selection of these traits. To achieve this global objective, the following objectives have been outlined:

1. Conduct a QTL detection analysis for four body conformation traits in Churra sheep, utilizing a medium-density SNP-array and two analysis methods, namely Linkage Analysis (LA) and genome-wide association study (GWAS), in order to identify regions that influence body conformation traits.
2. Annotate the detected genes and QTL regions, based on the lattermost sheep reference genome, Oar_ram_v2.0, and perform a comparative analysis with QTL previously identified in sheep for body conformation traits and other related traits such as growth and carcass related traits.

3. Identify the best functional candidate genes that could be further investigated through complementary -omic analyses, including whole-genome and transcriptomic sequencing, to find genetic markers that could be used to enhance the efficiency of classical and genomic selection for body conformation traits in Churra sheep.

2. Literature review

2.1. Importance of dairy sheep and traits of interest in dairy sheep breeding programs

Dairy sheep production is a significant agricultural sector in Mediterranean countries due to the favorable climatic conditions and terrain. These countries, such as Spain, Italy, Greece, and Portugal, have a long tradition of raising sheep for production of milk, which is mainly used for manufacturing of cheese and other dairy products (Pulina et al., 2018). The Mediterranean climate, characterized by mild temperatures with hot, dry summers and mild winters, provides optimal conditions for the production of high-quality forage for sheep. Furthermore, the hilly and mountainous terrain in some areas of these countries is frequently unsuitable for other forms of agriculture, making sheep farming a practical and profitable option (El Aich et al., 2020). The dairy sheep industry in the Mediterranean region typically uses local breeds such as the Sarda breed in Italy, Churra in Spain, and the Lacaune breed in France. These breeds, in general, have been selected over centuries for their ability to produce high-quality milk, and they are well adapted to the local environment and feeding systems (Pacetti et al., 2021). Sheep milk is highly valued in the Mediterranean region for its rich flavor and nutritional properties, and it is used to produce a wide variety of dairy products such as cheese, yogurt, and butter (Lanza et al., 2019). Many of these products are traditional and have been produced for centuries, and they are often protected by geographical indications or other quality designations (Spano et al., 2018). Despite challenges such as competition from other dairy products and changing consumer preferences, there is still a strong demand for traditional sheep milk products, and the industry continues to evolve to meet the changing needs of consumers.

Selection objectives in dairy sheep breeding typically focus on improving **milk production traits** to enhance the profitability and efficiency of dairy sheep farming. The specific selection objectives may vary based on the breed, production system, and regional preferences. Breeders need to consider multiple traits simultaneously to achieve a balanced and sustainable improvement in dairy sheep breeding programs. (de la Fuente et al., 1995).

At the practical level, milk yield and milk composition are the primary selection objectives in all the breeds, whereas some functional traits related to morphology conformation and udder health may also be considered in the selection index of dairy sheep breeding programs. Increasing milk yield is a primary objective in dairy sheep breeding. The first goal is to selectively breed individuals with higher milk production potential to achieve greater milk yields per lactation cycle. Apart from milk yield, the composition of sheep milk, mainly related to the contents of fat, protein and lactose is also essential as sheep milk is mainly derived for high-quality cheese manufacturing. Because

of that many selection schemes of dairy sheep breeds also include milk composition as selection objective, in many cases considering milk protein percentage as selection criterium.

In relation to functional traits, the most important functional traits in dairy sheep are those related to mammary morphology. The first studies on these traits in sheep, carried out in the early 1950s, confirmed their great importance in milk production (Emediato et al., 2008). Initially, selection for mammary morphology traits was implemented in certain sheep breeds to improve their suitability for mechanical milking (Casu et al., 2010). Later, it was found that there was a correlation between these traits and the presence of somatic cells in milk, and therefore a relationship between these traits and susceptibility or resistance to subclinical mastitis. Hence, the study of the structure and form of the mammary glands in dairy sheep plays a crucial role in understanding milk production capacity, udder health, and overall milk quality. The morphology of the mammary gland directly affects milk yield, as well as the efficiency of milk extraction during milking. Knowledge of mammary morphology helps farmers select breeding stock with desirable udder characteristics, such as well-attached and evenly distributed teats, which contribute to efficient milking and reduced risk of udder-related problems. Additionally, understanding the mammary morphology allows for early detection of any udder abnormalities or diseases, enabling timely intervention and treatment to maintain udder health and milk production (Caja et al., 2006; Gigli et al., 2016). The undesirable correlations between udder morphology traits and milk production (Marie-Etancelin et al., 2001) have determined that many breeds such as Lacaune, Sarda, Churra and Latxa, include these traits in their selection index by using the linear scale proposed by De la Fuente et al. (1996). Udder health related traits, such as low somatic cell count (SCC) and resistance to mastitis, are crucial in dairy sheep breeding. Healthy udders result in better milk quality, reduced veterinary costs, and improved animal welfare. Based on this, the Lacaune breeding scheme includes the SCC as selection objective since 2007 (Barillet, 2007). Other breeds, such as Spanish Churra have not introduced this trait due to the low heritability estimated in the specific breed (Othmane et al., 2002), or are introducing it recently, for example the Spanish Assaf breed (<https://assafe.es/>).

In addition, to udder morphology traits, also breeding for desirable **body conformation traits**, such as good stature, rump width, feet angle, rear legs-rear view, promotes optimal milk production and longevity in dairy sheep. Body conformation traits in dairy sheep play a vital role in assessing the overall physical structure and functionality of these animals.

The importance of body conformation traits and their possible relationship with milk production was initially reported by studies in dairy cattle after the 1980s. Studies in Holstein heifers demonstrated a positive correlation between milk production-related traits and certain body morphological traits (Lin et al., 1987). Other studies in dairy cattle reported a favorable correlation between somatic cell count and global body condition traits (Kadarmideen, 2004) and described positive genetic and phenotypic correlations between body morphological traits and udder conformation traits in heifers (Brotherstone, 1994). For milk production traits, a positive correlation was observed for a large number of body morphological traits, with hindquarter length

being the trait with the strongest correlation with milk yield traits (Lin et al., 1987). These studies in cattle have spurred similar research in other species, such as sheep.

They serve as important indicators of an individual sheep's ability to produce milk efficiently and maintain good health (De la Fuente et al., 2011). The importance of body morphology traits is related to optimal health, overall productivity levels and flocklife. In dairy cows, some body conformation traits have been related to reproductive efficacy (Boelling et al., 2001), whereas those describing feet and leg confirmation have shown positive correlations with functionally productive life (Caraviello et al., 2004). Stature and body depth may influence feed intake and thus milk production in lactating animals (Veerkamp, 2002; Barillet, 2007). Because of their moderate positive correlations with animal longevity (Dekkers et al., 1994), type traits are considered in cattle breeding schemes. Some authors have suggested that stature and body depth may influence feed intake and thus milk production in lactating animals (Veerkamp, 2002; Barillet, 2007). Based on all this, and the linear scale suggested by De la Fuente et al., (2011), body conformation traits are included as selection criteria in the breeding scheme of some dairy sheep breeds such as Churra. Based on the genetic parameters reported by De la Fuente et al. (2011), no significant correlated genetic response is expected in body conformation traits when selecting for milk production, milk composition and SCC traits, whereas positive and moderate-high phenotypic and genetic correlations were found between the general body score and the udder shape composite trait (De la Fuente et al., 2011).

Nowadays, with the new challenges that livestock breeding faces due to climate change, increase of protein supplementation prices, etc, novel traits are being studied for their future potential to get included as selection objectives. However, previous assessment of genetic parameters and response to selection are needed for these novel traits, which include milk coagulation traits (Sánchez-Mayor et al., 2019; Pelayo et al., 2021), feed efficiency (Hu et al., 2022), lactation persistency (Jonas et al., 2011), resilience traits (Sánchez-Molano, 2020), heat-stress tolerance (Jacobs et al., 2020), etc. In general, selection for these novel traits in sheep would contribute to maintaining a consistent milk supply and to improve flock sustainability, which is becoming very important nowadays. Dairy sheep breeds that are well-suited to local environmental conditions and can efficiently utilize pasture resources are desirable. Selecting for adaptability traits, such as heat tolerance, resistance to diseases, and efficient feed conversion, would ensure the sustainability and resilience of the flock. In this sense, the previously mentioned potential relationship of body traits with individual functional longevity could be of great interest because selection for type traits could improve the real functional production potential of the ewes which is very difficult to be measured in practice (Manca et al., 2017).

2.2. Evolution of gene mapping studies in sheep

Genomics is an interdisciplinary field that studies the genomes of different species, encompassing structure, function, evolution, mapping and editing. Two major branches of genomics are structural genomics, which focuses on DNA sequence variations and their effects, and functional genomics, which examines gene expression and genome regulation. In animal breeding, such as with sheep, genomics aims to identify genetic variations, gene expression patterns, and regulatory mechanisms that directly influence economic traits.

While some livestock traits of interest are single-gene controlled, such as wool color or halothane hypersensitivity, most of these traits are complex and influenced by numerous genes or loci known as QTLs, such as milk production, meat composition, morphological traits, and disease resistance.

The interest in detecting these QTLs holds profound implications for both scientific research and practical applications in agriculture. The investigation and identification of QTLs serve as a critical gateway to a better understanding of the underlying biology that governs these complex traits. This deeper comprehension not only enriches our knowledge of livestock genetics but also offers significant opportunities for practical utilization, particularly in the genetic selection (Georges, 1999).

The first compelling reason for detecting these QTLs lies in the enhanced understanding of the biological mechanisms underpinning livestock traits. By pinpointing the genetic regions associated with a specific trait, we gain insights into the genetic architecture and molecular pathways that control these traits. This knowledge, in turn, provides a solid foundation for further research, allowing us to unravel the complexities of how these traits are inherited and expressed at the genetic level (Carlborg, 2004). The interest in QTL detection also translates into practical advantages for the agricultural industry. One of the most exciting prospects is the ability to leverage genetic markers associated with QTLs for the improvement of livestock. The identification of these markers empowers breeders and farmers to make decisions in their selection and breeding programs. This not only accelerates progress but also enhances the precision of genetic selection in livestock breeding, leading to more efficient and productive agricultural practices (Dekkers, 2004).

During the early studies on QTL mapping in livestock, which primarily used linkage analysis and microsatellite markers, the goal was to use molecular information in selection programs through Marker Assisted Selection (MAS) (Georges et al., 1995). MAS involves using information from markers linked to QTLs in addition to pedigree and phenotypic data. For efficient use of molecular information from QTLs, the identification of the specific gene and mutation responsible for the genetic effect initially mapped as a QTL, called QTN (Quantitative Trait Nucleotide), is necessary. This strategy is called Gene Assisted Selection (GAS) and examples include using the *DGATI* gene genotype in New Zealand dairy cattle and genotyping polymorphisms of the ovine *PRNP* gene to

increase resistance to Scrapie disease. Classical QTL mapping using LA and microsatellites was not very effective in identifying causative mutations despite many studies. NGS technologies were developed as a result of the high level of competition for increased sequence yields towards the end of the Human Genome project.

The evolution of sheep (*Ovis aries*) genomics can be traced back to the early 2000s when the first efforts were made to sequence the sheep genome. At that time, genomics was a relatively new field, and sequencing the large, complex genome of a livestock species was a significant challenge (McEwan et al., 2005). In 2012, the first sheep genome sequence was published, which provided a valuable resource for understanding the biology and genetics of sheep (Jiang et al., 2014). Since then, there has been rapid progress in sheep genomics, with the development of new sequencing technologies, analytical tools, and resources (Clarke and McEwan, 2016). One of the key advances in sheep genomics has been the development of high-density SNP (single nucleotide polymorphism) genotyping arrays. These arrays allow for the simultaneous genotyping of thousands of genetic markers, providing a powerful tool for studying the genetics of complex traits (Kijas et al., 2015).

Another major development in sheep genomics has been the identification of genes and genetic variants associated with important traits, such as wool quality, meat production, and disease resistance. This has been made possible by advances in high-throughput sequencing and bioinformatics tools, which allow for the efficient identification and analysis of genetic variation (Oddy et al., 2016). In addition to identifying genes and variants associated with specific traits, sheep genomics has also been used to study the evolutionary history of sheep and their wild relatives (Hiendleder et al., 2002). Studies have shown that sheep domestication occurred around 10,000 years ago, and that modern sheep breeds are descended from multiple ancestral populations (Pérez-Pardal et al., 2018).

A virtual draft of the sheep genome was initially created using bacterial artificial chromosome end sequences, a sparse marker map, and the sequences of cow, dog, and human genomes (Dalrymple et al., 2007). This virtual genome was further developed using NGS technologies, leading to the first draft of the sheep reference genome. Sequencing advancements allowed for the development of genotyping platforms like SNP chips, which are genomic tools containing thousands of SNPs spaced at constant distances and provide dense coverage of the entire genome in domestic species like sheep. The availability of SNP chips has not only increased the potential for gene mapping, but also allowed for the implementation of GS, a variant of MAS proposed in 2001 by Meuwissen et al. (2001) based on genotyping information. NGS technologies have enabled the characterization and quantification of "omic" sciences such as genomics, transcriptomics, and epigenomics. Overall, the evolution of sheep genomics has greatly enhanced our understanding of sheep biology and genetics and has led to the development of new tools for genetic improvement in the sheep industry. As sequencing technology continues to advance, it is likely that we will see even more applications of genomics in sheep breeding and management.

One of the primary methods to search for genes that influence economically important complex traits in domestic species is the candidate gene approach. This approach involves studying genes that are believed to be involved in physiological pathways related to the trait of interest based on their known function. The approach aims to identify natural variants of the genes that exist in the population and partially explain the variability of the phenotype alongside wild forms of the gene. An example of this is the growth hormone gene (*GRH*) (Soria and Corva, 2004), which is a candidate gene for growth rate or weaning weight (Casas, 2006). The initial studies that utilized the candidate gene strategy in sheep primarily focused on analyzing coding genes responsible for milk proteins, such as alphaS1-casein (Piredda et al., 1993; Chianese et al., 1996) or beta-lactoglobulin (Barillet et al., 2005; Erhardt, 1989), due to their impact on cheese yield related traits. The MEGA-ULE group conducted further studies using this strategy to assess the effect of several SNPs in genes responsible for enzymes involved in fatty acid synthesis on the composition of these milk components (García-Fernández et al., 2010a; 2010b). Additionally, the candidate gene strategy can also be implemented based on knowledge obtained from QTL mapping studies performed on the same or different species. For instance, the MEGA-ULE group investigated the potential influence of genes carrying causal mutations in dairy cattle on milk production traits in Churra sheep (García-Fernández et al., 2011). The genes studied were *GHR*, *DGATI*, and *ABCG2*, which respectively code for growth hormone, acyl-CoA-diacylglycerol acetyltransferase I enzyme, and breast cancer resistance protein. The findings from the research revealed significant associations only for the *ABCG2* gene. This suggests that the genetic architecture of dairy traits may differ between sheep and cattle, and even between breeds of the same species, as noted later by Marina et al. (2021). Despite its usefulness, the candidate gene approach has several limitations, primarily due to incomplete knowledge of the physiological processes that influence the expression of genes that impact complex phenotypes (Gutiérrez-Gil, 2004).

Gene mapping based on microsatellite markers, also known as genome scans, is a technique used to identify and map the locations of genes in the genome of an organism. Microsatellites, or simple sequence repeats (SSRs), are short, repetitive DNA sequences that can be highly variable among individuals. These markers are distributed throughout the genome and can be used to track the inheritance of genetic traits. In a genome scan, a set of microsatellite markers, typically around 200 or more, are used to examine the association between the presence of specific markers and the expression of particular traits or diseases.

In the context of dairy sheep, genome scans based on microsatellite markers have been employed to investigate various aspects of sheep genetics, including milk production (Gutiérrez-Gil et al., 2009a), reproduction, and disease resistance (Gutiérrez-Gil, 2009b). Furthermore, in a study conducted by Gutiérrez-Gil et al. (2011), a genome scan was carried out to identify QTL associated with body conformation traits in Spanish Churra dairy sheep. Microsatellite markers were employed to investigate the genetic basis of these traits, providing valuable insights into the genetic architecture of body conformation in Churra sheep.

Since 2010 advances in animal genomics have revolutionized the field of animal breeding and genetics, enabling more efficient and targeted breeding programs. Some of these key advancements in animal genomics include genome-wide association studies (GWAS), GS, NGS, functional genomics, genomic editing and integrative genomics.

The development of these advancements has only been possible based on the results of the sequencing projects of the non-model livestock species during the first decade of the 21st century, and which were the result of the large development that sequencing technologies showed at the end of the Human Genome Project (Womack, 2005). The mentioned sequencing projects resulted in the identification of thousands of punctual mutations in the genome of the different species known as single nucleotide polymorphisms (SNPs) that allowed the development of the high-throughput genotyping technologies known as SNP-array or SNP-chip technology where about 60,000 (medium-density) or 600,000 (high-density) SNP markers can be interrogated at the same time for a given DNA sample. The SNP-chips are the basis of gene-mapping studies based on the Genome-wide Association Study (GWAS) methodology, which avoids some limitations of the experimental designs needed by classical gene-mapping studies based on Linkage Analysis (LA) and improve remarkable the mapping accuracy of studies trying to identify genetic variants related with phenotypes of economic interest. GWAS has allowed the identification of specific regions in the genome associated with particular traits or diseases (Bolorma et al., 2011; Zhang et al., 2013). By comparing the genomic profiles of animals with contrasting phenotypes, researchers can pinpoint genetic variations that contribute to desired traits. GWAS has facilitated the discovery of numerous genetic markers associated with economically important traits, enabling more targeted selection decisions (Sharma et al., 2015).

The progressive cost reduction of high-throughput genotyping in commercial populations using SNP-chips has made possible the practical implementation of the GS theory, theoretically presented by Meuwissen et al., (2001). The advent of GS has further revolutionized animal genomics. It involves using high-density genetic markers, like SNP-chips, to predict the genomic values of individuals for various traits (Savić an Milenković, 2017), even at a very young age, without relying solely on traditional pedigree and phenotypic data. By genotyping animals and analyzing their genomic data, including single nucleotide polymorphisms (SNPs), breeders can estimate Genomic Estimated Breeding Values (GEBVs). The development of GS has represented the most significant advancement in genetic improvement within the dairy cattle industry over the past three decades. In traditional classical selection methods, estimated breeding values (EBV) are determined through progeny testing and the evaluation of genetic information from offspring, typically assessed at 5 years of age for selection candidates. In contrast, GS utilizes genomic estimated breeding values (GEBV), which can be estimated as early as 3 months of age based on SNP-chip data. Normally, there is a strong correlation of around 70-80% between EBV and GEBV, indicating the reliability of genomic data. However, by incorporating causal mutations into the chip, we have the potential to further enhance the accuracy of GEBV, potentially reaching levels as high as 85%-90%. This advancement holds promise for more precise and efficient breeding

strategies as it offers several advantages, including a shortened generational interval, increased selection accuracy, and the ability to select for complex, economically important traits that are challenging to measure directly. It has particularly transformed livestock breeding programs, including those for sheep, by accelerating genetic progress and promoting the development of more productive and resilient animal populations.

On the other hand, NGS technologies have revolutionized the speed and cost-effectiveness of DNA sequencing. They allow the sequencing of entire genomes, including non-model species, at a much faster pace. NGS enables the discovery of novel genetic variants, improves genome assembly and annotation, and enhances the understanding of the genetic basis of complex traits (Dunisławska et al., 2017). Functional genomics focuses on understanding the functions and interactions of genes within an organism. Techniques such as transcriptomics, proteomics, and metabolomics enable the study of gene expression patterns, protein functions, and metabolic processes. Functional genomics provides insights into the biological mechanisms underlying phenotypic traits and helps identify candidate genes for further exploration. For instance, a causal mutation for mastitis resistance was identified in Lacaune sheep by using whole genome resequencing (WGR) as a complementary strategy to a GWAS analysis (Rupp et al., 2015). In addition, recent advances in gene-editing technologies, such as CRISPR-Cas9, have revolutionized the field of animal genomics. These tools allow precise modifications of the genome by adding, deleting, or modifying specific DNA sequences. Genomic editing offers immense potential for improving desirable traits, enhancing disease resistance, and reducing the prevalence of genetic disorders in livestock populations (Bhat et al., 2017). Integrative genomics involves combining genomic data with other types of biological information, such as transcriptomics, epigenomics, and phenotypic data. This multi-omics approach provides a more comprehensive understanding of the complex interactions between genes, the environment, and phenotypic traits. It enables the identification of key regulatory mechanisms and networks underlying important traits (Ritchie et al., 2015).

It is expected that all these advances in animal genomics may significantly help to reach a better understanding of the genetic architecture of traits of economic interest in livestock populations, and improve breeding strategies, facilitating the development of more productive and resilient livestock populations. Considering the current challenges of animal production nowadays, animal genomics could be essential to pave the way for more efficient and sustainable animal production systems, benefiting both producers and consumers.

2.3. Dairy sheep in the Spanish region of Castilla y Leon and the Churra milk selection program

Spain is one of the leading countries in the world in terms of dairy sheep production, and the genetic improvement of dairy sheep has played a significant role in this success (Mantecón and Juárez, 2015). Considering the last reports from the Spanish Ministry of Agriculture, Fisheries and Food, Spain is the fifth country in the world in terms of sheep's milk production and the second in terms of sheep's cheese production, accounting for 27.3% of sheep's milk produced in the European Union in 2020 (MAPA, 2022). In this context, it is important to highlight the importance of the Community of Castilla y León, which, with about 2.4 million dairy sheep, represents 39.7% of the production of the dairy sheep sector in Spain (MAPA, 2022).

Churra sheep is the most important autochthonous milk-producing population in the region of Castilla y León, although it can be considered of double aptitude based on its milk production specialization and the related production of suckling lamb meat and is reared in semi-extensive production systems based on grass-based rearing. This local breed shows good characteristics of hardiness and adaptation to the environment and is one of the most adapted breeds to the prevailing climatic conditions of the region. However, nowadays a significant decline in Churra breed census is being observed due to the displacement determined by foreign breeds with higher milk production potential, such as the Assaf breed, which is a stabilized cross between Awassi and Milchschaf breeds, and the French Lacaune breed (De la Fuente et al., 2006; Ugarte et al., 2001).

The Spanish Association of Sheep Breeders (ANCHE) is responsible for coordinating the national selection and breeding programs for milk production of Churra sheep in Spain. This program was designed in 1984, with the intention of avoiding the disappearance of this breed and increasing its competitiveness in the sector and began to be implemented in 1986 group (de la Fuente et al., 1995), with the technical support provided by the group of Animal Breeding of the University of León (MEGA-ULE). The selection program aims to increase the genetic merit of the Churra sheep population for milk production through advanced breeding techniques, such as artificial insemination and embryo transfer, as well as through the selection of superior sires and dams (ANCHE, 2021).

The Churra selection program for milk production was initially based on two primary objectives, the increase of milk production while maintaining or improving milk quality traits such as fat and protein content, which are directly related with the cheese yield. In this way the program focused on the parameters that most directly influence the profitability of livestock farms (de la Fuente et al., 1995). Later, in 2003, two new groups of functional traits were introduced as secondary objectives of the Churra selection program and considered in the estimation of the genetic index of the sires used for artificial insemination. These were mammary morphology traits (udder depth,

udder attachment, teat position, teat size, and udder shape) (de la Fuente et al., 1996) and body morphology traits (stature, rump width, feet angle, rear legs and general body score) (de la Fuente et al., 2011). Due to the European regulations (Commission Decision 2003/100/CE), the resistance to scrapie is also considered in this selection program. The classical milk production selection program, whose updated version can be found in the Spanish national database of livestock breeds (ARCA, 2023), relies on performance monitoring and the pedigree information to obtain the animals' estimated breeding values (EBV).

2.4. The use of molecular genetic information in Churra sheep

In addition to the tasks related with the coordination of the Churra genetic program, ANCHE routinely collaborates with the MEGA-ULE group in research projects defined with the aim of providing solutions to practical problems of the sheep dairy sector through the application of genetic studies. The initial projects performed in this sense by the MEGA-ULE group, which has hosted and provided the data analyzed in the present Master's Thesis, were related to the study of genetic parameters and the study of environmental factors influencing traits of economic interest in dairy sheep (Othmane et al., 2002; Gutierrez-Gil et al., 2009a, 2009b; Carriedo et al., 2009). Later, by applying progressively the advances that have taken place in the field of animal genomics, the MEGA-ULE group has conducted numerous studies aimed at identifying genes and mutations with effects on the traits of interest in the Churra breed selection program for milk production. The objective of these studies, in general, has been to generate molecular information that could be used as an additional source of information to enhance the efficiency of classical genetic selection, based on the estimation of classical breeding values. Nowadays, considering the progressive implementation of the GS in the Churra selection scheme, it is expected that the potential molecular information of interest generated through research projects could be more efficiently addressed to the improvement of the accuracy of the GEBVs.

The research projects and studies undertaken by the MEGA-ULE group during the last 20 years follow the evolution of the technologies and analysis approaches used in the animal genomics field during those years, specifically those available for the sheep species. Hence, considering the interest of identifying genetic variants associated with the traits of interest in the Churra sheep breeding program, the first studies of this group implemented the candidate gene strategy, where a gene or a set of genes are selected for detection of genetic variation, assuming a potential association with the traits of interest based on their known biological function (García-Fernández et al., 2011; Morán Pérez, 2016). Then, trying to avoid the major limitation of the gene candidate approach, related with our limited knowledge on the biological mechanisms underlying the phenotypes of interest, the group performed genome-wide scan studies of the sheep genome based on microsatellite markers to identify genomic regions associated with the traits of interest, known as Quantitative Traits Loci (QTL). Hence, analyzing a commercial population of Churra dairy

sheep, this group was the first one reporting a genome scan for milk production traits in dairy sheep (Gutiérrez-Gil et al., 2009a), whereas later studies reported QTLs for mastitis resistance mammary and body morphology, and nematode resistance (Gutiérrez Gil et al., 2007, 2008, 2009b, 2011). However, the limited gene mapping accuracy of these genome scans determined the lack of results with a direct potential practical application.

The mentioned limitation was solved some years later based on the availability in 2009 the Illumina Ovine SNP50 BeadChip® (50K-SNP-chip). This genomic tool had been developed by the International Sheep Genomic Consortium (ISGC) based on the information generated through the first attempts of sequencing of the sheep genome and provided an efficient tool to undertake research on the ovine genomic architecture of complex traits (International Sheep Genomics Consortium, 2008). Using the 50K-SNP-chip in a population of 1,680 half-sister ewes from 16 families, the MEGA-ULE group reported QTLs associated with various milk production traits (García-Gómez et al., 2012a, 2012b), gastrointestinal nematode infection resistance (Atlija et al., 2016), mammary morphology (Justo, 2017; Vrcan, 2022) and subclinical mastitis resistance (Gutiérrez-Gil et al., 2018). The higher marker density provided with the 50K-SNP-chip substantially increased the QTL detection power and the more accurate redefinition of the confidence interval. In the case of milk production traits, the GWAS analysis performed with this genomic tool identified a potential causal mutation, or QTN (from Quantitative Trait Nucleotide) for the OAR3 QTL previously reported based on the microsatellite-based genome scan (García-Gómez et al., 2012).

In any case, the practical implementation of the results of these gene-mapping studies into the classical genetic selection program of Churra sheep is difficult, even in the case of the causal mutation for milk protein content detected in the *LALBA* gene (García-Gómez et al., 2012a, 2012b). In the last years, and through the support of the Ministry, ANCHE is starting to apply the Genomic Selection (GS) approach by genotyping with a medium density SNP-chip a subset of the candidates for selection and this information is used to estimate genomic breeding values (GEBV) at an early age before progeny testing results are available to define the list of candidates. In the present situation, as previously mentioned, the results of gene-mapping studies could be efficiently exploited if they were included as additional information in the framework of the GS program that is in development for Churra sheep. An initial assessment of the use of a low-density SNP-chip and genotype imputation to implement GS in Churra sheep has been reported by Marina et al., (2022).

2.5. Importance of body morphology traits in Churra sheep program

Traits describing body conformation play a significant role in estimating mobility, productivity, longevity, and reproductive effectiveness in dairy cattle. Considering the importance in cattle, the body morphology traits was included in 2002 as selection objective in the Churra sheep breeding program. Currently, the integration of body conformation traits in sheep breeding programs represents a promising avenue for improving various aspects of sheep production.

The MEGA-ULE group designed a study to estimate the genetic parameters of body conformation traits and evaluate their correlation with mammary morphology traits, somatic cell count, milk yield, and composition in the Churra sheep (de la Fuente et al., 2011). This study showed the existence of phenotypic correlations between the traits of rump width (-0.36), feet angle (0.29), and overall conformation (-0.34) with milk production. These correlations would indicate that more productive sheep have a more horizontal feet angle, wider rump, and a lower score for overall body conformation (de la Fuente et al., 2011). In the Churra sheep breed, body conformation traits, as well as mammary morphology traits, are scored on a nine-point linear scale from 1 to 9 according to the scoring method determined by de la Fuente et al. (1996). This work will focus on the study of traits related to body morphology. Among them, there are four simple traits (stature, rump width, rear legs - rear view, and feet angle). De la Fuente et al. (2011) defined each trait and its evaluation method. The **stature** refers to the height to the tallest point of the rump and is related to the animal's size. The highest score assigned is 9, while the lowest is 1. The medium score of 5, which refers to the medium size, is most desired. **Rear legs**, as a trait, refer to the vertical straightness of the rear legs. As this trait determines mobility and movement, straight legs, with a score of 9, are preferable. "**Feet angle** is assessed by considering the angle formed by an axis that crosses the three phalanges of the hind articulation in comparison with the horizontal axis." Horizontal phalanges, as well as vertical phalanges, are not desired. The ideal score is 5. Width between hind-quarters is called **rump width** and it is preferred to be the widest possible, with a score of 9. It is relevant because it is related to the width of the pelvic canal, and thus with parturition (de la Fuente, 2011).

Based on the heritability estimates for body conformation traits, it can be concluded that genetic selection will effectively enhance these traits in dairy ewes. The heritability values for udder traits are similar to those of body conformation traits, indicating that genetic selection can effectively improve both types of traits. Moreover, selecting for milk yield, protein and fat percentages, and SCC traits in Churra ewes is not expected to result in a significant correlated genetic response in body conformation traits. Considering the genetic parameters of body conformation traits, which align with the requirements of the linear scale and are easily interpretable by classifiers, it is feasible to improve them through selection in dairy sheep (de la Fuente, 2011).

The importance of longevity and type traits in dairy cattle lies in their influence on lifetime productivity, sustainability, and animal welfare. Longevity, which refers to the cow's lifespan in

the herd, is a valuable trait for reducing production costs and improving profitability. Type traits, which pertain to body conformation and structure, affect mobility, health, and overall animal well-being. There is a growing interest in using type traits to enhance functional longevity, as these characteristics can contribute to a cow's ability to lead a productive and healthy life. By improving type traits that support longevity, the dairy industry can achieve more sustainable and economically viable practices while prioritizing animal welfare (Hu et al., 2021).

The dairy industry is gradually shifting its focus towards more comprehensive breeding goals as it adapts to intensive, large-scale, and mechanized practices on a global scale. There is a growing emphasis on the breeding value, lifetime productivity, sustainability in the face of climate change, and environmental concerns for dairy cattle. This shift has led to a more balanced approach, encompassing various breeding objectives such as longevity, health, animal welfare, milk yield, milk quality, and environmental sustainability. In many developed countries, longevity has already been integrated into breeding programs, acknowledging its importance to dairy production (Hu et al., 2021).

In addition to the remarkable strides made in the field of genomic selection (GS), the identification of genetic markers tailored specifically for body morphology presents a promising frontier. The integration of these markers into the existing SNP-chip, the cornerstone of the initial GS program, holds the potential to revolutionize our approach to selecting animals with desirable body conformation traits. By enhancing the efficiency of selection for these traits, this innovative step not only ensures the production of livestock with optimal body structures but also paves the way for improved mobility, productivity, and animal welfare in the agricultural sector. As genomics continues to unlock new avenues of precision in animal breeding, the prospect of tailoring genetic markers for body morphology offers exciting opportunities for the future of livestock production (Goddard and Hayes, 2009).

3. Materials and methods

3.1. Population, Phenotypes and Genotypes

The studied sheep population, as previously described by García-Gómez et al. (2012a), consisted of 1,696 individuals of the Spanish Churra sheep breed and included 1,690 ewes distributed into 16 half-sib families and the 16 sires of those families, all of them artificial insemination (IA) sires from the selection nucleus of the National Association of Breeders of Selected Churra Sheep (ANCHE). The average size of the family ranged from 29 to 277 daughters per ram (García-Gómez et al., 2012a). All the ewes in that population had available phenotypic records for four linear body conformation traits routinely recorded by ANCHE within the milk production breeding scheme of the breed: stature, rump width, rear legs, and feet angle. These traits are measured in the linear scale described by De la Fuente et al., (2003).

As previously described by García-Gómez et al. (2012a), the complete population had available genotypes generated by the MEGA-ULE group and ANCHE with a medium-density SNP-array, the Illumina OvineSNP50 BeadChip, which will be referred here from now on as 50K-SNP-chip. The analysis of the raw genotypes, which contained 1,696 individuals and 54,241 SNPs, started with implementing a quality control (QC) analysis in two phases using the PLINK software. In the first phase, the QC per individual, the individuals with more than 10% missing genotypes were removed (call rate >90%). After that the second phase of QC filtering, QC per SNP, was performed by applying the following criteria: call rate >95%; minor allele frequency (MAF) >0.05; correspondence with Hardy-Weinberg equilibrium (HWE), p value > 0.00001. After this QC, a total of 1,678 animals and 40,291 SNPs remained for the following analyses. The initial marker positions of the Chip-50K provided for this work were based on the Oar_v3.1 version of the sheep reference genome. Hence, also using PLINK, the corresponding processing steps were performed on the genotype dataset to update marker positions to the latest version of the sheep genome, Oar_Ramb_v2.0 (https://www.ncbi.nlm.nih.gov/assembly/GCF_016772045.1/). These update physical positions were then used to create the map files needed for both the LA and the GWAS analyses.

3.2. Phenotypes used for QTL mapping

The dependent phenotypic variables used for QTL mapping analysis were the Yield Deviation (YD) estimated, following Vanraden and Wiggans (1991), from the raw phenotypic records available for the five body morphology traits under study. Hence, the YDs for the each of the four traits under study were estimated as the deviation from the average of the raw phenotypic data corrected for the fixed environmental effects and the environmental permanent effect by using multivariate repeatability models (Garcia-Gamez, 2012a). The fixed effects used in YDs

calculations refer to the herd-test-day, birth order, the number of weeks in milk production and three covariates nested to birth order: the age of the ewe at parturition, the milk production in the previous lactation and the number of born lambs.

3.3. QTL mapping analysis

In this work we have implemented two different QTL mapping strategies. First, we have performed a genome-wide scan based on Linkage Analysis (LA) using the QTLMap software, which was created and developed at the National Institute for Agricultural Research in France (INRA) (Filangi et al., 2010). The genetic map with the marker order and positions was based on the sheep reference genome Oar_Ramb_v2.0 physical map. As described in García-Gómez et al. (2012a), the physical distances were converted into genetic distances using the 1cM ~1Mb equivalence. For this LA analysis, the option *calcul --4* for single traits of the software was applied to each of the four traits under study. The QTL and the polygenic effects of the sires were assessed and the chromosome-wise significance thresholds were defined using 1,000 permutations at 0.1 cM steps. Following Atlija et al. (2016), for each trait, a genome-wise significance threshold was also defined based on the chromosome-wise significance thresholds by correcting the total chromosome number analyzed. The within-family linkage analyses were performed for each QTL detected in LA across-family scan in order to identify the appropriate segregating families. To determine the confidence intervals (CI) for the QTL detected by performing the LA, likelihood ratio test (LRT) values were converted into logarithm odds ratio (LOD) values (Beraldi et al., 2007), and the corresponding confidence intervals (CI) were then defined using the 1-LOD drop-off method described by Botstein and Lander in 1989.

Beside the linkage-based analyses, a genome-wide association (GWA) analysis was carried out using the Genome-wide Complex Trait Analysis (GCTA) software (Yang et al., 2011), following the linear mixed model formula:

$$Y = Q_0 + Wu + e$$

where Y is the ewes' vector of the phenotypes (YD), Q_0 represents the intercept of the regression model, W is the SNP markers' incidence matrix, u is the vector of random SNP effects, and e is the vector of residual effects. We assume that u and e are normally distributed with a mean of zero and variance of $\sigma_u^2 \mathbf{I}$ and $\sigma_e^2 \mathbf{I}$, respectively, being \mathbf{I} the identity matrix.

The software GCTA was initially created to calculate the proportion of phenotypic variance for a complex trait that can be explained by all of its genome-wide SNPs (Yang et al., 2011). When estimating the SNP effects, the GCTA software corrects for the family-related population substructure. To assess the genome-wise and chromosome-wise significant thresholds for the GWA analyses, we applied the Bonferroni correction by implementing the method described by Gao et al. (2008). This method considers that the markers analyzed across the genome are not independent and was previously implemented in other studies analyzing the genotype dataset here

analyzed (García-Gómez et al. 2012a; Atlija et al. 2016). Hence, firstly, the number of effective tests (M_{eff}) was calculated using the LD estimate for each chromosome (M_{eff_C}) using the following parameters for the *indep-pairwise* command: window size in SNPs, 50; the number of SNPs to shift the window at each step, 5; and the r^2 threshold, 0.2. After that, the adjusted significance level for each chromosome, was calculated as follows:

$$\alpha_C = \alpha_e / M_{eff_C}$$

where α_C stands for the adjusted significance level for each chromosome (chromosome-wise significance threshold), the α_e is the error rate of type I (0.05), and M_{eff_C} is the number of markers that were independently analyzed per chromosome. Later the number of independently analyzed SNPs across the genome was then corrected to determine the genome-wise significance threshold, based on the α_C . Hence, a total of 16,520 SNP markers were identified as independently analyzed across the genome and a value $\log(1/P\text{-value}) = 5.519$ was defined as the genome-wise significance threshold for the GWAS analysis. Based on this, only the SNPs reach at least corresponding the chromosome-wise significant threshold were considered as significant markers and included in the subsequent analyses.

3.4. Overlapping with previously reported QTL and gene annotation

For the significant QTL identified by LA, to determine their physical positions in the sheep reference genome, we converted the CI flanking interval positions (in centimorgans) to base pairs (bp) assuming the 1cM ~1Mb equivalence. These genomic intervals, which were considered as target genomic intervals (TGIs), were then subjected to gene and QTL annotation using the GALLO R package. This package offers a systematic approach to annotate positional candidate genes in genomic regions of interest (Fonseca et al., 2020). For gene annotation, the annotation file for the Oar_Ramb_v2.0 ovine reference genome was obtained from https://www.ncbi.nlm.nih.gov/data-hub/genome/GCF_016772045.1/. For the QTL annotation, the gff file from Sheep QTLdb correspondent to the ARS-UI_Ramb_v2.0 of the ovine genome was used. The GALLO package was also used to perform a QTL enrichment analysis for each trait annotated in the Sheep QTLdb within the TGIs defined using a genome-wise approach. The enriched QTLs were defined based on a False-Discovery Rate (FDR) <0.05 and a total number of QTLs reported in the Sheep QTLdb higher than one.

Similarly, for the significant SNPs identified by GWAS analyses, we defined corresponding TGIs. These TGIs encompassed 2.50 Mb intervals, with a 1.25 Mb region at each side of the significant SNP. TGIs defined based on the GWAS analysis were also considered for gene and QTL annotation conducted using the GALLO R package, following some procedures described above.

4. Results

4.1. QTL regions detected

The LA genome scan revealed eight QTLs significant at the 5% chromosome level for four body morphology traits: stature, rump width, rear legs (rear view), and feet angle, whereas no QTLs reached genome-wide significance. The details of the characterization of QTL regions identified is presented in Table 4.2.1. On the other hand, the GWAS analysis performed identified 27 chromosome-wise significant QTLs associated with all the studied traits, as presented in Table 4.3.1. Further information on the significant findings from each analysis is provided below.

4.2. Results of the LA analysis performed for body conformation traits

The regression analysis, conducted across families, examined stature, rump width, rear legs (rear view), and feet angle across the 26 ovine autosomes. This analysis identified eight QTLs that were significant at the chromosome-wise level. Among these QTLs, one was located on OAR (Ovis aries chromosome) 3 (peak of 186.65 cM) impacting stature. Additionally, two QTLs on OAR5 (peak at 20.07 cM) and OAR23 (peak at 19.34 cM) were associated with rump width. Two QTLs were found on OAR2 (peak at 211.52 cM) and OAR18 (peak at 18.82 cM) for rear legs (rear view), while QTLs on OAR1 (peak at 11.66 cM) and OAR14 (peak at 14.88 cM) affected feet angle. Table 4.2.1 provides details of the significant QTLs identified by the across-family LA analysis, including their corresponding confidence intervals estimated by the 1-LOD drop-off method. The results of the within-family analyses are also provided in Table 4.2.1. Additionally, Figure 4.2.1 illustrates Manhattan plots representing the LRT statistical values obtained from the across-family LA analysis for each trait across the ovine autosome.

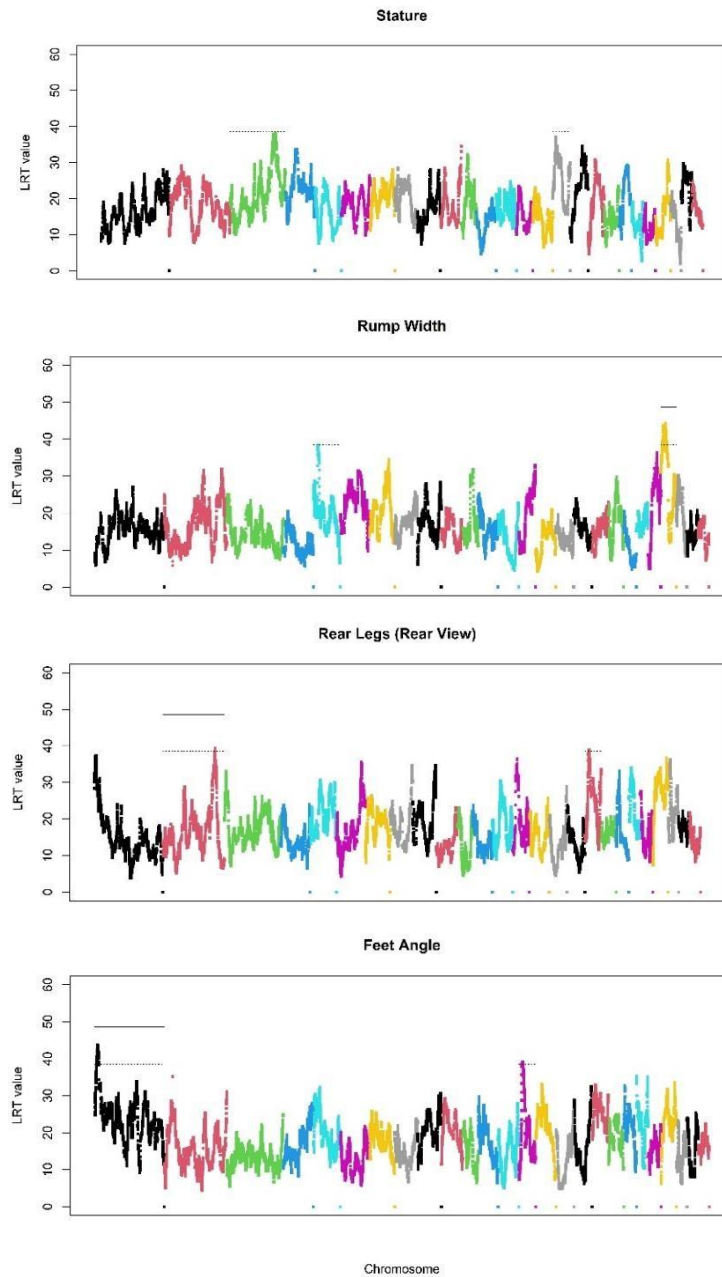


Figure 4.2.1. Results of the linkage analysis performed at the across-family level for the four body conformation traits under analysis. The autosomic chromosomes of sheep analyzed, from 1 to 26, are consecutively shown in different colours. For the chromosomes harboring a significant QTL, the chromosome-wise (dashed line) and genome-wide significance thresholds are indicated as horizontal lines.

Table 4.2.1. Significant chromosome-wise QTLs detected by linkage analysis (LA) for the body conformation traits analyzed in the present work.

Trait ^a	OAR ^b	Across-family analysis			Within-family analysis		
		Pos of max LRT (cM) ^c	P-value ^d	CI (cM) ^e TGI (Mb) ^f	Number of overlapping QTLs (QTL ID) ^g	Segregating family identifier (Pos of max LRT, cM) ^h	Size effect trait units (SD units) ⁱ
Stature	3	186.65	<0.05	18.15-18.82	1 (14014)	Fam. 3362 (192.85) Fam. 3535 (158.15)	0.111 ± 0.089 (0.59) 0.224 ± 0.165 (1.19)
	16	12.57	<0.05	11.8-13.5	2 (14306, 14307)	Fam. 3535 (12.57)	0.261 ± 0.131 (1.38)
Rump width	5	20.07	<0.05	16.2-22.5	2 (12934, 211652)	Fam. 5041 (16.37) Fam. 3362 (5.47)	No -0.015 ± 0.014 (-0.06)
	23	19.34	<0.05	18.6-22.4	7 (13963, 14311, 14312, 14313, 14314, 14335, 14336)	Fam. 5041 (2.04) Fam. 2387 (54.44)	No -0.155 ± 0.016 (-0.57)
Rear legs (Rear view)	2	211.52	<0.05	210.1-213.6	0	Fam. 3362 (113.72) Fam. 1671 (200.62) Fam. 2387 (129.22) Fam. 3305 (207.22) Fam. 2976 (206.02)	0.095 ± 0.070 (0.23) -0.581 ± 0.069 (-1.40) -0.409 ± 0.084 (-0.99) 0.064 ± 0.075 (0.15) 0.077 ± 0.069 (0.19)
	18	18.82	<0.05	18.1-22.4	3 (12924, 12926, 147165)	Fam. 2387 (28.52) Fam. 3331 (49.62) Fam. 3296 (16.22)	-0.395 ± 0.091 (-0.95) 0.226 ± 0.089 (0.55) -0.165 ± 0.105 (-0.40)
Feet angle	1	11.66	<0.05	9.5-17.2	1 (14276)	Fam. 5041 (17.46) Fam. 3535 (171.06) Fam. 3331 (41.56) Fam. 2406 (162.46) Fam. 1444 (142.96)	No -0.172 ± 0.046 (-0.37) -0.177 ± 0.030 (-0.38) -0.408 ± 0.017 (-0.87) -0.147 ± 0.019 (-0.31)
	14	14.88	<0.05	11.6-19.0	1 (14302)	Fam. 5041 (19.98) Fam. 3331 (15.88) Fam. 3153 (41.88)	No -0.189 ± 0.026 (-0.40) -0.215 ± 0.038 (-0.46)

^a Analyzed traits.

^b OAR ovine chromosome.

^c Position of the chromosome (in centiMorgans) at which the maximum LRT of the LA is reached in the analysis involving 16 half-sib families included in this work (across-family analysis) or the individual analysis of the segregating families (those showing a Pc-value <0.05 in the within-family analysis), respectively.

^d Pc-value chromosome-wise significant P-value established through 1,000 permutation analysis. (Pg-value genome-wise significant P-value, only if significant) The genome-wise significant QTL are highlighted in bold font.

^{e,h} CI confidence interval (in cM) estimated from the position of the max LRT for the across-family analysis, respectively, following the 1-LOD-drop-off method (García-Gómez et al., 2012)

^f TGI Target genome interval (Mb) defined as the corresponding genomic regions, according to the reference sheep assembly Ramb_v2.0, to the CI estimated for LA significant QTL.

^g Number of overlapping QTL/Associations annotated in SheepQTLdb in relation to traits of interest for this study for the corresponding QTL region identified in this work. The unique QTL identifier of SheepQTLdb is provided in brackets.

ⁱ Estimated size effect of the QTL identified in within-family analysis expressed in trait units (Yield Deviation) and in phenotypic SD of the trait (in brackets)

4.3. Results of the GWAS analysis performed for body conformation traits

The GWAS analysis performed identified 27 SNPs exceeding the 5% chromosome-wise significance level. These SNPs influenced all four traits under study. In addition, no SNPs reached the 5% genome-wide significance threshold (Figure 4.3.1., Table 4.3.1.). Regarding the chromosome distribution and traits affected by the significant associations identified, six chromosome-wise significant SNPs for stature were distributed on five chromosomes (OAR4, OAR6, OAR11, OAR20 and OAR26) and seven chromosome-wise significant associations were identified for rump width (OAR3, OAR6, OAR10, OAR19, OAR22, OAR24). For rear legs (rear view), there were eight significant SNPs that reached chromosome-wise significance, distributed on six chromosomes (OAR1, OAR2, OAR9, OAR10, OAR21 and OAR 25). Finally, six chromosome-wise significant SNPs were identified and distributed on five chromosomes (OAR4, OAR5, OAR20, OAR22 and OAR24) for feet angle (Table 4.3.1., Figure 4.3.1.). The allelic substitution effects of all the significant SNPs identified ranged from -0.228 (for on OAR20), to 0.233 SD unit (for on OAR4) (Table 4.3.1.). As mentioned in the Methods section, for each significant SNP, a TGI was defined considering the LD extent previously reported in Churra sheep (see details in Table 4.3.1.).

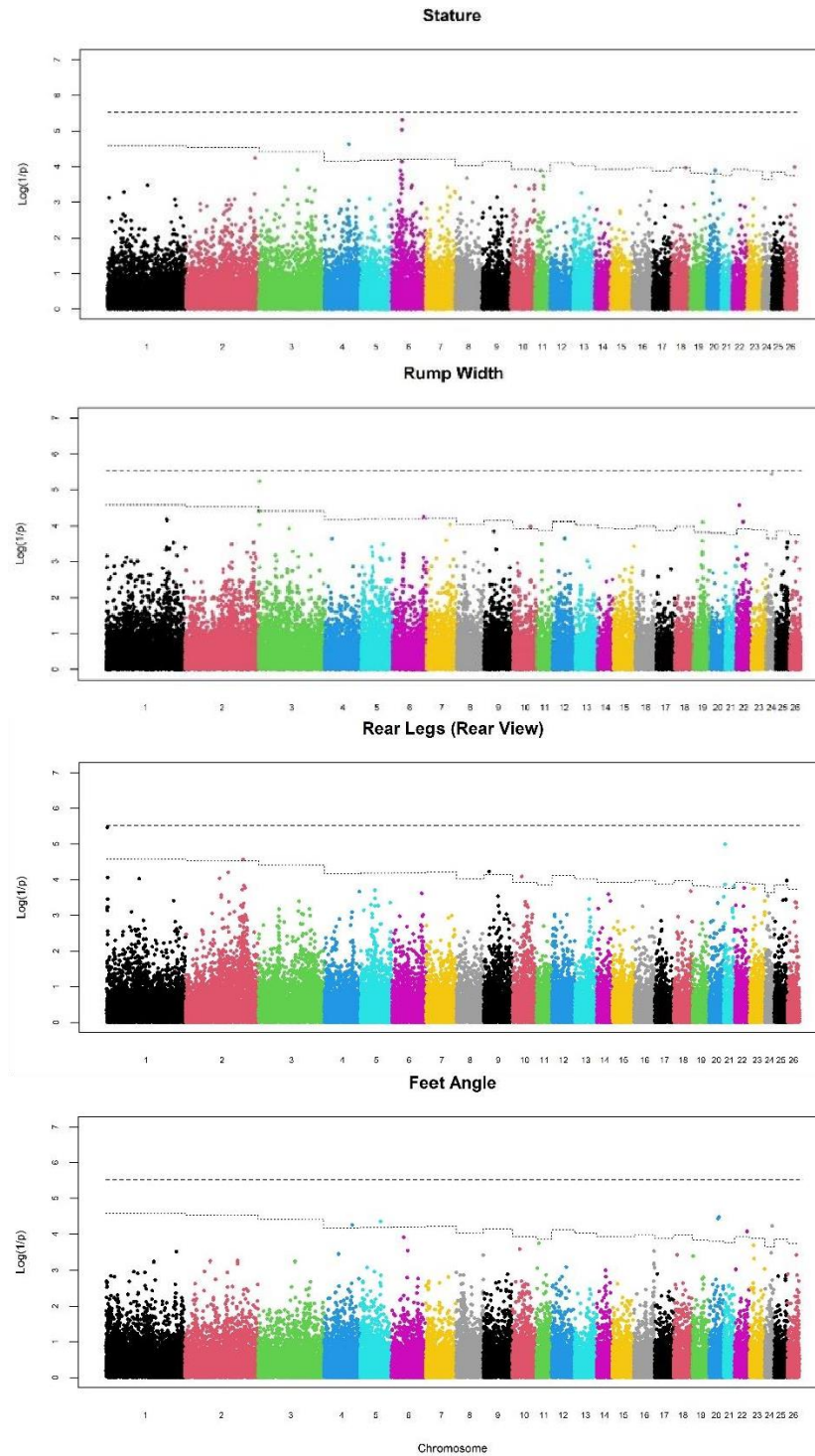


Figure 4.3.1. Graphical representation of the statistical profile obtained by the GWAS analysis performed for body conformation traits in the present work. The autosomic chromosomes of sheep analyzed, from 1 to 26, are consecutively shown in different colours. Horizontal thicker dashed line and horizontal thinner dashed line represent the genome-wise threshold [$-\log_{10}(\text{P-values}) > 5.52$], and the chromosome-wise threshold, respectively.

Table 4.3.1. Chromosome-wise SNPs significantly associated with body conformation traits identified by GWAS

OAR ^a	Trait	SNP name	Top SNP position ^b	Allele substitution effect trait units (SD units) ^{c,d}	Nominal P-value	P _q -value (P _q -value)	TGI (Mb) ^f	Number of overlapping QTLs (QTL ID) ^g
1	Rear Legs	s46580.1	2689885	0.086 ± 0.018 (0.208)	3.43E-06	0.015	2.56-2.81	1 (14276)
2	Rear Legs	OAR2_207906714.1	197459645	-0.060 ± 0.014 (-0.145)	2.70E-05	0.112	197.33-197.58	4 (14280, 14254, 14279, 14253)
3	Rump Width	OAR3_4044086.1	4153155	0.055 ± 0.012 (0.204)	5.90E-06	0.022	4.03-4.28	0
4	Stature	s31422.1	82805508	0.044 ± 0.010 (0.233)	2.38E-05	0.049	82.68-82.93	0
	Feet Angle	OAR4_98852126.1	94870088	0.107 ± 0.027 (0.227)	5.56E-05	0.114	94.75-94.10	0
5	Feet Angle	s46021.1	72097117	-0.107 ± 0.026 (-0.227)	4.42E-05	0.079	71.97-72.22	1 (12934)
6	Stature	OAR6_40955920.1	37465730	0.033 ± 0.008 (0.175)	9.29E-06	0.018	37.34-37.59	27 (14284, 14261, 57680, 193060, 193064, 193073, 193074, 57663, 57643, 95743, 95748, 95746, 14285, 95775, 14282, 14332, 95757, 95767, 95771, 14283, 14260, 14286, 14333, 127004, 95756, 95766, 95768)
	Stature	s56396.1	38605646	0.031 ± 0.007 (0.164)	4.93E-06	0.010	38.48-38.73	13 (13950, 14284, 14261, 13934, 57641, 57655, 14285, 14282, 14332, 14283, 14260, 14286, 14333)
	Rump Width	OAR6_119546066.1	106250133	0.041 ± 0.010 (0.152)	5.71E-05	0.110	106.13-106.38	3 (14285, 14282, 14332)
9	Rear Legs	OAR9_19107267.1	18498211	0.061 ± 0.015 (0.147)	5.89E-05	0.096	18.37-18.62	3 (14290, 14291, 14289)
10	Rump Width	OAR10_64413399.1	62610720	0.041 ± 0.011 (0.152)	1.05E-04	0.142	62.49-62.74	4 (14293, 14294, 14292, 14295)
	Rear Legs	OAR10_34110882.1	33805371	-0.060 ± 0.015 (-0.145)	8.14E-05	0.110	33.68-33.93	5 (218576, 14293, 14294, 14292, 14295)
11	Stature	OAR11_29443457.1	28029464	-0.036 ± 0.009 (-0.190)	1.28E-04	0.112	27.90-28.15	5 (57792, 14297, 14296, 16017, 16018)
19	Rump Width	OAR19_34614508.1	33033938	0.048 ± 0.012 (0.178)	8.01E-05	0.076	32.91-33.16	1 (14310)
20	Stature	s69570.1	29515770	-0.043 ± 0.011 (-0.228)	1.27E-04	0.104	29.39-29.64	5 (13790, 13783, 13788, 13784, 14151)

Table 4.3.1. continue. Chromosome-wise SNPs significantly associated with body conformation traits identified by GWAS

OAR ^a	Trait	SNP name	Top SNP position ^b	Allele substitution effect trait units (SD units) ^{c,d}	Nominal P-value	P _c -value (P _q -value) ^e	TGI (Mb) ^f	Number of overlapping QTLs (QTL ID) ^g
20	Feet Angle	OAR20_35185147.1	32191614	-0.105 ± 0.025 (-0.223)	3.74E-05	0.031	32.07-32.32	0
	Feet Angle	OAR20_38909849.1	35845272	-0.069 ± 0.017 (-0.146)	3.32E-05	0.027	35.72-35.97	5 (14151, 13795, 16614, 14151, 16615)
21	Rear Legs	OAR21_8294093.1	7192930	-0.088 ± 0.020 (-0.213)	1.01E-05	0.006	7.07-7.32	0
	Rear Legs	s62668.1	7700964	-0.060 ± 0.016 (-0.145)	1.37E-04	0.086	7.58-7.83	0
	Rear Legs	s52755.1	42591452	0.058 ± 0.015 (0.140)	1.59E-04	0.100	42.47-42.72	1 (169405)
22	Rump Width	OAR22_12892429.1	11031179	-0.044 ± 0.010 (-0.163)	2.67E-05	0.023	10.91-11.16	0
	Rump Width	OAR22_30511327.1	26767888	-0.042 ± 0.011 (-0.156)	7.81E-05	0.066	26.64-26.89	0
	Feet Angle	OAR22_48708041.1	43967950	-0.068 ± 0.017 (-0.144)	8.49E-05	0.072	43.84-44.09	0
24	Rump Width	s16016.1	25115046	0.049 ± 0.010 (0.181)	3.59E-06	0.002	24.99-25.24	9 (13947, 13953, 13960, 13926, 13931, 13937, 13686, 13687, 14315)
	Feet Angle	s02082.1	33953904	-0.076 ± 0.019 (-0.161)	5.99E-05	0.032	33.83-34.08	4 (13686, 13687, 14315, 14008)
25	Rear Legs	s37537.1	41902925	-0.062 ± 0.016 (-0.150)	1.06E-04	0.080	41.78-42.03	2 (12925, 211622)
26	Stature	s03810.1	37810996	0.029 ± 0.007 (0.153)	1.03E-04	0.069	37.69-37.94	1 (14185)

^a OAR ovine chromosome

^b Position of the significant SNP identified by the GWAS analysis based on the Oar_rambv2 version of the Ovine Genome Assembly (<https://www.ncbi.nlm.nih.gov>)

^{c, d} Magnitude of the allele substitution effect, and standard error, in trait units (Yield Deviations) and in phenotypic standard deviations (SD) units (in brackets)

^e Corrected P-values at the 5 % chromosome-wise level (and 5 % genome-wise level) obtained after applying a Bonferroni correction considering the number of independent markers analyzed for each chromosome and for the whole genome, respectively.

^f TGI Target genomic interval defined for the GWAS significant associations as 250 Kb long intervals centred on the significant SNP. The genes within that interval were extracted as positional candidate genes. In this case, none of these genes was identified as a functional candidate by the candidate gene survey performed.

^g Number of overlapping QTL/Associations annotated in SheepQTLdb in relation to traits of interest for this study for the corresponding QTL region identified in this work. The unique QTL identifier of SheepQTLdb is provided in brackets.

4.4. Correspondence with previous studies and potential candidate genes

Using the GALLO R package, we conducted a systematic extraction of positional candidate genes, resulting in a total of 1,558 annotated positional candidate genes identified from the TGIs defined based on significant results from both LA and GWAS significant genomic regions.

To gain a better understanding of the genetic architecture of the body morphology traits under consideration, we incorporated multiple sources of biological information. To achieve this, we conducted QTL annotation and enrichment analyses using the SheepQTLdb information for all significant regions detected through both LA and GWAS analyses. Figure 4.4.1. provides insights into the proportion of different trait types (milk, meat and carcass, health, production, reproduction, and exterior) and different exterior traits of the QTLs and Associations annotated in the SheepQTLdb that overlapped with the significant regions detected in the LA analysis. This information is represented both as a pie chart (Figure 4.4.1.A) and a bar plot (Figure 4.4.1.B). Notably, the two most frequent QTL types for the LA QTLs were Meat and Carcass (51.61%) and Health (16.13%) (Figure 4.4.1.A). Figure 4.4.2. presents the same analyses regarding the significant TGIs based on the GWAS results reported in this thesis. Notably, the most prevalent QTL types differed between Linkage Analysis (LA) and GWAS findings. GWAS-identified QTLs exhibited different trends from LA-identified, with the majority falling into the Meat and Carcass category (40%) followed by Milk (20.48%), as shown in Figure 4.4.2.A.

Additionally, we conducted a detailed analysis of Exterior QTL type to assess the frequency of specific traits within this category. Exterior traits encompass various aspects of animal appearance, including udder morphology, coat color, horns, conformation, behavior, and breech traits. The analysis revealed that, in the case of LA QTLs, the most frequently observed exterior trait was horn type (4.2%), as outlined in Figure 4.4.1.B. In contrast, the GWAS-detected QTLs predominantly featured Stature (0.5%) as the most frequent exterior trait, as illustrated in Figure 4.4.2.B.

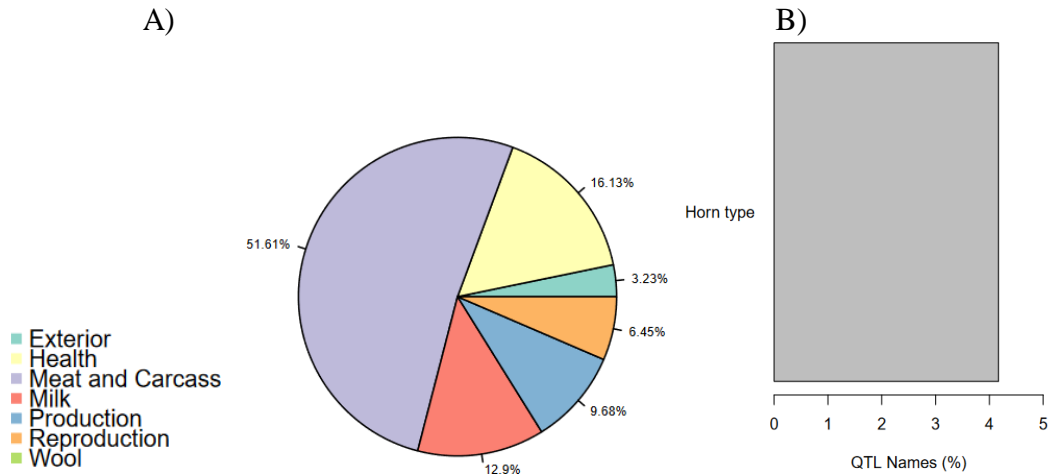


Figure 4.4.1. Percentage of QTL type (pie chart) and traits related to exterior QTLs (bar plots) overlapping with the significant QTL identified in the present work by LA, based on the QTL annotation analyses performed with the GALLO software.

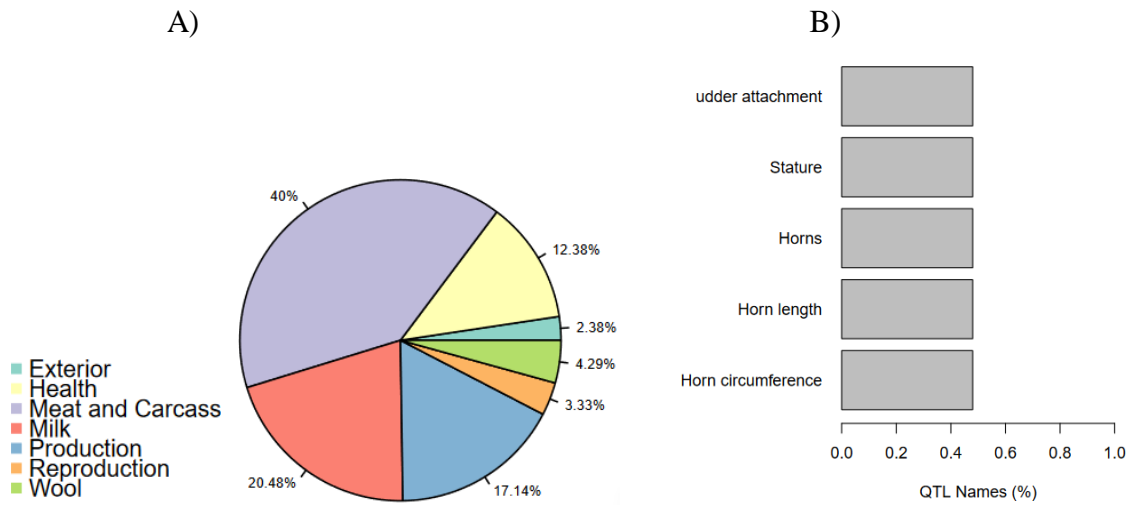


Figure 4.4.2. Percentage of QTL type (pie chart) and traits related to exterior QTLs (bar plots) overlapping with the significant QTL identified in the present work by GWAS, based on the QTL annotation analyses performed with the GALLO software.

To assess the significance of the published QTL for type traits within the identified regions of interest identified through Linkage Analysis (LA), we conducted a QTL enrichment analysis using the GALLO R package. Figure 4.4.3. visually represents the results of this analysis. On the x-axis, a richness factor is represented, which is calculated as the ratio of the number of QTL annotated in the candidate regions for the different traits to the total number of each QTL in the reference database considered. The darkest red shade in the circle indicates the most significantly enriched terms from QTL annotation, while the size of the circle represents the traits with the highest number of QTLs that overlap with our candidate regions. In the case of the LA QTLs, these regions were significantly enriched for QTLs associated with the testes weight and two traits related with carcass composition (muscle weight in carcass, lean meat yield percentage) (Figure 4.4.3.A). In the case of the significant regions detected by GWAS, the hot carcass weight trait showed the most significant enrichment, and body weight was the trait with the largest numbers of QTLs overlapping with our candidate regions (4.4.3.B).

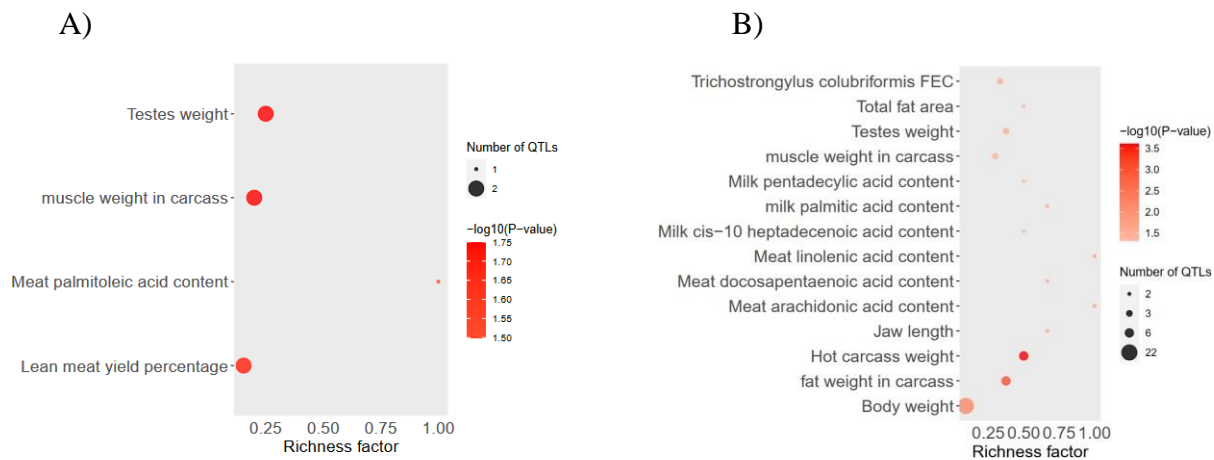


Figure 4.4.3. Bubble plots displaying the most significant results of the type QTL enrichment analysis results performed with the QTLs annotated in SheepQTLdb within the candidate regions identified in the present work by LA (A) and GWAS (B) analyses.

In addition, based on the QTL annotation results obtained, we performed a deeper analysis of the genetic effects annotated in the QTL regions of interest identified in this work by LA and GWAS, in case the corresponding reporting studies could be informative for the interpretation of our results. For that, from the initial lists of 24 and 210 genetic effects (QTL/Associations) extracted with the GALLO software from the LA and GWAS target genomic intervals defined in this work, we selected those that were associated with traits related with growth traits, carcass composition and body composition, whereas also QTL for reproduction traits and to resistance to footrot disease, specifically, were also considered for the potential relationship with the body type traits under

study. In this way, a total of 17 and 98 QTL/Associations were found to overlap, respectively, with the LA and GWAS QTL regions identified in this study. The number of these genetic effects overlapping with each of the genetic effects identified in this work is indicated in Table 4.2.1. and Table 4.3.1. for LA and GWAS respectively (see column 'Number of overlapping QTLs').

5. Discussion

Besides traditional breeding methods, Genomic Selection is now being utilized to enhance the desired traits in dairy sheep. While this approach is not yet regularly integrated into Spanish sheep breeding programs, there is an expectation that this may change in the coming years. SNP-chip data could be employed not just to improve milk production traits but also other functional characteristics such as udder and body morphology. Additionally, leveraging genetic markers known to be linked to the traits targeted for improvement through Genomic Selection has the potential to increase the accuracy of estimated genomic breeding values (Teng et al., 2020).

In recent years, GWAS have emerged as a robust method for identifying genomic regions influencing the phenotypic variation in traits among different cattle breeds. In some cases, these studies have even pinpointed the specific genetic mutations responsible. This study utilizes a high-throughput SNP-array GWAS, with a specific focus on body morphology traits in dairy sheep. Additionally, it incorporates a genome scan using LA.

While associated regions were identified by both analytical methods, LA and GWAS, employed in this study, they yielded differing results. QTL mapping has traditionally relied on LA based on the family data available from the studied populations and that was the dominant approach for many years. However, with the availability of SNP-chips, offering more comprehensive genetic maps, this method has largely given way to GWAS analyses, which harness LD information across the entire population. Nevertheless, it has been demonstrated that a hybrid approach, known as combined LDLA analysis, can yield a wealth of information in specific populations characterized by a family-based structure. This approach leverages both linkage information derived from within-family analyses and LD information obtained at the population level (García-Gómez et al., 2013).

In this study, focused on analyzing a commercial population of half-sib dairy ewes of Churra sheep, a total of eight chromosome-wise significant QTL regions were identified using LA. No QTLs reached genome-wide significance by this analysis. In addition, when employing the GWAS method, we identified 27 chromosome-wise significant regions. A thorough comparison of these findings leads to two important observations: first, the GWAS method represents a substantial improvement in QTL detection compared to the traditional LA approach. Second, in our family-based design, classical LA can only identify QTL when multiple sires are heterozygous (Qq) at the same QTL. Consequently, many associations between markers and traits that do not adhere to this assumption, yet still possess a genuine connection at the population level, may not be detected by LA but can be identified through GWAS. In any case, we consider that the two analyses performed together offer a global picture of the QTL segregating in the studied population at both the family and the population levels.

This study did not identify any genome-wide significant QTL although several chromosome-wise significant regions were identified. The lack of genome-wide significant results can be related to the statistical power of our analyses (Marina et al., 2020). In any case, when we compared our results with the information annotated in the SheepQTLdatabase, we found that many of the chromosome-wise significant QTLs overlapped with previously reported QTLs for traits related to body morphology and body growth in the ovine species (as shown in Table 4.2.1. and Table 4.3.1.). This correspondence with previously reported QTLs in many cases adds supports to the validity of the chromosome-wise significant results reported here. In the results section, a comprehensive table was shown to systematically pinpoint the regions with the highest number of overlapping QTLs, making them a key focus of this Master's Thesis. Hence, among the detected QTL regions, we will present a deeper discussion of those that showed a higher correspondence level with previously reported sheep QTLs.

Within the context of the LA results, two regions drew our attention (as indicated in Table 5.1.). The first one, located on chromosome 23 and associated with rump width, which stood out with a total of seven overlapping QTLs, making it a promising area for further study, given its alignment with prior research. The second region of interest was the rear legs (rear view) QTL on chromosome 18, which showed three overlapping QTLs.

The rump width QTL detected on chromosome 23 was coincident with a QTL for Average Daily Gain (ADG) previously reported by Raadsma et al. (2009a). The other six reported QTLs in this genomic region of chromosome 23 were associated to body weight and carcass composition related traits. This connection suggests a potential genetic link of this QTL for rump width with general growth traits, such as ADG and body weight, and also carcass composition traits, such as hot carcass weight, carcass fat percentage, muscle weight in carcass and lean meat yield percentage. The relation of this genomic region with ADG, linked to postnatal growth, adds an additional layer of complexity to our research, shedding light on how body type traits may be interconnected with growth traits.

Interestingly, a high-density SNP-chip GWAS (630K) for body size traits reported in Qira black sheep identified significant markers associated with chest width in an exon of the *ELP2* gene (elongator acetyltransferase complex subunit 2) and an intron of the *MOCOS* (molybdenum cofactor sulfurase) genes (Tao et al., 2021). These two genes were included in the list of annotated genes within the TGI of the chromosome 23 QTL for rump width. Although we have not found a direct relationship of these two genes with growth or morphology related traits, it is interesting that this QTL overlaps with previously reported QTL genes for body size traits in sheep.

Also, among the 16 genes annotated within the TGI for this chromosome 23 QTL we also consider of interest *SLC39A6* and *RPRDIA*, which are related to zinc ion transmembrane transporter activity and RNA polymerase II complex binding, respectively. Zinc transporters are responsible for controlling zinc homeostasis and are essential for maintaining optimal cellular functions. Broadly

speaking, members of the ZnT family, categorized as mammalian cation-diffusion facilitator (CDF) proteins, function as efflux transporters. They lower cytosolic zinc levels either by transporting zinc out of the cell or into internal compartments. In contrast, ZIP family proteins act as influx transporters, increasing cytosolic zinc levels by drawing zinc into the cytosol either from extracellular fluid or intracellular vesicles. *SLC39A6*, specifically, is responsible for facilitating the uptake of zinc ions into cells (Hara et al., 2017). Dysregulation of zinc levels can have a profound impact on health, affecting growth, development, and the immune response, while the deficiency of zinc can lead to the immune dysfunction and growth retardation (Hara et al., 2017). Zinc plays a crucial role in processes such as growth, cell specialization, the immune system's functioning, neurological activities, and the production of proteins (Hara et al., 2017). The *RPRD1A* gene encodes for a protein that plays a role in regulating the cell cycle and transcription processes. It interacts with a cell cycle inhibitor called cyclin-dependent kinase 4 inhibitor B. Additionally, it can form dimers with another protein called "regulation of nuclear pre-mRNA domain-containing protein 1B" creating a framework that engages with the C-terminal segment of RNA polymerase II subunit B1. This interaction governs multiple facets of transcription (GeneCards, 2023).

The overlapping QTLs in the significant region on chromosome 18 identified for rear legs were those associated with two specific traits: testes weight (TESTWT), reported by Fullard et al., (2006), and foot rot susceptibility (FROT), reported by Mucha et al., (2015). We think that the QTL effect associated with foot rot susceptibility in sheep might be linked to the rear leg trait due to the nature of foot rot infection. Foot rot is a contagious bacterial disease that affects the hooves of sheep, causing lameness and discomfort. When sheep are afflicted with foot rot, they tend to alter their gait and movement patterns to avoid pain and discomfort in their affected feet (Sheep Connect SA, 2020). This change in movement and weight distribution can lead to stress and strain on the rear legs as the sheep attempt to alleviate pressure from the infected hooves. Over time, this altered gait and movement can affect the development and structure of the rear legs, potentially leading to issues related to bone and joint health. The QTL associated with foot rot susceptibility may influence the severity of the disease in affected sheep, which in turn affects their mobility, potentially impacting the overall health and structure of their rear legs (Sheep Connect SA, 2020).

One of the genes included within the region of chromosome 18 associated with the rear legs trait was the *ACAN* gene. This gene encodes the production of the aggrecan protein. Aggrecan belongs to a category of proteins called proteoglycans, characterized by the presence of multiple sugar molecules attached to them. It serves as the predominant proteoglycan within cartilage, a resilient and flexible tissue that forms a substantial part of the skeletal structure during the initial developmental stages (Aspberg et al., 1999). The *ACAN* gene mutations lead to the development of skeletal dysplasia. It also serves as a controller of height (Manzari et al., 2019). Moreover, this gene is linked to functions of the nervous system. These aspects collectively exert an influence on the rear leg traits in sheep, primarily through their impact on growth and developmental processes.

In the context of the significant QTLs detected in this work by the GWAS analysis, two regions on chromosome 6, both associated with stature (as shown in Table 5.1.), revealed a very outstanding level of correspondence with previously reported ovine QTLs. The first one, including the genomic interval of 36.96-37.98 Mb, with 27 overlapping QTLs, and the second one, within the 38.06-38.79 Mb interval, with 13 overlapping QTLs. These two QTL signals for the stature trait overlapped with several QTLs reported on sheep chromosome 6 for body weight, carcass composition traits (hot carcass weight, fat weight in carcass, muscle weight in carcass, carcass fat percentage), ADG and bone traits (bone area and total bone). Within the first of these QTL signals, Matika et al. (2016) reported QTL for bone area (BOA) and total bone (TOTBONE) in a Scottish Blackface lamb population. Several candidate genes were identified for this QTL region, including *SPP1* (secreted phosphoprotein 1), *MEPE* (matrix extracellular phosphoglycoprotein), and *IBSP* (integrin-binding sialoprotein), which are related with bone formation, and also the *LCORL* and *NCAPG* genes, which are associated with stature in humans and many animal species (Matika et al., 2016).

Interestingly, our GWAS analysis identified two separate significant markers on chromosome 6 which were the most significant effects detected in this analysis and were close to reach the genome-wide significance threshold. The region corresponding to the 36.96-37.98 Mb interval on chromosome 6, contains 10 annotated genes, among which we can find *ABCG2*, *SPP1*, *LAP3* and *FAM184B*. Based on previous studies these positional candidate genes are likely to control many different traits, such as reproduction traits (*ABCG2*), milk production traits (*ABCG2*), growth traits (*SPP1*, *LAP3*, *FAM184B*) and bone traits (*SPP1*, *LAP3*). The TGI defined for the other significant marker identified for stature on chromosome 6, the 38.06-38.79 Mb genomic region, only contains one annotated gene, *LCORL*, which based on the previously mentioned associations reported for the *LCORL-NCAPG* locus with stature and height in humans and animals appears as a promising functional candidate gene. An assessment of the potential functional links of all these positional candidate genes with the stature trait is presented below.

The *ABCG2* gene is a member of the ATP-binding cassette family, which includes genes responsible for encoding proteins that facilitate the movement of molecules across cellular membranes. Within the intestines, the *ABCG2* protein plays a role in expelling a compound known as urate into the urine (Merriman, 2017). This gene has been associated with milk production traits (Árnyasi et al., 2013) and litter size in sheep (Bozhilova-Sakova et al., 2021). The association of the *FAM184B* gene with traits related to meat, body weight, and body composition in sheep has been reported by Yuan et al (2021). *FAM184B* affects growth, body size, meat quality and carcass traits, (Liu et al., 2022). On its side, *SPP1* was originally identified as a major sialoprotein in bone. It aids in osteoclast binding to the mineralized bone matrix and influences bone remodeling. *SPP1* is also related to cytokine activity and extracellular matrix binding, as well as the *LAP3* gene. Several studies in cattle have identified *LAP3* as a potential candidate gene for various characteristics, including calving (Bongiorni et al., 2012), bone weight (Miao et al., 2018), and

body size (An et al., 2019). Furthermore, there is a strong indication that the *LAP3* gene plays a role in muscle development in sheep as well. Results from the La et al., (2019) indicate that *SPP1* and *LAP3* are related to growth traits in sheep and hold promise as candidate genes for improving the body weight of sheep during breeding. Ge et al. (2022) have uncovered that *LAP3* negatively regulates myoblast proliferation and positively regulates myoblast differentiation.

Table 5.1. Potential functional candidate genes identified in the present work for the QTL regions showing a highest level of correspondence with previously reported QTL according to SheepQTLdb.

Method	Trait	OAR	Corrected Pc-value (Pq-value)	TGI (Mb)	Number of positional annotated genes within TGI	Functional candidate genes highlighted by the literature review	Study of reference
LA	Rump width	23	<0.05	18.6-22.4	20	<i>ELP2</i> <i>SLC39A6</i> <i>RPRD1A</i>	Tao et al., (2021) Hara et al., (2017)
	Rear legs (rear view)	18	<0.05	18.1-22.4	67	<i>ACAN</i>	Aspberg et al., (1999)
GWAS	Stature	6	0,018	37.34-37.59	10	<i>FAM184B</i> <i>SPP1, LAP3</i> <i>ABCG2</i>	Yuan et al., (2021) La et al., (2019) Árnyasi et al., (2013)
		6	0,01	38.48-38.73	1	<i>LCORL</i>	Gutiérrez-Gil et al., (2012)
	Rump width	24	0,002	24.99-25.24	6	<i>GTF3C1</i>	Li et al., (2022)

In relation to the *LCORL* gene (see Table 5.1.), it is important to consider that recent advancements in the field of genetics related to stature have uncovered a relatively straightforward genetic makeup in livestock species, which differs from the complexity observed in humans. The genetic region recognized as the *NCAPG-LCORL* locus, which has conventionally been linked to the height of adult humans, has been found to play a role in the body weight and height of cattle and horses (Saatchi et al., 2014; Metzger et al., 2013). Additionally, it has been linked to selective adaptations in dogs and pigs (Vaysse et al., 2011; Rubin et al., 2012). All these findings underscore the significant influence of this genetic locus on the growth and body size of mammals, highlighting their potential utility in selective breeding for desirable traits. However, it is crucial to note that this locus has been associated with unfavorable traits, such as delayed puberty or susceptibility to

neuropathic diseases. This emphasizes the need to carefully distinguish between causality and mere association in genetic studies (Takasuga, 2015).

The study from Gutiérrez-Gil et al., (2017) describing a high resolution analysis, based on whole genome sequencing, of regions previously identified as selection signals between Churra and Merino provided support for the idea that the *NCAPG-LCORL* genetic locus could have a direct impact on various sheep production traits, potentially including aspects like growth and carcass traits. Previous studies comparing the carcass features of Churra and Spanish Merino sheep at the same age of slaughter have indicated that Merino sheep tend to have notably higher measurements in terms of hot carcass weight, conformation score, carcass yield, total muscle, and bone percentage in comparison to Churra sheep (Martínez-Cerezo, 2005; Campo et al., 2008). This study marks the first instance where specific mutations in this region have been suggested as potential influencers of production traits in sheep.

In cattle, the *NCAPG-LCORL* region has been linked to various traits of economic interest, including carcass weight (Setoguchi et al. 2009), birth weight (Eberlein et al. 2009; Snelling et al. 2010), weaning and yearling weight (Snelling et al. 2010), peri-pubertal weight gain (Weikard et al. 2010), and an increase in body frame size (Setoguchi et al. 2011). Numerous QTLs have been identified in previous studies suggesting the relevance of the bovine *NCPG-LCORL* locus on a wide range of traits on interest in cattle production, including stature, body size, growth, and carcass traits. For example, this locus has been linked to body size, carcass weight, and feed efficiency by Lindholm-Perry et al., (2011). Also, Gutiérrez-Gil et al., (2012) identified a QTL related to bone traits in close proximity to the *NCAPG-LCORL* region. Also, *LCORL* has been specifically associated with bone weight, as documented by An et al., (2020) and Liu et al., (2021). *LCORL* has been identified as the potential gene associated with a calving ease QTL effect by Bongiorno et al. (2012) and Sahana et al. (2015). Moreover, this gene has been linked to traits like carcass and meat quality, as well as components related to feed efficiency in cattle, which could have a direct connection to the size of the animals (Zhang et al., 2016; Anton et al., 2018; Wang et al., 2020).

Additionally, as previously mentioned, multiple QTLs, revealed through GWAS and the Illumina Ovine SNP50 BeadChip, have been identified in sheep, containing promising candidate genes like *SPP1* and *LCORL* associated with bone-related and meat quality traits (Matika et al., 2016), which agree with the involvement of the reported effects of the *LCORL* gene in relation to human skeletal size and infant height growth (Wood et al., 2014; Helgeland et al., 2019). *LCORL* is expressed in various tissues of bovine fetuses and calves, including the liver, intestine, and extraembryonic tissue (Lindholm-Perry et al. 2011).

With regard to the other significant QTL regions detected by GWAS, and using the overlapping QTL effects as criteria, we could mention the significant effect detected on chromosome 24 for rump width, which overlapped with nine QTL annotated in the SheepQTLdb. The positional candidates included in the TGI region of this QTL included genes encoding for interleukin

receptors, the *IL21R* and *IL4R*, which are associated with the immune system and play essential roles in the regulation of immune responses (Lin et al., 2018). Although immune traits may be related with body traits, the link is not direct. Hence, among the other annotated genes included in the TGI of this QTL for rump width, we found the *GTF3C1* gene, which is predicted to play a role in the DNA binding process. We have found a potential link between this gene and body type traits in the research conducted by Li et al. (2022), which presents genomic and metabolomic analyses to identify genetic mechanisms underlying with carcass merit traits in a multibred population of beef cattle. Among the long list of candidate genes identified, playing roles in numerous biological functions related to carcass merit traits, the study identifies the *GTF3C1* gene as a potential candidate for the acetic acid metabolite in relation to the HCW trait (Li et al., 2022). This connection implies that the *GTF3C1* gene might also have relevance to body and carcass traits in sheep. Another study in lambs has shown that the acetic acid have a potential to enhance growth, carcass characteristics, and meat quality (Li et al., 2023). In this study, when lambs were subjected to a diet with high-concentration of acetic acid, those individuals fed either variation of guanidine acetic acid (GAA) addition experienced accelerated growth. Both types of GAA (UGAA and CGAA) led to increased final body weight, enhanced carcass quality with reduced backfat thickness, and improved water-holding capacity (Li et al., 2023).

Overall, the present study has successfully identified several significant QTL regions for body conformation traits in Churra sheep. The number of QTL detected, by using the two different analysis approaches implemented, provides support for the idea that complex interactions between multiple genes, rather than individual genes, play a role in determining body conformation in dairy sheep. Our findings mark the initial step in identifying genetic variations that directly influence the various phenotypic traits observed in Churra sheep body morphology. Integrating this information into Churra sheep breeding programs would necessitate identifying specific genetic causal variants or SNPs that are strongly associated with body conformation traits. Such variants are expected to enhance the accuracy of predicting genomic breeding values for body conformation, as well as indirectly affecting the health, productivity, and longevity of the sheep of the targeted populations. The results presented in this study can be viewed as the beginning of a new research line for the MEGA-ULE group, which involves combining functional genomic data with the analysis of genetic variations to better understand the biological mechanisms that control sheep body conformation.

6. Conclusion

This study conducted a gene mapping analysis in a commercial population of Spanish Churra sheep to investigate body conformation traits using a medium-density SNP array. Utilizing two analytical approaches, LA and GWAS, a total of 35 QTLs were identified. The systematic comparison of the genomic intervals containing the identified QTLs with previously reported QTLs in sheep for morphological, growth and carcass related traits provide substantial support for the authentic association of these highlighted genomic regions with body conformation traits. For the regions showing the highest level of correspondence with previously reported ovine QTLs for traits related with body morphology, we have performed a deeper evaluation to identify the most promising functional candidate genes. Hence, this study offers a collection of potential functional candidate genes for the detected QTL effects that could be added to enhance, through the initial genomic selection program of this breed, the improvement of body conformation traits. This can have positive effects on the animals' overall health, longevity, and production capabilities. Future studies should be planned within commercial populations to verify the potential associations proposed here, aiming to supply valuable genomic information that can enhance the predictive capacity of genomic selection programs in Spanish Churra sheep in the future.

7. References

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Appendix

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8. Biography

Valentina, born on January 6, 1998, in the city of Zagreb, embarked on a remarkable academic journey that would shape her into an engineer in the field of agricultural genetics and breeding. Valentina's educational path began at the General Gymnasium in Sesvete, Croatia, where she honed her intellectual curiosity and developed a strong foundation in various subjects. Graduating in 2016, she laid the groundwork for her future studies. Valentina enrolled in the Faculty of Agriculture at the University of Zagreb in 2016, specializing in Organic Agriculture. During her undergraduate years, Valentina's commitment to academic growth led her to expand her horizons beyond her homeland. In pursuit of enhancing her knowledge and embracing diverse cultures, she participated in the Erasmus+ Placement Program, spending a transformative semester at the ETSEA campus in Lleida, Spain. Here, Valentina not only enriched her Spanish language skills but also gained insights into international agricultural practices. Having completed her Bachelor's degree in Organic Agriculture in 2020, Valentina pursued further studies. She embarked on a Master's program in Animal Genetics and Breeding at the same esteemed institution, the Faculty of Agriculture, University of Zagreb. A pivotal moment in Valentina's academic journey occurred when she secured an Erasmus+ traineeship at the Faculty of Veterinary at the University of Leon in Leon, Spain. Valentina joined the MEGA-ULE research group within the Department of Animal Production. Guided by mentors and experts, she delved into the detection of genomic regions underlying complex traits utilizing bioinformatics tools such as ENSEMBL, Animal QTLdb, GCTA software, QTLMap software, plink software, and the Gallo R package. Valentina's experience in the research group refined her analytical skills and helped her ability to interpret and compare results. She conducted QTL analysis, linkage analysis, and GWAS. Beyond academia, Valentina's journey was enriched by her engagement in volunteering and various student jobs, both in Croatia and abroad. These experiences cultivated not only professional skills but also social acumen, shaping her into a well-rounded individual poised for success in both her research pursuits and collaborative endeavors.