A REVIEW: DNA MARKERS ASSOCIATED WITH PRODUCTION TRAITS IN DIFFERENT CATTLE BREEDS

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Abstract

The main objective of modern livestock breeding is to find an efficient and a fast way to increase productivity and quality, and the application of technologies based on DNA markers have a great influence on the livestock and also contributes further to the mapping of the genomes of different species of economic importance. Past decade research carried out at the DNA level had a main goal: to identify the genes responsible for the expression of quantitative characters and the detection of places of interest, the latter becoming markers of DNA or SNP type that can be used in the selection process and improvement programs of dairy farms.
In Romania, several studies were performed in order to identify the associations of the genetic markers with the main traits of milk production by Vlaic *et al*, (2001; 2003; 2005); Creangă *et al*., (1996, 2002, 2003; 2007, 2008, 2010); Bâlteanu *et al*., (2007a; 2007b, 2008; 2010a; 2010b, 2013); Bugeac et al., (2013a, 2013b, 2013c, 2015, 2019), respectively for the quality of meat by Carşai *et al*, (2009, 2010, 2013).

Key words: (DNA markers, dairy milk, beef, cattle, polymorphism)

INTRODUCTION

Several studies to date have shown significant associations between a series of mutations in the coding or non-coding regions of DNA (genetic markers) and diseases with genetic substrate or phenotypic characters of interest (external or production). The explanation for this phenomenon is related to the fact that mutations can affect the expression or the expression product of the genes that control these characters. In cattle, more than 80% of the qualitative traits are determined by autosomal recessive genes (Healy *et al,* 1996). Genomic association studies aimed at decoding the genetic substrate of quantitative traits and highlighted the involvement in their genetic determinism of several chromosomal regions containing functional genes. Using the information provided by SNP markers, markers for milk production were identified in cattle (Mai *et al*, 2010; Cole *et al*, 2011), markers for meat production (Casas *et al*, 2000; Casas *et al*, 2001; Snelling *et al*, 2010; Snelling *et al*, 2011.

Genetic markers associated with beef production in cattle.

The candidate genes for quantitative character loci for milk and meat production are selected based on the associations that are established between biochemical or physiological processes and the respective quantitative character. Subsequently, these loci are tested as loci of a quantitative character (QTL). The aim is to identify DNA markers positively associated with different palatability characteristics. For example, genes specifying thyroglobulin, leptin, and calpastatin have been shown to influence the tenderness, juiciness, and marbling of meat, carcass quality, and body weight (Carşai *et al*, 2010).

Calpastatin (CAST) - is located on bovine chromosome 7 and is responsible for the meat tenderness, this being one of the essential criteria for the selection of beef cattle, because this trait is desired by consumers. Studies have shown that calpastatin is one of the main factors that higly associated with fragility of meat because it regulates the activity of m-Calpain and µ-Calpain enzymes. These proteolytic enzymes are responsible for the breakdown of muscle fiber, producing post-mortem tenderness of the meat (Koohmaraie *et al*, 1996). Significant associations with meat tenderness have been identified in several breeds of cattle (Barendse *et al*, 2007).

µ-Calpain (CAPN1) is a proteolytic enzyme of skeletal muscle, it is encoded by the CAPN1 gene located on chromosome 29. In *Bos taurus*, an SNP associated with meat tenderness has been

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identified (Page *et al*, 2002). Other SNPs associated with this trait have been identified in *Bos indicus* (Riley *et al*, 2003; White *et al*, 2005).

Myostatin (MSTN). The myostatin gene, located on bovine chromosome 2, has been identified as being responsible for the genetic determinism of this trait and is responsible for muscle hypertrophy in cattle. Phenotypically speaking, animals with two copies of the mutant allele responsible for the double croup have a higher birth weight (McPherron *et al*, 1997). Studies have shown that animals that are homozygous have difficulty giving birth as opposed to heterozygous ones (Casas *et al*, 1999). Double-legged animals have been shown to have 30% more muscle mass than others. The flesh of these animals is also much tenderer (Arthur, 1995). This marker is currently used in selection programs for the production of heterozygous individuals.

Thyroglobulin (TG) - is a glycoprotein hormone synthesized by the follicular cells of the thyroid where it is stored and which is subsequently iodinated, whose coding gene is located on bovine chromosome 14. Thyroglobulin is the carrier of triodothyronine (T3) and thyroxine (T4) stored in the lumen of the gland, so that T3 and T4 have been associated with the degree of meat marbling in Wagyu cows. TG5 polymorphism is located in the leader sequence at the 5 'end of the thyroglobulin gene and has been associated with intramuscular fat content in cows fed ad libidum (Carşai *et al*, 2010). Cows homozygous or heterozygous for the T delta allele (CT or TT) have a much higher degree of marbling than those homozygous for the C delta allele (Barendse, 1997). Individuals with the T allele in the genotype have superior growth performance and a higher degree of meat marbling. The selection assisted by this marker will not aim at a lower amount of subcutaneous fat but at a higher degree of marbling of the meat.

Leptin (Lep) - is a hormone synthesized in adipose tissue and secreted into the blood, with implications for regulating body weight, bone formation, fertility and immune functions of the body. Leptin, called the obesity gene is associated with risk factors in cardiovascular disease, infertility, etc. and is located on bovine chromosome 4. This hormone also plays an important role in glycogen synthesis, glucose transport and muscle lipid disposition (Carşai *et al*. 2010), and the larger the adipocytes, the more leptin mRNA will be present.

Genetic markers associated with milk production in cattle

Polymorphisms within key gene structure, such as: pituitary growth factor (PIT1), somatotropic growth hormone (GH), insulin-like growth factor 1 (IGF1), prolactin (PRL), prolactin receptor (PRLR), growth hormone receptor (GHR), signal transmission and transcription activation factor 5 (STAT5), AcylCoA diacylglycerol acyltransferase 1 (DGAT1), alpha-lactoalbumin (LALBA) may have a positive or negative effect on the development of the mammary gland, its capacity lactogenic and implicitly on the quantitative and qualitative production of milk. The effects of some polymorphisms identified in some of these loci on the quantity and quality of milk have been evaluated in several studies performed on various breeds of cattle. For example, the SNPs of the diacylglycerol acyltransferase 1 (DGAT1), stearoyl-CoA dasaturase 1 (SCD1), fatty acid synthetase (FASN), lipoprotein receptor (OLR1), prolactin (PRL), signaling factor 5 and activation genes of transcription 5A (STAT5A) growth hormone receptor (GHR) are closely correlated with the composition of cow's milk in several breeds (Mele *et al*, 2007; Moioli *et al*, 2007; Schennink *et al*, 2009; Sun *et al*, 2009).

Pituitary growth factor (PIT1 or POU1F1) is a transcription factor synthesized in the hypothalamus (Moody *et al*, 1995). The gene encoding the 291 amino acid protein is located in cattle on chromosome 1. PIT 1 plays an important role in activating in the anterior pituitary gland the expression of genes encoding prolactin and growth hormone (Bona *et al*, 2004). PIT1 is also involved in regulating the expression of the gene encoding the beta subunit of thyrotropin-releasing hormone (TSH) - a hormone essential for the activity of the thyroid gland. Polymorphisms in the structure of the PIT1 gene can lead to deficiencies in the synthesis of growth hormone, prolactin, and thyrotropin-releasing hormone (Radovick *et al*, 1992). Mutations in the structure of the PIT1 gene can have positive or negative influences on milk production, which is why it is currently considered an extremely valuable genetic marker in improving animal production.

Similar studies indicate the same associations between PIT1 A and milk production, respectively the percentage of milk protein, but also with a lower percentage of fat (Zwierzchowski *et al*, 2002; Mattos et al., 2004 Hori-Oshima et al., 2003). However, there are a number of studies (Dybus *et al*, 2004; Zakizadeh *et al*, 2007; Trakovická et al., 2014) performed on different breeds of cattle in which no positive associations between PIT1-Hinf1 polymorphisms and the productive properties of milk.

Somatotropic hormone (GH) is synthesized and secreted by somatotropic cells in the anterior

pituitary gland under the influence of PIT1 (Bona *et al*, 2004). In cattle, the gene encoding GH is located on chromosome 19 and encodes a 191 amino acid protein (Hediger *et al*, 1990). It has a role in stimulating cell division and differentiation as well as an essential role in regulating metabolism. GH stimulates the secretion of secretory epithelial cells from the mammary gland by activating in the liver the gene encoding insulin-like epithelial growth factor 1 (IGF1). Experiments performed on cattle have shown that exogenous administration of GH increases the amount of milk by 10-15% (Zhou *et al*, 2008) by stimulating the proliferation of lactogenic breast epithelium. This is done indirectly by stimulating the expression of the IGF1 gene in the liver. GH also acts directly in the mammary gland by binding to its receptor on the secretory mammary epithelial cell (GHR). It has a stimulating effect on genes that are involved in the synthesis of major proteins in milk, by their transcriptional activation mediated by STAT5 factor. On the other hand, by stimulating the synthesis of α-lactoalbumin by the LALBA gene, there is an increase in the amount of the enzyme lactose - synthetase, the enzyme involved in the synthesis of lactose. Therefore, there is an increase in the amount of milk because the proportion of lactose depends on the amount of water absorbed from the cytoplasm during the synthesis of milk droplets (Yang *et al*, 2005; Zhou *et al*, 2008). Some polymorphisms significantly associated with milk production have been reported at the GH gene locus. Exon 5 identified a G / C type polymorphism located at the restriction site of the AluI enzyme and which causes the substitution of a leucine (L) with a valine (V) at position 127 of the mature protein (Lucy *et al*, 1993; Zhang *et al*, 1993). Dybus (2002) studied in the Polish Frisian breed the associations between this polymorphism from exon 5 and milk production. The LL genotype was associated with a higher amount of fat and protein. Yardibi *et al*, (2009) studied the same polymorphism in South Anatolian and East Anatolian Red bull breeds. In both breeds the LL genotype was associated with a higher amount of milk and a higher percentage of fat compared to the other two genotypes. In contrast, in other studies, the VV genotype was associated with a higher amount of milk and a better composition (Zwierzchowski *et al*, 2002).

Insulin-like growth factor 1 (IGF1) is a protein hormone consisting of 70 amino acids with a molecular weight of 7.5 kDa, being similar to insulin; the gene encoding it is located in cattle on pair 5 chromosomes. IGF1 is produced mainly in the liver by the direct action of GH, being the primary mediator of the effects of this hormone in tissues. IGF1 is one of the most potent activators of stimulating cell growth and proliferation and a potent inhibitor of apoptosis (cell death). It is produced throughout its life, reaching its maximum level during puberty. Its effects of stimulating cell division and differentiation have repercussions on every cell in the body. IGF1 is also produced locally in the mammary gland (through the direct action of growth hormone), where it stimulates together with estrogen hormones the development of galactophore channels and the growth of the mammary epithelium by stimulating cell divisions and inhibiting apoptosis (Plath-Gabler *et al*, 2001). At the IGF1 gene locus, Siadkowska *et al*, (2006) studied in the Polish Holstein breed the associations between a C472T-type polymorphism located in the 5 'untranslated region of the gene and milk production. The CT genotype was significantly associated with a higher amount and percentage of fat and protein in milk compared to the CC genotype. Mehmannavaz *et al*, (2010) studied the effect of the same polymorphism in the Holstein Iranian breed. The CT genotype was associated with a higher amount of milk and fat compared to the other genotypes.

Prolactin (PRL), also known as luteotropic or lactotropic hormone (LTH), is a protein hormone made up of 199 amino acids with a molecular weight of 24kDa; the gene encoding it is located in cattle on the chromosomes in the pair 23. PRL is synthesized and secreted by lactotropic cells in the anterior pituitary lobe, but is produced in small amounts and in other tissues including the mammary gland (Le Provost *et al*, 1994). Together with GH, PRL stimulates the growth and development of breast tissue, triggering and maintaining lactation and the synthesis of milk components by regulating the expression of genes encoding the 6 major proteins in milk and genes involved in the synthesis of lactose and fats. The increase in serum concentration of PRL circulating during pregnancy, leads to an increase and differentiation of the lactogenic tissue in the mammary gland, which is correlated after birth with an increase in milk production. Activation of the gene encoding PRL is done under the influence of the transcription factor PIT1, which binds to the gene promoter and thus promotes its expression in the pituitary gland. Several polymorphisms have been identified at the PRL gene locus, of which an A / G type substitution in exon 4 leads to a restriction site for the RsaI enzyme (Lewin *et al*, 1992; Mitra *et al*, 1995). Dybus *et al*, (2004) studied in the Polish Frisian breed the associations between this polymorphism and milk production, the AA genotype being associated with a higher percentage of protein. Brym *et al*, (2005a) studied

the associations between an A8398G polymorphism in exon 4 and milk production in the Polish Holstein and Jersey breeds. The AG genotype was associated with a higher amount of milk while the GG genotype was associated with a higher percentage of protein in milk. Miceikiene et al. (2006) studied the polymorphism of the prolactin gene in four cattle breeds in Lithuania. Genotype AA was associated with a higher percentage of fat in milk compared to genotypes AB and BB. Ghasemi et al, (2009) studied the same RsaI type polymorphism from exon 4 in the Montbeliard breed, the AA genotype being associated with a higher amount of milk. Similar results regarding the milk fat percent were reported also for Chinese Holstein (Echeverry *et al*, 2011; Lewin *et al*, 1992; Chung *et al*, 1996; Dybus, 2002; Brym *et al*, 2005) and Russian Red Pied cattle (Alipanah *et al*, 2007).

The prolactin receptor (PRLR) and the *growth hormone receptor (GHR)* are trans-membrane proteins with an essential role in the uptake and further transmission of prolactin and growth hormone signals to STAT5 family cytoplasmic transcription factors. The genes encoding the two receptors are located in cattle on chromosomes in pair 20 in the proximity of each other (Georges *et al*, 1995; Arranz *et al*, 1998). These two genes are specifically expressed in all target tissues where the two hormones act, especially in the mammary gland in the case of the prolactin receptor, respectively in the liver and in the mammary gland in the case of the growth hormone receptor. Both receptors have three domains: extracellular (through which it binds to hormones whose effect mediates it), transmembrane and intracellular (through which it interacts with STAT5A factor). Binding of prolactin and growth hormone to their specific receptors causes their homodimerization, leading to phosphorylation activation of STAT5A factor that has intracellular localization (Herrington *et al*, 2001). At the locus of the PRLR gene, Brym *et al*. (2005b) studied the associations between the Polish Holstein and Jersey breeds between an A205C polymorphism from intron 9 and milk production. The CC genotype was associated with a higher amount of milk and a higher percentage of protein compared to AA and AC genotypes. Viitala *et al*, (2006) studied a polymorphism that causes the substitution of a serine (S) with an asparagine (N) at position 18 of the prolactin receptor in the Finnish Ayrshire cattle, this polymorphism being associated with a higher amount of fat and protein in milk.

At the GHR gene locus, the existence of polymorphisms with a marked effect on milk production has been suggested in various studies (Georges *et al*, 1995; Arranz *et al*, 1998). In particular, an exon 8 T / A polymorphism, which results in the substitution of a phenylalanine (Phe) with a tyrosine (Tyr) at position 279 of the mature protein, has been significantly associated with this character. In the Friesian and Jersey Holstein breeds this polymorphism was significantly associated with a higher percentage of protein and fat and to a lesser extent with a higher amount of milk (Blott *et al*, 2003). Viitala *et al*, (2006), respectively Sun *et al*. (2009), studied this polymorphism in the Finnish Ayrshire and Holstein breeds in China, respectively. In the Finnish Ayrshire breed, the presence of Tyr (allele A) was associated with a higher percentage of protein and fat (Viitala *et al*, 2006), and in Holstein with a higher percentage of protein (Sun *et al*, 2009).

Signal transmission and transcription activation factor 5 (STAT5) is an intracellular protein first identified in the lactating mammary gland, and later identified in other tissues. Two protein forms of this factor have been identified called STAT5A and STAT5B, which have a homology of 93% in terms of amino acid sequence. They are encoded by two extremely similar genes in structure (Darnell *et al*, 1997) and located in close proximity to each other on the chromosomes in pair 19 of taurine (Seyfert et al., 2000). The gene encoding STAT5A factor is expressed at all stages of mammary gland development and shows few changes in its expression profile at these stages. In the epithelial cell of the mammary gland, the factor STAT5A has a double role. It acts as an essential intracellular mediator of the transmission of prolactin signals in the cell nucleus, where by binding specific regions of the promoters of genes encoding major proteins in milk determines their transcriptional activation (Wakao *et al*, 1994). It is also the main mediator of the action of growth hormone on some target genes (Argetsinger *et al*, 1996). It mediates under hormonal influence and transcriptional activation of genes involved in the synthesis of fats, lactose and other components of milk. At the locus of the STAT5A gene, Brym *et al*, (2004) studied a G9501A polymorphism in intron 9 in the Jersey breed. The GG genotype was associated with a higher amount of milk and a higher percentage of fat, while the AA and AG genotypes were associated with a higher percentage. higher protein. Flisikowski *et al*, (2004) studied in the Polish Frisian breed a T12743C type polymorphism from exon 16. The TC genotype was associated with a higher amount of milk and a higher percentage of dry matter,

protein and lactose in milk compared to the TT genotype. Sadeghi *et al*, (2009) studied the same polymorphism in the Italian Holstein breed, the CT genotype being associated with a higher amount of protein in milk. Selvaggi *et al*, (2009) studied the Italian Brown breed a C6853T polymorphism from exon 7, the CC genotype being associated with a higher amount of milk and a higher percentage of protein compared to CT and TT genotypes.

AcylCoA diacylglycerol acyltransferase 1 (DGAT1) is a protein with an enzymatic role consisting of a polypeptide chain of 425 amino acids, with a molecular weight of 47 kDa; the gene encoding it is located in cattle on chromosomes in pair 14 (Coppieters *et al*., 1998; Riquet *et al*, 1999). In milk, fats are present in the form of small spherical or elliptical globules made up of triglycerides, which represent 98-99% of total fat. Their synthesis in the lactating mammary gland is catalyzed by the key enzyme DGAT1 (Winter *et al*, 2002). This essential metabolic process takes place in the smooth endoplasmic reticulum where this enzyme uses as substrate for their synthesis diacylglycerol and acetyl coenzyme A. DGAT1 is the main enzyme involved in the synthesis of adipocyte triglycerides (Kuhn *et al*, 2004).

At the locus of the DGAT1 gene, an AA / GC polymorphism was identified in exon 8 which has the effect of substituting a lysine (K) with an alanine (A) in the mature protein (Winter et al., 2002; Kuhn et al., 2003).). The effect of this polymorphism on milk production has been studied in cattle populations in New Zealand (Spelman *et al*, 2002), Israel (Weller *et al*, 2003), the Netherlands (Grisart *et al*, 2004), Germany (Sanders et al. et al., 2006), Poland (Pareek et al., 2005), Simmental in Czech Republic (*Hasunova et al*, 2014), and France (Gautier et al., 2007; Näslund et al., 2008). It has been found that the effects of this amino acid substitution are to increase the amount and percentage of fat in milk. Following an expression study, it was highlighted that the lysine-containing variant has a higher enzymatic activity than the alanine-containing variant (Grisart *et al*, 2004).

ATP-G2 binding box (ABCG2). The ATP G2 binding cassette (ABCG2) is a gene that carries drugs, having mainly a protective function against xenotoxin. Xenotoxins are transferred from mother to infant through milk. The ABCG2 gene is located in a specific quantitative trait (QTL) linkage region for milk production and milk composition, making it a functional candidate gene for associations with milk production traits. The ABCG2 gene is located in the *Bos* genus on chromosome 6.

CSN2 gene encoding Kappa-casein. K-CN is a very important milk protein consisting of 169 amino acids. There are 2 cysteine residues, which can form intra- and inter-molecular disulfide bridges (S-S), giving rise to several polymeric forms. These cysteine residues, under the influence of heat, can also form disulfide bridges with free SH groups of β-lactoglobulin. It is located on chromosome 6 (6q31). K-CN is completely soluble in the presence of calcium ions. It is the only casein that can be associated with a carbohydrate co-factor, the most common carbohydrates with which it is associated are galactose, galactosamine and N-acetylneuraminic acid (Creamer *et al*, 1998). The most common are variants A and B of K-CN. Almost all studies aimed at testing the associations between the genetic variants of K-CN, have shown that the BB genotype is associated with a higher content of milk in total protein and casein compared to the AA genotype: BB> AB> AA (Jakob *et al*, 1994).

Milk from the AE and EE genotypes also showed a less firm curd, which was also found in other breeds: Holstein Friesian breed - BB> AB> BE> AA / EE / AE; Angler breed - BB> BE> AB> EE> AA / AE (Oloffs *et al*., 1992); Fleckvieh breed: BE> AB> AA> AE (Jakob *et al*, 1994); Schwarzfleckvieh breed - AB> AA> AE (Jakob et al., 1994). Lodes *et al*, (1997) found the following order of genotypes regarding clot firmness: BB / BC> AC / AB> AA> BE> AE. The same decrease in clot firmness is found in the case of BG and AG genotypes: BB> AB> AA> BG> AG (Erhardt *et al*, 1993).

As can be seen above, the reports regarding the firmness of the curd are contradictory. Interestingly, the AC and BC genotypes, which have the longest clot formation time, have a coagulation firmness almost as good as the BB genotypes (Lodes *et al*, 1997).

Due to the economic importance of cheeses, the potential for turning milk into cheese is an important improvement objective. Many studies in this direction have been carried out so far. However, it is quite difficult to compare different experiments, which aimed to study the amount of cheese obtained, due to the different protein and fat content of raw milk, experimental conditions and different statistical interpretation of experimental data.

In other more recent studies, the same differences were found in terms of the amount of cheese obtained in favor of the BB genotype. In two Gouda cheese experiments, only a small difference was observed for the conversion of total milk nitrogen into cheese component nitrogen, which was 3% for BB genotypes and 2.7% for AA genotypes (Van den Berg *et al*, 1992).

In an experiment conducted in order to obtain Cheddar-type cheese, 6.2% more cheese was obtained from milk from BB genotypes compared to AA genotype (Fitzgerald *et al*, 1997). In another experiment to obtain Cheddar and Mozzarella cheese, Walsh *et al*, (2008) obtained 5.5% differences in favor of the BB genotype.

CSN2 encoding beta casein. β-CN is a protein consisting of 209 amino acids and is encoded by the CSN2 gene which is located on bovine chromosome 6. This protein has a hydrophobic character, and at room temperature it is sensitive to calcium ions. By the action of plasmin β-CN is cut into 3 positions, giving rise to the 3 γ-caseins. The β-CN family constitutes approximately 45% of the casein in cow's milk and represents a complex polymorphism due to the action of plasmin (Eigel *et al*, 1984) and high genetic variability (Formaggioni *et al*, 1999). At the β-CN locus, the A2A2 genotype was significantly associated with the Canadian Holstein breed with a higher amount of milk but less fat compared to the A1A1 genotype (Ng-Kwai-Hang 2006; Cieslinska *et al*, 2019). In another Holstein study, cows with the A1A1 genotype produced significantly more milk compared to other genotypes (Bovenhuis, 1992). Milk from BB genotypes at the β-CN locus showed the shortest clot formation time, compared to other genotypes: BB <A1B <A2C <A2B <A1A1 <A1A2 / A2A2 (Lodes *et al*, 1997). The other genotypes at this locus differ only slightly in coagulation time, except for the CC genotype which has a significantly shorter time compared to genotypes A1A1, A1A2, A2A2 (Delacroix - Buchet *et al*, 1994). Genotypes at the β-CN locus (with the exception of the CC genotype) do not appear to differ significantly in terms of clot firmness. At standardized pH, milk from CC genotypes was found to form a weak clot, compared to genotypes A1A1, A1A2, A2A2: CC <A1A1 / A1A2 / A2A2 (Delacroix-Buchet et al., 1994). The same seems to be true for the BB genotype. In other experiment, in order to obtain Beaufort cheese from the French Tarentaise breed, the amount of cheese from genotypes A1A1, A1A2, respectively A2A2 was 15% higher than in the case of CC genotype. The C allele, which has a high frequency in this breed (17%), has been associated with a spicy taste of cheese and a harder consistency of it (Delacroix-Buchet et al., 1994).

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