

## Original Research

## Polymorphism of FABP3 gene and its relationship with productive performance in Holstein cows

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**ABSTRACT:**

This study was conducted at the Al-Salam Station in dairy cows / Private Sector (Latifia township, 25 km south of Baghdad). Forty samples of Holstein cows were collected from the period of lactation during 2017 – 2018 for conducting the study. Determination of the polymorphisms of FABP3 gene (SNP: rs210042291) and the relationship with some of productive performance in Holstein cows were investigated. The polymorphisms of cows were determined in the SNP (rs210042291) of the studied fragment of FABP3 gene (458 bp) by using sequencing method, which were GG, GA and AA and their distribution ratios were 80.00, 15.00 and 5.00 % respectively. The differences between these percentages were highly significant ( $P<0.01$ ), and allele frequency were 0.88 and 0.12 for both G and A respectively. The results of the present study showed that the total milk production, the lactation period and the length of the peak of production for the Holstein cows were significantly ( $P<0.01$ ) affected by the polymorphism of FABP3 gene (SNP: rs210042291) for the cows with heterozygous genotype GA. There seen significant differences ( $P<0.01$ ) in the period from birth to the peak of production, with the values of  $46.82\pm 0.76$  and  $39.63\pm 0.96$  day for the genotypes GA and AA respectively. The percentages of fat and protein were significantly affected ( $P<0.05$ ) by polymorphisms of FABP3 gene (SNP: rs210042291), reaching the highest fat and protein percentage of cows milk with homozygous genotypes (GG and AA), and the lowest fat and protein percentage of cow's milk with heterozygous genotype (GA), while the percentage of lactose, non-fat solids and specific gravity of milk were not significantly affected by the polymorphisms of FABP3 gene (SNP: rs210042291). ). It was concluded from the study of the FABP3 gene polymorphism that these markers could be utilized for the genetic improvement strategies for the cattle to increase their economical income by polymorphism selection and crossing which gave a good performance.

**Keywords:**

FABP3 gene, Productive performance, Holstein cows.

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## INTRODUCTION

The aim of dairy industry has been to identify efficient and economical method of increasing milk production and its constituents without increasing the size of herd, improving production traits or productivity and quality of milk in dairy animals. Selection of dairy cattle with desirable genotypes has been the basis of livestock improvement, identification of molecular markers is a promising technique which can strengthen the current conventional breeding methods for quantitative trait selection, if proven these variations identified in genes are associated with the quantitative traits of interest, it may hasten the genetic gain in dairy animals (Ratwan *et al.*, 2017; Yadav *et al.*, 2017). Further, selection intensity with respect to certain quantitative traits were relatively higher through indirect selection, such as marker-dependent approach (Bhattacharya and Gandhi, 1997). Thus overall genetic progress can be improved for desired trait at a considerably higher rate. Single Nucleotide Polymorphisms (SNPs) have observed positive and significant associations with milk fat secretion among different breeds of dairy cows. It was note that milk fat is a major contributor to the energy density of whole milk and affects the physical and manufacturing properties of various dairy products, and fat content in milk is genetically variable in an animal and many physiological and environmental factors also affect milk fat secretion in dairy cows (Ratwan *et al.*, 2017).

Milk lipids are synthesized from fatty acids

which bind to specific proteins such as it fatty acid binding proteins (FABPs) (Yardibi *et al.*, 2013). They are a family of small cytoplasmic proteins, nine members of the family have been identified so far (FABP1-FABP9) and they are differentially expressed in different tissues (Chmurzynska *et al.*, 2006). They play an important role in fatty acid uptake, transport and metabolism, heart-type fatty acid binding protein (H-FABP/FABP3), whose molecular weight is 15 kD. It is present in several tissues, particularly the ones with high fatty acids demand, such as heart muscle, skeletal muscles, mammary gland during lactation, liver or adipose tissues (Calvo *et al.*, 2004; Lanier and Corl, 2015). The FABP3 gene is involved in fatty acid transport from the cell membrane to intracellular sites (Roy *et al.*, 2003). The most abundant isoforms of FABPs expressed in the lactating bovine mammary gland are FABP3, FABP4 and FABP5 (Jensen, 2002). The polymorphisms in FABP3 gene affect the selectivity of fatty acid uptake from the blood and fatty acid transport inside the mammary epithelial cells, resulting in differences in milk fatty acid composition (Jensen, 2002; Soyeurt *et al.*, 2006). The association of some of a single nucleotide polymorphism has been determined of FABP3 with milk production traits in Dairy Animals (Calvo *et al.*, 2004; Kulig *et al.*, 2010; Yadav *et al.*, 2018). In Iraq, it is the first study of this our kind, and our objective were therefore to identify genotypes of Single Nucleotide Polymorphisms (SNPs: rs210042291, rs136297772 and

**Table 1. Number and percentages of genotypes and allele frequency of FABP3 gene (SNP: rs210042291)**

Genotype	Number	Percentages(%)
GG	32	80.00
GA	6	15.00
AA	2	5.00
Total	40	100%
Chi-Square value ( $\chi^2$ )	---	64.60 **
Allele		Frequency
G		0.88
A		0.12

\*\* (P<0.01)



Figure 1. The studied fragment (458 bp) of FABP3 gene and SNPs (rs210042291, rs136297772 and rs132781540) studied according to UCSC and NCBI browsers.

rs132781540) in the studied fragment of FABP3 gene by using sequencing method, determined of polymorphism and allelic frequency, and to evaluate its association with productive performance in Holstein cows.

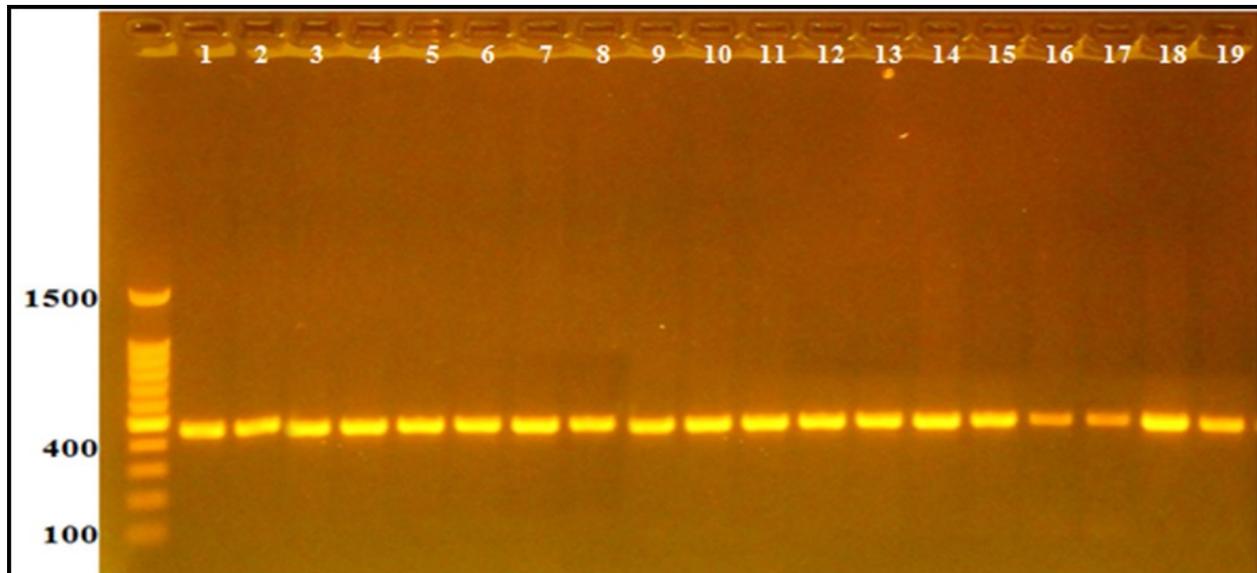
#### MATERIALS AND METHODS

This study was conducted at Al-Salam Station for dairy cattle / Private Sector (Latifia district, 25 km southern Baghdad), for the period from 1/1/2017 to 1/1/2018 to determine the genotype of FABP3 gene and its association with some productive and reproductive traits in 40 Holstein cows as well as extraction ratios for distribution of genotypes in herd and allele frequency. Samples of milk produced from dairy cows were taken in the morning for the analysis of milk components in the research and development division of the Abu Ghraib dairy factories using an electrical device called Ultrasonic Milk Analyzer (Master LM2), to estimate some milk components, as well as the monthly analysis of the milk components which is played by the station by agreement with the milk marketing lab of Jawhara company in Latifiyah (Al-Janabi *et al.*, 2018). Genetic

examination of the blood was done in the laboratory of scientific progress. Polymerase chain reaction (PCR) procedure was utilized to amplify the required fragment to finish the molecular detection and polymorphism of FABP3 gene and as per the sequencing technique, a samples from the studied fragment of FABP3 gene (PCR Product) of all cows samples was sent by a scientific office outside of Iraq and specifically to Macrogen company in the United States of America to obtain the real sequences of the nitrogen bases of the studied fragment of FABP3. The primers were selected as shown below for the purpose of conducting molecular detection and for the polymorphism analysis of the gene resulting from the presence of mutations (SNPs) of FABP3 gene (Wu *et al.*, 2005). Note both of the SNPs identified in the studied fragment of FABP3 gene were located in intragenic regions (the third intron) on chromosome 2 (Figure 1).

Forward = 5'- GTGAGTTGAGGAAGGGGCTGTG -3'

Reverse = 5'- TAGGTCTCCACCTCTTGTCTTCAG -3'



**Figure 2. Extraction fragment (458 bp) of the FABP3 gene by PCR technique**

The gene fragment was studied and their location have been confirmed and verified by electronic browsers for vertebrate genome: National Center for Biotechnology Information (NCBI), University of California Santa Cruz (UCSC) and Ensembl Genome Browser. The sequences of FABP3 gene is available in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) under Accession No. 281758.

The data were analyzed statistically using the Statistical Analysis Program - SAS software (SAS, 2012), to study the effect of the genetic polymorphism of FABP3 gene (SNP: rs 383276104) according to the mathematical model below. Significant differences between the averages were compared with the application of the least square mean method.

$$Y_{ijk} = \mu + G_i + O_j + e_{ijk}$$

$Y_{ijk}$ : view value (k) of genotype (i) and the month of birth (j),  $\mu$ : the general average of the traits,  $G_i$ : effect of genetic polymorphism of FABP3 gene (GG and GT),  $O_j$ : effect of month of birth (April, May and June),  $e_{ijk}$ : random error which is distributed naturally at an average of zero and variance of  $\sigma^2_e$ . The Chi-square- $\chi^2$  test was used to compare the percentage distribution of genotypes.

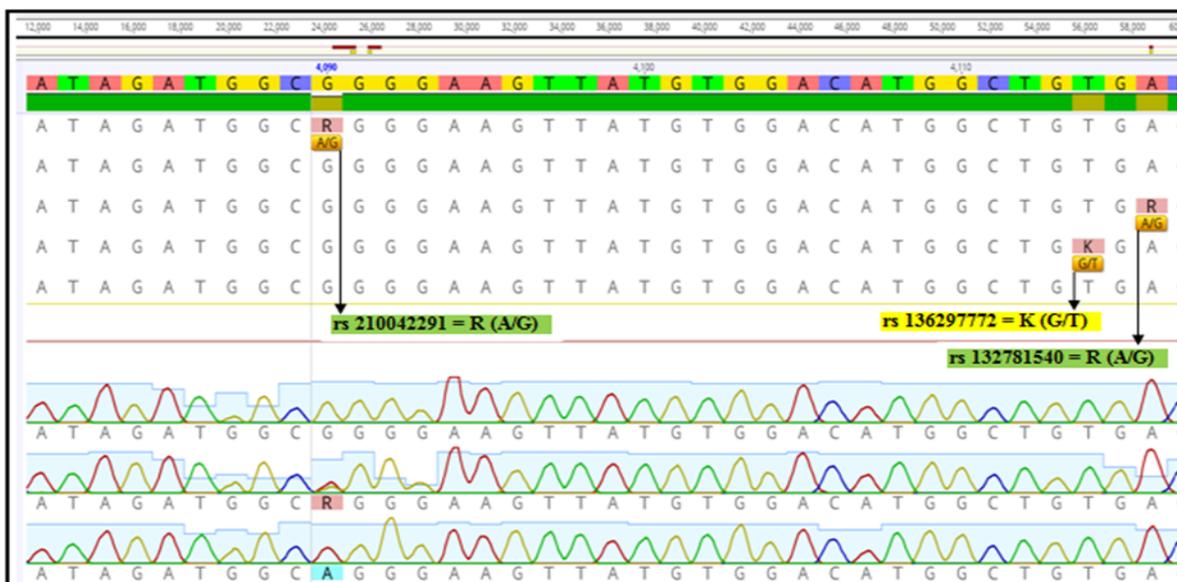
## RESULTS AND DISCUSSION

### DNA extraction and Determination of the genotypes of FABP3 gene

FABP3 gene was extracted with polymerase chain reaction (PCR) technique by used the PCR kit and primers, a total DNA samples and adjust your thermal cycles, the samples were then migrated from each sample model and imaging the output of the migration to make sure the extraction process is successful and required fragment size 458 bp for FABP3 gene, the size of the DNA fragments were used as a marker 1500-100 bp DNA ladder (Figure 2). The polymorphisms of the experiment cows were determined of SNP (rs210042291) in the studied fragment of FABP3 gene (458 bp) by used sequencing method (Figure 3). Note that both SNP (rs136297772 and rs132781540) did not show in the sample of Holstein primiparous cows imported from Germany, only SNP (rs210042291), whose relationship to production performance has been studied.

### Distribution of genotypes and allele frequency of FABP3 gene (SNP: rs210042291)

Table 1 showed then number and percentage of FABP3 gene polymorphism. There were a highly significant different ( $P < 0.01$ ) between distribution ratio of



**Figure 3. The sequencing products of SNP (rs210042291) in the studied fragment of FABP3 gene. The genotype GG = Homozygous (Wild); The genotype GA = Heterozygous; The genotype AA = Mutant**

FABP3 gene (SNP: rs210042291) in the studied cows sample which reached to 80.00%, 15.00 % and 5.00 % for the genotypes GG, GA and AA respectively. The reports demonstrated that the homozygous genotype (GG) is prevailing a more prominent than the heterozygous genotype (GA) and the scarcity of mutant genotype (AA) in the study animals. The consequences of past investigations on the same studied fragment and position of FABP3 gene in beef cattle demonstrated that percentage frequency of genotypes were 57.20, 36.21 and 6.58 % for the genotypes GG, GA and AA respectively (Wu *et al.*, 2005). While percentage of genotypes frequency in Jersey cows were 6.00, 36.00 and 58.00 % for the genotypes GG, GA and AA respectively (Kulig *et al.*, 2010). Yardibi *et al.* (2013) showed that there

were significant difference between number of genotypes, which were in East Anatolian red cattle breed (16, 30 and 4), and South Anatolian red cattle breed (5, 0 and 45) for the genotypes GG, GA and AA respectively, when they studied on same fragment and position of FABP3 gene, and they used 50 animals for both breeds. The results (Table 1) showed that the allele (G) return of FABP3 gene in the sample of Holstein cows was 0.88, while the allele (A) frequency was 0.12, indicating that the allele (G) is dominant in imported German Holstein cows. The results of previous studies on the same studied fragment and position of FABP3 gene in beef cattle showed that the allele frequency was 0.7531 and 0.2469 for both the allele G and A respectively (Wu *et al.*, 2005). But the allele frequency in East Anatolian red

**Table 2. Relationship of FABP3 gene (SNP: rs210042291) polymorphism in total milk production and lactation period**

Genotype	Number of cows (Total number 40)	Mean ± SE	
		Total milk production (kg)	Lactation period (day)
GG	32	1613.37±25.64 <sup>b</sup>	167.42±1.67 <sup>b</sup>
GA	6	1869.02±57.33 <sup>a</sup>	187.31±2.05 <sup>a</sup>
AA	2	1526.48±44.54 <sup>b</sup>	160.73±2.68 <sup>b</sup>
Significance level		**	**
**(P<0.01) Means with different letters in same column differed significantly			

**Table 3. Relationship of FABP3 gene (SNP: rs210042291) polymorphism with the period from birth to the peak of production and the length of the peak of production**

Genotype	Number of cows (Total number 40)	Mean $\pm$ SE	
		The period from birth to the peak of production (day)	The length of the peak of production (day)
GG	32	42.79 $\pm$ 0.81 <sup>ab</sup>	41.57 $\pm$ 0.77 <sup>b</sup>
GA	6	46.82 $\pm$ 0.76 <sup>a</sup>	50.78 $\pm$ 0.91 <sup>a</sup>
AA	2	39.63 $\pm$ 0.96 <sup>b</sup>	42.35 $\pm$ 1.27 <sup>b</sup>
Significance level		**	**

\*\*( $P < 0.01$ ) Means with different letters in same column differed significantly

cattle breed was 0.62 and 0.38, and South Anatolian red cattle breed was 0.10 and 0.90 for both the allele G and A respectively (Yardibi *et al.*, 2013). While the allele frequency in Jersey cows was 0.24 and 0.76 for both the allele G and A respectively (Kulig *et al.*, 2010).

#### **Relationship of FABP3 gene (SNP: rs210042291) polymorphism with milk production and lactation period**

There was highly significant ( $P < 0.01$ ) changes in total milk production between the genotypes of FABP3 gene (SNP: rs210042291), the lows with heterozygous genotype (GA) accomplished the highest milk production rate (1869.02 $\pm$ 57.33 kg), contrasted with dairy animals with the homozygous polymorphism (GG and AA), which were recorded as 1613.37 $\pm$ 25.64 and 1526.48 $\pm$ 44.54 kg respectively, which did not significance differ between them which were recorded as 1613.37  $\pm$  25.64  $\pm$  and 1526.48 $\pm$ 44.54 kg respectively, which did not differ significantly between them. The results of previous studies confirmed on that there is a significant relationship between polymorphism of FABP3 gene with milk production traits (Kulig *et al.*, 2010; Yadav *et al.*, 2018). The results in Table 2 showed highly significant differences ( $P < 0.01$ ) between genotypes of FABP3 gene (SNP: rs210042291) in the lactation period. The mean lactation period was 187.31  $\pm$  2.05 day for cows with heterozygous genotype (GA), compared to cows with the homozygous genotypes (GG and AA), which were recorded 167.42 $\pm$ 1.67 and 160.73 $\pm$ 2.68 day respectively, which did not differ sig-

nificantly between them. Accordingly, the relationship of the GA polymorphism of FABP3 gene with milk production and the lactation period recommends that this marker might be valuable for animal selection based on the contrast at the DNA level, direct markers that were distinguished and connected with milk production and the lactation period in breeding projects of Holstein cows.

#### **Relationship of FABP3 gene (SNP: rs210042291) polymorphism with the period from birth to the peak of production and the length of the peak of production**

Profoundly significant differences ( $P < 0.01$ ) for the period from birth to peak of production and the length of the peak of production with various genotypes of FABP3 (SNP: rs210042291) (Table 2), showed that cows with genotype GA had the best rate in the longest period from birth to the peak of production (46.82 $\pm$ 0.76 day), contrasted with cows with genotype AA, which enrolled lowest rate (39.63 $\pm$ 0.96 day). While cows registered with genotype GG had median rate (42.79 $\pm$ 0.81 day), which did not differ significantly from both genotypes GA and AA. As cows achieved with genotype GA had the best rate in the length of the peak (50.78 $\pm$ 0.91 day), compared to cows with the homozygous genotypes (GG and AA), which were recorded as 41.57 $\pm$ 0.77 and 42.35 $\pm$ 1.27 day respectively, which did not differ significantly between them. It has been attributed to the fact that the cows with the heterozygous genotype (GA) gave the highest production so it had the longest the

**Table 4. Relationship of FABP3 gene (SNP: rs210042291) polymorphism with milk composition**

Genotype	Number of cows (Total number 40) Number of samples (Total number 120)	Mean $\pm$ SE				
		Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)	Specific gravity of milk
GG	32 (96 sample)	3.77 $\pm$ 0.62 <sup>a</sup>	4.52 $\pm$ 0.05 <sup>a</sup>	3.36 $\pm$ 0.03 <sup>a</sup>	8.39 $\pm$ 0.09 <sup>a</sup>	1.031 $\pm$ 0.02 <sup>a</sup>
GA	6 (18 sample)	3.15 $\pm$ 0.24 <sup>b</sup>	4.42 $\pm$ 0.12 <sup>a</sup>	2.84 $\pm$ 0.05 <sup>b</sup>	8.44 $\pm$ 0.21 <sup>a</sup>	1.033 $\pm$ 0.66 <sup>a</sup>
AA	2 (6 sample)	3.91 $\pm$ 0.57 <sup>a</sup>	4.52 $\pm$ 0.04 <sup>a</sup>	3.26 $\pm$ 0.05 <sup>a</sup>	8.41 $\pm$ 0.06 <sup>a</sup>	1.029 $\pm$ 0.02 <sup>a</sup>
Significance level		*	NS	*	NS	NS

\*(P<0.05) Means with different letters in same column differed significantly  
NS: Not significant.

peak of production compared to cows with homozygous genotypes (GG and AA) in this study. The studies have indicated that dairy cows that have higher initial production and higher production yield and more ability to continue produce milk at near peak levels throughout the lactation are expected to have higher milk yields per lactation (Seangjun *et al.*, 2009).

#### **Relationship of FABP3 gene (SNP: rs210042291) polymorphism with milk composition**

Table 4 demonstrated that a significant difference (P<0.05) in fat and protein percentage in milk between the genotypes of FABP3 gene (SNP: rs210042291), the cows with the homozygous polymorphism (GG and AA) recorded the highest fat percentage (3.77 $\pm$ 0.62 % and 3.91 $\pm$ 0.57 %) and the most astounding protein content (3.36 $\pm$ 0.03 % and 3.26 $\pm$ 0.02 %) at both polymorphism (GG and AA), contrasted with dairy animals with the heterozygous (GA), which recorded the least fat and protein content (3.15 $\pm$ 0.24 % and 2.84 $\pm$ 0.05 %) respectively. The reports in Table 4 indicated non-significant differences in the level of lactose, non-fat solids and specific gravity of milk between the genotypes of FABP3 gene (SNP: rs210042291). The cows with the homozygous genotype (GG) recorded 4.52 $\pm$ 0.05, 8.39 $\pm$ 0.09 and 1.031 $\pm$ 0.02 of lactose, non-fat solids and specific gravity of milk respectively and dairy animals with heterozygous genotype (GA) recorded 4.42 $\pm$ 0.12, 8.44 $\pm$ 0.21 and 1.033 $\pm$ 0.06 of lactose, non-fat solids and specific gravity of milk respectively,

while cows with mutant genotype (AA) recorded 4.52 $\pm$ 0.04, 8.41 $\pm$ 0.06 and 1.029 $\pm$ 0.02 of lactose, non-fat solids and specific gravity of milk respectively. Kulig *et al.* (2010) showed that there were statistically significant differences between the mean values of protein and fat content in Jersey cows with different FABP3 genotypes. The AA cows were characterized by significantly higher protein content in milk than the GA and GG cows. Similarly, milk fat content in the AA cows was significantly higher compared with the GA and GG cows. Moreover, the GA cows were characterized by a significantly higher fat content compared with the GG cows.

#### **CONCLUSION**

FABP3 is an important candidate gene for milk production traits. The results of the present study confirmed mainly the significant impact of polymorphisms in FABP3 gene on productive performance. Polymorphism (GG, GA and AA) of FABP3 gene (SNP: rs210042291) has been associated with both milk yield and its composition traits. It is generally accepted that the FABP3 has an important role in the milk fat synthesis of dairy cow mammary epithelial cells, therefore, can be considered as a strong candidate gene for economically important production traits in Holstein cows. Thus, selection of dairy cows for FABP3 could be a major deterrent in improving dairy economics. Since, our study was biased by small sample size, obviously,

all information concerning the tested polymorphisms in FABP3 gene (SNP: rs210042291) should be confirmed during tests using different breeds of cows and a larger number of animals, that would increase the reliability of the obtained results. It is therefore recommended to analyze a larger population of Holstein cows. It could be concluded that it is the first study, which are characterized by a precise description of the studied fragment of FABP3 gene and its size, and determination of the genotypes of single nucleotide polymorphism (SNP: rs210042291) for FABP3 gene on the basis of the browsers of the genome of vertebrates, the FASTA for FABP3 gene and used sequencing method compared to all previous studies, which used PCR-RFLP technique.

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