Identification of lethal recessive genetic variants in Holstein cattle

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Abstract. Artificial insemination is the main method of herd reproduction in cattle breeding and is associated with the risk of spreading genetically determined diseases. Widely used high-yield bulls are often carriers of fertility haplotypes and other harmful genetic variants. The traditional approach to identifying genetic factors associated with lethal recessive variants that cause defects or death is to track the common ancestors of sick animals using pedigrees and is unable to detect harmful genetic variants that cause the death of embryos. A homozygous harmful phenotype leads to early death of the developing embryo, the only observed consequence of this is lower fertility of the parents. With the development of genomic technologies, it has become possible to identify mutations that lead to embryonic death at different stages of fetal development. The purpose of the work was to perform an analytical review of the literature on the identification of lethal recessive genetic variants in cattle. The haplotypic approach is considered as the main method of detecting harmful mutations. The essence of the method is to search for segments in the genome, the actual homozygosity of which tends to zero, unlike the expected one. Haplotype analysis revealed lethal recessive genetic variants and causal mutations in Holstein cattle, common in populations with a frequency of 0.07-47.75%. Considering the occurrence of lethal genetic variants in cattle populations, the disclosure of the basis of genetically determined diseases will make it possible to screen animals and eliminate carriers from the breeding process.

1 Introduction

The main method of reproduction in cattle breeding is artificial insemination. Outstanding sires can produce thousands of offspring in a short time. Replication of the genotypes of such bulls is associated with the risk of spreading genetically determined diseases and can cause significant economic damage [1]. First of all, this is due to the fact that most anomalies are inherited recessively and phenotypically manifest only in a homozygous state in the form of embryo death [2].

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A small number of popular breeders may dominate the breeding herd. Consequently, the effective population size (Ne) decreases, which leads to an increase in the frequency of harmful alleles. Hereditary defects usually include unique variants of the "founders" [3]. Such variants can be observed with high frequency in a certain population and originate from a single influential ancestor. Usually, these types of harmful alleles are eliminated from the population by selection, which can be effective for dominant harmful alleles that reduce the fitness of heterozygous animals. Nevertheless, due to the low frequency, recessive harmful alleles are usually masked from natural selection by a dominant harmless allele and therefore easily transmitted to the next generation [4]. This effect is called balancing selection. The recessive lethal allele demonstrates pleiotropic effects on productive traits with moderate frequency in the population. Several cases of balancing selection in different cattle breeds have been described. Thus, the deletion of 660 kbp caused embryonic death in homozygous individuals, at the same time it was associated with the level of milk productivity and milk composition in heterozygous individuals among northern red breeds [5] and signs of meat and fattening productivity in animals of the Belgian blue breed [3]. Identifying balancing selection by lethal alleles can be a difficult task, since the only observed consequence is lower parental fertility and the absence of affected live-born individuals.

The identification of harmful genetic variants in farm animals is a difficult task, which is currently being solved by applying traditional breeding methods or modern genomic breeding strategies. The low efficiency of elimination of harmful variants is often due to an unpredictable or poorly characterized relationship between genotype and phenotype. For example, when a homozygous harmful phenotype leads to early death of a developing embryo, the only observed consequence of this is lower fertility of the parents [6].

The traditional methodology for identifying genetic factors associated with lethal recessive variants that cause defects or death is to track the common ancestors of sick animals using pedigrees. Since this method is based on phenotypic data, it is not able to detect harmful genetic variants that cause embryo death [7]. In the presence of a large number of SNPs, combining genetic information with the pedigree of individuals makes it possible to uncover the genetic basis of rare diseases in cattle populations [8].

The purpose of the work was to perform an analytical review devoted to the identification of lethal recessive genetic variants in cattle.

2 Results

2.1 Identification of mutations leading to embryonic mortality using a haplotypic approach

Recessive defects causing embryo death can be detected by the absence of homozygous haplotypes. VanRaden and colleagues [9] determined haplotypes with a length of ≤ 75 markers in 58453 individuals of the Holstein breed, 5288 - Jersey, and 1991 - Brown Swiss breed based on data from genome-wide genotyping using BovineSNP50 BeadChip (Illumina, San Diego, California). Haplotypes were determined using the program findhap.f90 v.2 [10]. A total of eleven candidate haplotypes have been identified, and phenotypic effects have been confirmed for five of them. This method is a powerful tool for identifying harmful haplotypes that are common in a population without using phenotypic data, while its detection ability directly depends on the number of genotyped animals [9].

The proposed approach, supplemented by subsequent analysis, led to the identification of haplotypes HH1, HH3, HH4, HH5, HH6, and HH7 of Holstein cattle [11-17]. The method effectiveness depends on several parameters, of which the most important are: the

number of genotyped animals, the frequency of mutations in this population, the linkage disequilibrium between the haplotype and mutation [12]. The linkage disequilibrium depends on the mutation history and the informativeness of the haplotype. In dairy cattle populations, these mutations are usually spread by recently popular bulls and are surrounded by large chromosomal segments of ≥1 Mb in size. Thus, the use of medium-density SNP chips (containing 50 thousand or 80 thousand markers) provides sufficient variability of haplotypes, which guarantees a high linkage disequilibrium and a decrease in the number of false positive or false negative results. Obviously, older mutations surrounded by a shorter ancestral segment will require a higher density of markers to be detected. As a rule, samples with a capacity of 2,000 animals are sufficient to identify lethal haplotypes with a frequency of 10%, and more than 200,000 individuals are needed to obtain significant results for haplotypes distributed with a frequency of 1%. Thus, it is expected that a regular increase in genotyping databases will lead to the regular identification of new harmful haplotypes that segregate with low frequency in the analyzed populations [17].

Schmidt and co-authors [18] searched for candidates of lethal haplotypes in Nellore cattle. The construction of haplotypes was performed using the sliding window method implemented in the software findhap.f90 v3 [19]. The program identified haplotypes with a length of ≤ 200 markers, which were used for further analysis. Haplotypes with the highest frequencies (> 2%), which have never been observed in the homozygous state, were a priority for study because of their potential significance and to increase the detection probability. When calculating the expected number of homozygous individuals for each haplotype, two methods were used: a simple method — it was assumed that all members of the population were randomly mated over time, and the mating method - the actual mating model (observed natural mating and artificial intelligence) generating genotyped individuals in the population. To determine the expected area of the recessive lethal haplotype, conditions were accepted, according to Wu and colleagues [8]: the frequency of the haplotype carrier should be higher than 2%, the number of expected homozygous individuals according to the haplotype should be greater than 1, the probability of observing 0 homozygotes should be <0.6. All haplotypes satisfying these conditions were selected. GWAS was then performed to identify significant SNPs inside or near the regions of the candidate lethal haplotype. The authors found 30 potentially lethal haplotypes with a minimum frequency of 2% in the population.

Hoff and co-authors [20] searched for candidate lethal haplotypes and causal mutations in Angus cattle. Haplotypes were determined using 20-marker sliding windows throughout the genome. As a result of the research, it was found that seven loci include haplotypes that were not observed in homozygous form, despite a sufficiently high frequency and the expected homozygosity calculated on the basis of pedigrees.

Charlier and colleagues [21] applied a reverse genetics approach that takes advantage of the growing amount of full-exome and full-genome NGS data in livestock. The proposed approach consisted in analyzing available sequence data for predicted LoF variants and harmful missense mutations, genotyping large samples of relevant candidates, identifying suspected lethal mutations based on a significant decrease in homozygotes, confirming the corresponding supercandidates based on a significant decrease in homozygotes during carrier × carrier mating.

Häfliger and colleagues [22] report the results of large-scale reverse genetic screening of animals of the Simmental breed, which revealed 11 genomic regions with haplotypes (from SH1 to SH11) showing a significant decrease in homozygosity and allele frequency from 3.2 to 10.6%. A subsequent search for single-nucleotide variants and short indels in the sequenced genomes of 23 haplotype carriers made it possible to identify three ideally related candidate variants causing protein changes for haplotypes SH5, SH8, and SH9. Four

selected haplotypes SH5, SH7, SH8, and SH10 demonstrated a complete deficiency of the observed homozygous animals, while the rest showed a partial deficiency in the range from 85 to 96% of the expected homozygotes.

Jenko and colleagues [23] performed a search for haplotypes that carry presumably recessive lethal or semi-lethal alleles in 132725 genotyped five meat breeds in Ireland: Aberdeen Angus, Charolais, White-faced, Limousine, and Simmental. The genotypes were arranged in stages in sliding windows along the genome and five tests were used to identify haplotypes with no or reduced homozygosity. After that, an associative analysis of the identified haplotypes was performed with 44,351 insemination records, which indicated early embryonic losses, and records of postnatal survival. Thus, one haplotype was confirmed, which presumably carries a recessive lethal one (chromosome 16 in Simmental cattle) and two haplotypes carrying semi-lethal alleles (chromosome 14 in Aberdeen Angus cattle and chromosome 19 in Charolais). The analysis of the data presented in the literature indicates the success of the strategy used to identify recessive lethal mutations in cattle. Nevertheless, for some animal populations, it is not possible to study large samples due to the small number of breeds. Naji and co-authors [24] performed a search for haploblocks in cattle of the Tyrolean Grey breed (n=276). Haploblocks were identified using the GHap package (Utsunomiya and Milanesi, 2017) in the R software, a scan window of 500 KB in size and a sliding step of 100 kb. They found 5 haploblocks with an observed homozygosity of 0 and two haploblocks, the observed homozygosity of which significantly differed from the expected one in a smaller direction.

2.2 Recessive lethal mutations identified in Holstein cattle

One of the most common breeds in the world is the Holstein breed. The number of Holstein breed animals in the Russian Federation in 2022 amounted to 1428.43 thousand heads, which accounts for 54.43% of the total livestock [25]. In this regard, an important practical aspect is characterized by the study of recessive lethal mutations identified in Holstein cattle using the approach proposed by VanRaden and colleagues (2011) [9] and the assessment of the frequency of their spread in populations. We analyzed information on lethal embryonic mutations occurring in Holstein cattle, posted in the OMIA database (www.omia.org) (Table 1), the identification of which was carried out using the haplotypic approach.

Table 1. Lethal embryonic mutations occurring in Holstein cattle

Gene (haplotype)	Description	BTA	Mutation (genome assembly)	Source
APAF1 (HH1)	A nonsense mutation in the *APAF1* gene truncates about one third of the encoded APAF1 protein. The functional peptide APAF1 is necessary for the embryo development.	5	g.62810245C>T (APC-UCD1.2)	[15]
IFT80 (HH2)	The reading frame shift in *IFT80* (p.Leu381fs) disrupts the transmission of WNT and hedgehog signals and is responsible for the death of homozygous embryos.	1	g.107172616delT (ARS-UCD1.2)	[26]
SMC2 (HH3)	The polymorphism of F1135S changes phenylalanine to serine and causes a non-neutral, intolerant, and evolutionarily	8	g.93753358T>C (ARS-UCD1.2)	[14]

	unlikely replacement in the NTPase domain of the encoded protein. SMC2 plays an important role in DNA repair, chromosome condensation and segregation during cell division.			
GART (HH4)	Convincing evidence is presented for a possible causal missense mutation (G.1277227A>C; genome assembly UMD 3.1) in the *GART* gene, which encodes glycinamide ribonucleotide transformylase.	1	g.1997582A>C (ARS-UCD1.2)	[11]
TFB1M (HH5)	Deletion 138 kbp, covering the position from 93,233 to 93,371 kbp on BTA9 is a probable causal mutation.	9	Deletion of 138 kbp, covering the position from 93,233 to 93,371 kbp on chromosome 9 (BTA9) (UMD3.1)	[16]
SDE2 (HH6)	Replaces the initiator codon ATG (methionine) with ACG, since the gene is transcribed on the reverse chain... Initiation of translation from the nearest Met codon in the reading frame truncates SDE2 into 83 amino acids, including an 8 amino acid motif conserved in eukaryotes.	16	g.29020700A>G (ARS-UCD1.2)	[12]
CENPU (HH7)	A deletion of 4 bps in length (27: 14168130_14168133delTACT UMD 3.1) affects a highly conserved region in mammals.	27	g.15123637_15123640del (ARS-UCD1.2)	[17]
KIR2DS1 (HH13)	The *KIR2DS1*:p.Gln159* mutation has been proposed as a probable causal variant of the HH13 haplotype.	18	g.62758881G>A (ARS-UCD1.2)	[27]
RIOX1 (HH25)	Deletion *RIOX1*; c.396_425delGGC GCA GAC CCC GGC GGC ACG CTT GGT GGA has been proposed as a probable causal variant of haplotype H25.	10	g.84938408_84938437del (ARS-UCD1.2)	[27]
PCDH15 (HH35)	The *PCDH15*; c.2599C>G mutation has been proposed as a probable causal variant of haplotype 35.	26	g.5325675C>G (ARS-UCD1.2)	[27]
APOB (HCD)	The mutation is the insertion of a mobile LTR (ERV2-1) element with a size of 1.3 kbp into the coding sequence of the *APOB* gene, which leads to shortened transcripts and aberrant splicing [p.Gly135ValfsX10).	11	1.3 KB insertion on BTA11:77,958,995 (UMD3.1)	[16]
RABGGTB	The missense mutation	3	g.69060748T>C (ARS-	[21]

	p.Tyr195Cys has been proposed as a recessive potentially lethal mutation.		UCD1.2)	
RNF20	Nonsense mutation, stop codon p.Lys693* is proposed as a recessive potentially lethal mutation.	8	g.91297797A>T (ARS-UCD1.2)	[21]
TTF1	Nonsense mutation, stop codon p.Arg527∗ has been proposed as a recessive potentially lethal mutation.	11	g.102463944G>A (ARS-UCD1.2)	[21]
FANCI	A 3.3 KB deletion removing exons 25-27 of the *FANCI* gene	21	g.20773899_20777226del (ARS-UCD1.2)	[28]

As shown in Table 1, haplotypes have been identified in the Holstein breed, as well as the positions of a number of lethal recessive mutations have been mapped. Almost all of them lead to embryonic death at various stages of development. Phenotypically, this is manifested in an increase in the interbody period, a decrease in fertility in general.

The frequency of lethal recessive mutations in populations of Holstein cattle of various origins is shown in Table 2.

Table 2. The frequency of lethal recessive mutations in populations of Holstein cattle.

Gene (haplotype)	Frequency, %	Source	Gene (haplotype)	Frequency, %	Source
APAF1 (HH1)	2,90	[29]	*TFB1M (HH5)*	2,35	[36]
	2,82	[36]		1,9	[12]
	2,5	[31]		1,8	[17]
	1,4	[32]		3,24/1,41 bulls/cows	[41]
	2,1	[33]		12	[42]
	47,75	[34]	*SDE2 (HH6)*	0,07-0,9	[36]
	2,04 / 1,83 bulls/cows	[35]		1,2-1,4	[12]
	0,39-7,27	[36]		0,30	[37]
	1,7	[12]		1,6	[17]
	3,45	[37]		2,2	[38]
	1,6	[17]	*CENPU (HH7)*	0,03-0,29	[36]
	4,5	[38]		0,29	[37]
IFT80 (HH2)	1,66	[39]		1,1	[17]
	4,6	[9]		2,2	[38]
	0,8	[12]	*KIR2DS1 (HH13)*	4,1	[38]
	0,94	[40]		3,7	[11]
SMC2 (HH3)	3,75	[36]		1,81	[27]
	0,75-5,7	[36]	*RIOX1 (HH25)*	1,96	[27]
	3,1	[12]	*PCDH15 (HH35)*	2,44	[27]
	3,62	[37]	*APOB (HCD)*	2,2	[36]
	3,1	[17]		2,5	[39]
	4,7	[38]		5,57 / 2,06 bulls/cows	[35]
	1,14 / 2,98 bulls/cows	[35]		11	[42]
GART (HH4)	0,59	[36]		8,09	[43]
	0,04-5,15	[36]	*RABGGTB*	2,13	[21]
	4,4	[12]	*RNF20*	1,82	[21]
	1,31	[37]	*TTF1*	3,52	[21]

	4,2	[17]	*FANCI*	7	[38]
	7,2	[38]		2,89/4,13 bulls/cows	[35]
	1,30/1,04 bulls/cows	[35]		2,76	[39]

The analysis of the occurrence of mutations persisting in various populations of cattle (Table 2) showed that the frequency of the HH1 haplotype varied in the range of 0.39-7.27, but in the group of bulls of Polish origin it reached 47.75% [34]. In the Russian Federation, the frequency of the HH1 haplotype was 1.4-2.04%. The HH2 haplotype was found with a frequency of 0.8-4.6%. In the Russian Federation, there is no data on the spread of this mutation in herds, since the causal mutation became known relatively recently. The HH3 haplotype was recorded with a frequency of 0.75-5.7%. In the Russian population, its frequency was 1.3% in bulls and 1.04% in cows [35]. According to the HH4 haplotype, frequencies from 0.59 to 7.2% were recorded. In Russia, this parameter was at the level of 1.30 and 1.04 for bulls and cows, respectively. The HH5 haplotype occurs with a frequency of 1.41-12%. In Russia, its frequency was 3.24% for bulls and 1.41% for cows. The causal mutation for the HH6 haplotype has become known relatively recently, therefore, there is still no information about the distribution of this haplotype in the Russian population of Holstein cattle. According to research by foreign scientists, it is common with a frequency of 0.07-2.2%. According to the haplotype HH7, it should be noted that it occurs with a frequency of 0.03-2.2%. The studies of Kovalyuk N.V. and co-authors [44] showed the absence of carriers of haplotype HH7 in the Holstein breed subpopulation of the Krasnodar Territory. The causal mutations of haplotypes HH13, HH25, and HH35 were among the last to be identified. Few studies have been conducted to determine the frequency of their occurrence in populations of Holstein cattle of various origins, it can be noted that the frequency of HH13 was 1.81-4.1%, haplotypes HH25 and HH35 were found in populations of Holstein cattle of Swiss origin with a frequency of 1.96 and 2.44%, respectively. The frequency of the HCD haplotype in Russian animals was 2,06-8,9% [35, 43], and abroad 2,2-2,5% [30, 39]. The following frequencies of occurrence were recorded for the *RABGGTB, RNF20*, and *TTF1* genes: 2.13, 1.82, and 3.52%, respectively. The mutation in the *FANCI* gene causing the brachyspina defect, according to various data, was 2.76-7%. In the Russian population of the Holstein breed, its frequency was noted at 2.89% in bulls and 4.13% in cows.

3 Conclusion

The development of genetic technologies has provided a wide range of methods and approaches for the identification of lethal recessive mutations in cattle. Genome-wide genotyping using chips of different densities and NGS sequencing provides opportunities for the identification of haplotypes with loss of homozygosity, which may be responsible for embryonic death, the phenotypic manifestation of which may be a decrease in fertility. The search for haplotypes with loss of homozygosity can be carried out without using phenotypic data. The determination of causal mutations makes it possible to develop molecular genetic tests for livestock screening and eliminate heterozygous carriers of harmful mutations from the breeding process. Given the prevalence of fertility haplotypes in Holstein breed populations of various origins, monitoring the genetic status of breeding animals, especially breeding bulls, is of great practical importance. Thus, the search for haplotypes with loss of homozygosity, mapping of causal mutations and DNA diagnostics of breeding material aimed at identifying genetically determined diseases will allow controlling the spread of harmful mutations in herds and increase the economic efficiency of breeding work.

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