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745. Characterization of homozygous haplotype deficiency compromising fertility traits in Manech Tête Rousse dairy sheep

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Abstract

With the objective to identify deleterious recessive mutations, we have scanned the genome of 5,271 French Manech Tête Rousse (MTR) dairy sheep using 50k SNP phased genotypes and pedigree data. Five deficient homozygous haplotypes, named MTRDHH1 to MTRDHH5, were identified. These haplotypes showed a highly significant deficit of homozygous animals ranging from 84 to 100%, and a carrier frequency ranging from 7.8 to 16.6%. By comparing at-risk matings (between carriers) and safe matings, some of these haplotypes were associated with a reduced success of artificial insemination (MTRDHH2), and/or an increased stillbirth rate (MTRDHH1 and 2) suggesting the segregation of recessive variants leading to embryo and/or perinatal lethality affecting fertility. Other haplotypes are likely to be associated with lamb morphological disorders or counter-selection based on breed standard. For each haplotype, the most probable candidate genes were highlighted based on their roles in lethal phenotypes, genetic disorders, hornless or coat color.

Introduction

In livestock under selection, the small effective population size and a non-negligible inbreeding rate could increase homozygosity and thereafter lead to the emergence of genetic disorders under the control of recessive deleterious variants. When homozygous, these variants very often lead to early embryo death, or to developmental disorders that affect fetuses and subsequently young individuals altering health and welfare (Georges *et al.* 2019). Detection of homozygous haplotype deficiency using reverse genetic screen is a useful method for mapping specific haplotypes associated with deleterious recessive mutations (VanRaden *et al.* 2011). The analysis is based on high throughput genotyping data usually available in the framework of genomic selection. For the first time in sheep, we recently validated this approach in Lacaune dairy sheep by the identification of eight independent deficient homozygous haplotypes (Ben Braiek *et al.* 2021). Complementary whole genome sequence studies have led to identify the causal variant in the *CCDC65* gene within one of these haplotypes (LDHH6, OMIA 002342-9940). Homozygous lambs for the *CCDC65* mutation suffered from a lethal respiratory syndrome (Ben Braiek *et al.* 2022). The objective of the present study is to search for homozygous haplotype deficiency in the Manech Tête Rousse (MTR) dairy sheep also having a large set of genotyping data from genomic selection. This opens the opportunity to discover new deleterious recessive mutations in sheep.

Materials & methods

Animal and genotyping data. The total dataset consists of 6,845 genotyped Manech Tête Rousse animals (82% male and 18% female) born between 1993 and 2021. Genotypes were obtained in the framework of the French dairy sheep genomic selection from Ovine SNP50 BeadChip (MD; n=3,889) and SheepLD (LD; n=2,956) (Illumina Inc., San Diego, USA). Quality control for each SNP was based on: (1) a call frequency >97%; (2) a minor allele frequency >1%; and (3) the respect of the Hardy-Weinberg equilibrium ($P>10^{-5}$). Phasing and imputation (from LD to MD SNP chip) of genotypes were done using *FImpute v2.2*. After quality control, the remaining 38,523 autosomal SNPs were mapped onto the *Ovis aries* genome assembly Oar v3.1.

Detection of homozygous haplotype deficiency. The genome of 5,271 genotyped animals belonging to trios (77 offspring with both parents genotyped and 4,799 offspring with sire and maternal grandsire genotyped) was scanned by a 20 SNP sliding window as described (Ben Braiek *et al.* 2021). Consecutive 20 SNP windows with the same parameters (haplotype frequency, significant homozygous animal deficit) were clustered to define larger region called 'Manech Tête Rousse Deficient Homozygous Haplotype' (MTRDHH). For each MTRDHH, the genotypical status (homozygous non-carriers, heterozygous and homozygous carriers) was determined for all genotypes available (n=6,845).

Analysis of fertility traits. Trait records of MTR matings between 2006 and 2019 were obtained from the national database and based on ewes with a genotyped sire and genotyped rams (maternal sire and ram both having a known status at each MTRDHH; n=330,844 matings). We analyzed two fertility-associated traits, artificial insemination success (AIS) and stillbirth rate (SBR). We have considered 'at-risk' a mating between a carrier ram and a ewe from a carrier sire and the other combinations were 'safe' mating. AIS and SBR were considered to differ significantly when the fixed effect 'mating type' had a *P*-value lower than 1.0×10^{-3} after correcting for multiple testing with a level of significance at 1% following the SAS GLIMMIX procedure previously described (Ben Braiek *et al.* 2021).

Identification of candidate genes in MTRDHH regions. We extracted all annotated genes located in the MTRDHH region extended by 1 Mb from each side from the ovine genome Oar_v3.1 using CLC export annotation function (QIAGEN CLC Main Workbench 7.9). All genes related to mortality/aging phenotypes when knocked-out in mice and/or associated to mammalian genetic disorders were extracted from Mouse Genome Informatics (MGI, http://www.informatics.jax.org), Online Mendelian Inheritance in Man (OMIM, https://omim.org) and Online Mendelian Inheritance in Animal (OMIA, https://omia.org) databases.

Results

Identification of deficient homozygous haplotypes in MTR dairy sheep. Using a reverse genetic screen in MTR genotyped population, we identified 5 independent regions showing a significant deficit of homozygous animals and named MTRDHH (Table 1). Among those regions, three haplotypes (MTRDHH1, 2 and 3) showed a complete deficit of observed homozygous animal while 9 to 13 were expected. The two other haplotypes (MTRDHH4 and 5) presented a partial deficit between 84 and 91% (some homozygous animals were genotyped). The length of these haplotypes ranged from 1.8 to 4.2 Mb and carrier frequencies

Table 1. List of Manech Tête Rousse deficient homozygous haplotypes.

Haplotype	OAR	Number of markers	Position (Mb)[1]	Carrier frequency (%)[2]	Number of homozygotes			
					Exp[3]	Obs[4]	Deficit	Poisson *P*-value
MTRDHH1	20	32	21.4-23.2	9.7	13	0	100%	2.9×10^{-6}
MTRDHH2	1	66	229.2-233.4	8.7	10	0	100%	3.8×10^{-5}
MTRDHH3	1	39	97.2-99.8	7.8	9	0	100%	9.6×10^{-5}
MTRDHH4	10	26	28.2-29.9	8.7	11	1	91%	4.5×10^{-4}
MTRDHH5	13	29	61.3-64.0	16.6	49	8	84%	5.3×10^{-13}

[1] Position on ovine genome assembly Oar_v3.1.

[2] Whole genotyped population (n=6,845).

[3] Expected.

[4] Observed.

ranged from 7.8 to 16.6% in the whole genotyped population. MTRDHH2 and 3 are located on the same chromosome (OAR1) but are not in linkage disequilibrium. The other haplotypes are located on different chromosomes, MTRHH1 on OAR20, MTRDHH4 on OAR10 and MTRDHH5 on OAR13. Consequently, the five MTRDHH detected are likely to host five recessive deleterious variants.

Impact of MTRDHH on the success rate of AI and on stillbirth rate. In order to identify the putative lethal effect of the MTRDHH, we studied two fertility binary traits (AIS and SBR) by comparing at-risk matings to safe matings. AIS (population mean= 60.9%) is a good proxy for embryonic loss, while SBR (population mean=7.5%) is a good proxy for perinatal lethality. As shown in Figure 1, MTRDHH1 has a significant negative impact on SBR (+7.5% in at-risk matings) and MTRDHH2 have negative effects on both AIS (-3.3%) and SBR (+4.3%). The other haplotypes showed no significant effect on the two tested traits.

Candidate genes for MTRHH regions. Among the 459 genes annotated in the five MTRDHH regions, 61 are implicated in lethal phenotype when knocked-out in mice. Among them, several are associated also with mammalian genetic disorders (Table 2). The remaining candidate genes are involved in important functions such as metabolism (*MMUT* and *SSR3*), DNA/RNA processing or transcription (*TFAP2B*, *DHX36* and *BRCA2*), or cell signaling (*PKHD1*, *SV2A*, *ECM1*, *KL* and *RXFP2*).

Discussion

In this study, we have confirmed the usefulness of reverse genetic screen to detect homozygous haplotype deficiency in sheep, even with a more reduced dataset of genotyped trios (MTR~5,000) compared to our previous work (Lacaune ~15,000, (Ben Braiek *et al.* 2021)). However, using the same parameters we identified less significant haplotypes (5 in MTR vs 11 in Lacaune) with the lower haplotype carrier frequency at 7.8% compared to 3.7% in Lacaune.

Among the five MTRDHH, three of them are totally deficient in homozygous animals leading to the hypothesis that these haplotypes host recessive lethal mutations. Accordingly, MTRDHH1 and 2 are associated with defect in fertility traits, AIS and/or SBR. This lets us think that when homozygous these haplotypes induce embryonic or fetal/neonatal lethality. To support this hypothesis, we found obvious candidate genes in these regions (*MMUT, TFAP2B, PKHB1, DHX36* and *SSR3*) all associated with
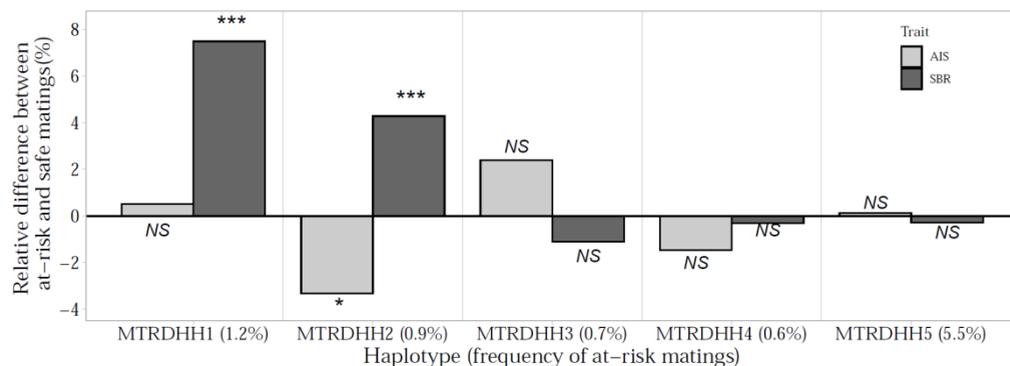
Figure 1. Effects of MTRDHH on the success rate of artificial insemination and on the stillbirth rate in at-risk matings compared to safe matings. *AIS*: artificial insemination success, *SBR*: stillbirth rate. Significant effects are indicated by the corrected *P*-value for multiple tests with a threshold set at α=1%: *$P > 1 \times 10^{-3}$; **$P > 1 \times 10^{-4}$; ***$P > 1 \times 10^{-5}$. NS: not significant.

Table 2. Candidate genes located in MTRDHH regions.

Haplotype (phenotype)[1]	Gene name/Phenotype disorder in mouse and human
MTRDHH1 (Neonatal lethality)	*MMUT:* neonatal, postnatal lethality [MGI:97239]/ methylmalonic aciduria [OMIM# 609058]; *TFAP2B:* neonatal, postnatal, preweaning lethality [MGI:104672]/ patent ductus arteriosus, Char syndrome [OMIM# 601601]; *PKHD1:* perinatal, postnatal, preweaning lethality [MGI:2155808]/ polycystic kidney/ liver disease [OMIM# 606702]
MTRDHH2 (Embryo/fetus lethality)	*DHX36:* embryonic lethality [MGI:1919412]; *SSR3:* neonatal lethality [MGI:1914687]
MTRDHH3 (Juvenile lethality)	*SV2A:* premature death [MGI:1927139]; *ECM1:* preweaning lethality, premature death [MGI:103060]; skin/mucosa/viscera lipoid proteinosis [OMIM# 602201]
MTRDHH4 (Juvenile lethality, morphological disorder)	*KL:* premature death [MGI:1101771]/ tumoral calcinosis [OMIM# 604824]; *BRCA2:* postnatal, prenatal lethality, premature death [MGI:109337]/ Wilms tumor, Fanconi anemia D1 [OMIM# 600185]; *RXFP2:* Polled/Horns phenotype in sheep [OMIA 000483-9940]
MTRDHH5 (Morphological disorder)	*ASIP:* Coat color in sheep [OMIA 000201-9940]; skin/hair/eye pigmentation [OMIM#600201]

[1] Supposed deleterious phenotype in sheep.

embryonic, perinatal or neonatal lethality when altered in mouse or in human (Table 2). In contrast, MTRDHH3 showing also a complete deficit in homozygous animals was not associated with alteration of AIS or SBR. Since lambs are genotyped around three months of age, this haplotype could affect lambs after birth in the preweaning period as supposed by premature death caused by the alteration of the two candidate genes in this region (*SV2A* and *ECM1*, Table 2).

MTRDHH4 and 5 showed a partial deficit in homozygous animals and no significant effect on fertility traits. We hypothesize that these deficits are likely due to either a defect after birth in the juvenile period (alteration of *KL* or *BRCA2* genes for MTRDHH4) or counter-selection based on breed standard. Indeed, horned female and black animals are unwanted in MTR selection scheme. Interestingly, MTRDHH4 hosts the *RXFP2* gene known to be implicated in horn/polled phenotype (Wiedemar and Drögemüller 2015), and *ASIP*, known to be involved in coat color determinism, locates in MTRDHH5 (Norris and Whan 2008).

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