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Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Germany

CHRISTINA WEIMANN, BIRGIT LEYHE-HORN, MATTHIAS GAULY and GEORG ERHARDT

Suitability of microsatellites BM1329 and OarAE101 as markers for the introgression of the Fec^B locus into different sheep breeds

Dedicated to Prof. Dr. Dr. h. c. mult. Horst Kräußlich on the occasion of his 75th birthday

Summary

Litter size in sheep can be improved by introgression of the Fec^B gene. Genetic markers closely linked to the Fec^B locus can be used to detect gene carriers. The efficiency of introgression can be increased by using marker assisted selection. Individuals of the prolific breeds Tyrolean Mountain sheep (n=41), Romanov (n=43), East Friesian Milksheep (n=35) and Merinoland sheep (n=265) were genotyped with the microsatellite markers OarAE101 and BM1329 linked to the Fec^B gene to estimate allele frequencies of both microsatellites in these breeds. The frequencies of the microsatellite alleles linked to the Fec^B gene in Merinoland sheep were 0.07 and 0.1 respectively. The alleles occured also in the other breeds (Tyrolean Mountain sheep, Romanov and East Friesian Milksheep) but with mostly higher frequencies. Furthermore the segregation of the microsatellite alleles was studied in families produced by mating Merinoland sheep ewes (Fec+/Fec+) with two heterozygous Booroola Merino rams (Fec+/Fec^B) in order to use these markers within an indirect gene test. The recombination rate between BM 1329 and OarAE101 was 13 cM (LOD score 14.6).

Key Words: sheep, Fec^B, indirect gene test

Zusammenfassung

Titel der Arbeit: **Eignung der Mikrosatelliten BM1329 und OarAE101 als Marker für die Einkreuzung des Fec^B Locus in verschiedene Schafrassen**

Eine Möglichkeit zur Steigerung der Wurfgröße in verschiedenen Schafrassen ist die Einkreuzung des Fec^B Gens. Um diese Einkreuzung nachhaltig durchführen zu können, ist die Anwendung von markergestützter Selektion unter Verwendung von Markern, die eng mit dem Fec^B Locus gekoppelt sind, von Vorteil. Tiere der fruchtbaren Rassen Tiroler Bergschaf (n=41), Romanov (n=43) und Ostfriesisches Milchschaf (n=35) sowie Tiere der Rasse Merinolandschaf (n=265) wurden mit den mit dem Fec^B gekoppelten Markern OarAE101 und BM1329 typisiert und die Allelfrequenzen geschätzt. In der Rasse Merinolandschaf betrugen die Allelfrequenzen für die zum Fec^B Locus gekoppelten Mikrosatellitenallele der Marker OarAE101 und BM1329 0,07 bzw. 0,1. Beide Allele wurden auch in den anderen Rassen nachgewiesen, jedoch in fast allen Fällen mit einer höheren Frequenz. Um zukünftig die Mikrosatelliten OarAE101 und BM1329 innerhalb eines indirekten Gentests nutzen zu können wurde die Segregation der Fec^B gekoppelten Mikrosatellitenallele dieser beiden Marker in der Rasse Merinolandschaf untersucht. Hierfür wurden Familien durch die Kreuzung von Merinolandschafen (Fec+/Fec+) mit heterozygoten Böcken (Fec+/Fec^B) der gleichen Rasse erstellt.

Die Rekombinationsrate zwischen den Markern OarAE101 und BM1329 betrug 13 cM (LOD score 14,6).

Schlüsselwörter: Schaf, Fec^B, indirekter Gentest

Introduction

Litter size in sheep has a low heritability coefficient and a large variability between and within breeds (ELSEN et al., 1994). Attempts to increase litter size by selection within breeds have been hindered by a slow selective gain. For identifying major genes

with effects on ovulation rate and litter size, a single autosomal segregation was discovered in the Booroola strain (DAVIS et al., 1982). The Booroola gene (Fec^B gene) is known for its large impact on prolificacy. One single copy of the gene resulted in +1.3 extra lambs in 2-year-old ewes (NIEUWHOF et al., 1998).
Introgression of the Fec^B mutation in other breeds requires extensive progeny testing of the rams or measuring the ovulation rate of the ewes by laparoscopy over three to five cycles. The use of genetic markers linked to the Fec^B locus would allow an early identification of Fec^B carriers and is independent from sex. Further on the use of marker assisted selection costs less time and money than the use of laparoscopy.
Linkage to Fec^B was first demonstrated with two microsatellite markers (OarAE101 and OarHH55) and epidermal growth factor (EGF) from human chromosome 4 (MONTGOMERY et al., 1993). Comparison of the human gene map with the gene maps of cattle and sheep, Fec^B could be mapped to sheep chromosome 6 (MONTGOMERY et al., 1994). BLATTMANN et al. (1996) assumed linkage between the microsatellite BM1329 and the Fec^B gene. That was confirmed by linkage mapping of this microsatellite between OarAE101 and EGF on sheep chromosome 6 (LORD et al.,1996). Actually, Fec^B is localized in a region of 10 cM between the two microsatellite markers BM1329 and OarAE101 (LORD et al., 1998).
The aim of this study was to determine the allele frequencies of microsatellites BM1329 and OarAE101 in four sheep breeds in order to test the suitability of these two microsatellites as markers for the introgression of the Fec^B locus into the Merinoland sheep. Furtheron the segregation of microsatellites alleles BM1329 and OarAE101 should be demonstrated in families of German Merinoland sheep in order to improve litter size and the recombination rate between BM1329 and OarAE101 was calculated.

Material and Methods

For the estimation of allele frequencies of microsatellites BM1329 and OarAE101 samples were collected from Merinoland sheep (n=265), Romanov (n=43), Tyrolean Mountain sheep (n=41) and East Friesian Milksheep (n=35). The Booroola Merino breeding program at the Research Station 'Oberer Hardthof' of the University in Giessen has been started in 1989. Heterozygous F_1 Booroola Merino rams were produced by inseminating eight Merinoland ewes with semen from a homozygous Fec^B carrier ram. The F_2 generation was obtained by mating three heterozygous Fec^B carrier rams with 107 Merinoland ewes. The ovulation rates of the F_2 daughters were recorded by repeated laparoscopy. In the last two years two further heterozygous Fec^B carrier rams were mated with 125 Merinoland ewes for segregation analysis. 35 heterozygous Fec^B carrying daughters of the produced F_1 generation were mated with non-Fec^B carriers. For the twopoint linkage analysis six halfsib families of crossbreds between Merinoland sheep breed and Texel (n = 390) were used.
DNA was isolated from white blood cells according to MONTGOMERY & SISE (1990). The PCR (15µl final volume) for amplifying the microsatellites BM1329 and OarAE101 was performed using 50 to 100 ng ovine genomic DNA and a Perkin Elmer Gene Amp PCR system 9600 cycler. The forward primer of each microsatellite was Cy5 fluorescense end- labelled (Amersham Pharmacia, Freiburg). The microsatellite

OarAE101 was amplified according to the conditions described by MONTGOMERY et al. (1993). The PCR for amplifying the microsatellite BM1329 was performed using 10 p*M* of each primer, 200 µ*M* of each dNTP, 0.4 units of *Taq* polymerase (Hybaid-AGS, Heidelberg), 1.5 m*M* $MgCl_2$, and PCR buffer (20 m*M* Tris-HCl, pH 8.55; 1.6 m*M* NH_4SO_4). Thermal cycling began with an initial cycle of 94°C (1.5 min), followed by 30 cycles of 94°C (1 min), 58°C (1 min), and 72°C (1 min), and concluded with a final extension at 72°C (5 min).

Microsatellite typing was done using an automated laser detection system (A.L.F. *express*, Amersham Pharmacia, Freiburg). The PCR products were run on a 5.5% Long Ranger gel (0,5 mm, 6M UREA). The fragment analysis were done using AlleleLinks v1.00 (Amersham Pharmacia, Freiburg). Twopoint linkage analysis between BM1329 and OarAE101 were done using CRI-MAP v.2.4 (GREEN et al., 1990).

Allele frequencies were estimated by counting the alleles of unrelated individuals of each breed.

Results

Between the four breeds great differences in the occurence and frequency of the different alleles of microsatellites BM1329 and OarAE101 were observed. Six alleles were identified with microsatellite BM1329, but only three of them were found in all breeds (Table 1).

Table 1
Allele frequencies of the BM1329 microsatellite in different breeds (Allelfrequenzen des Mikrosatelliten BM1329 in verschiedenen Schafrassen)

	Breed			
	Merinoland	Romanov	Tyrolean Mountain sheep	East Friesian Milksheep
n	265	40	41	35
alleles				
162	0.10	0.03	0.21	0.26
164	0.23	0.50	0.27	0.43
166	0.60	0.21	0.47	0.20
174	0.02	0.26	0	0.11
178	0.03	0	0.05	0
180	0.02	0	0	0

The Romanov and East Friesian Milksheep showed a high occurence of the allele 164, whereas in the breeds Merinoland sheep and Tyrolean Mountain sheep the allele 166 showed the highest frequency. Allele 180 occured in the Merinoland sheep breed only at a low frequency. With microsatellite marker OarAE101 10 alleles were obtained (Table 2). The two alleles 97 and 109 were present in all four breeds whereas the other 8 alleles showed breed specific alleles, for example allele 107 occurs only in Tyrolean Mountain Sheep. Allele 97 had a very high frequency in the Romanov breed and a very low one in the Merinoland sheep breed. Allele 101 was not present in Tyrolean Mountain Sheep and had very low frequency in Romanov whereas it was very frequent in the Merinoland sheep. Allele 109 had the highest frequency in Tyrolean Mountain Sheep and lower frequencies in the other breeds. The observed distribution

of microsatellites alleles within breeds follows Hardy-Weinberg expectation of equilibrium.

Table 2
Allele frequencies of the OarAE101 microsatellite in different breeds (Allelfrequenzen des Mikrosatelliten OarAE101 in verschiedenen Schafrassen)

	Breed			
	Merinoland	Romanov	Tyrolean Mountain sheep	East Friesian Milksheep
n	218	43	41	34
alleles				
97	0.07	0.72	0.35	0.10
99	0.02	0	0	0
101	0.65	0.01	0	0.35
107	0	0	0.04	0
109	0.16	0.20	0.42	0.21
111	0	0	0	0
113	0.06	0	0	0
115	0.04	0	0.07	0.16
117	0	0.07	0.10	0
119	0	0	0.02	0.18

In the microsatellite BM1329 allele 162 and in OarAE101 allele 97 was linked to the Fec^B allele which was shown in the Booroola/Merinoland families. The marker analyses were validated by the ovulation rate data of the F_2 daughters. Both microsatellites alleles linked to the Fec^B gene were rare in the Merinoland sheep population. The twopoint linkage analyses in six half sib families showed a genetic distance (sex averaged) of 13 cM with a LOD score of 14.6. The number of informative meioses were 233 for OarAE101 and 203 for BM1329 respectively.

Discussion

The aim of this study was to establish a genotyping system to identify Fec^B carriers in sheep using the microsatellite markers BM1329 and OarAE101. Both markers are mapped on ovine chromosome 6 flanking the Fec^B locus (http://www.ri.bbsrc.ac.uk).
In former investigations it was difficult to identify heterozygous Fec^B gene carriers clearly: for example VISSCHER et al. (1998) compared results from laparoscopy and genotyping and found a descrepancy of 4 %. In the studies of LORD et al. (1998) the control of laparoscopy results with three Fec^B flanking markers showed, that 6-13 % of the heterozygous Fec^B gene carriers were not identified by laparoscopy.
As the alleles 162 and 97 of the two markers BM 1329 and OarAE101 occur also in non Fec^B carriers, genotyping of the informative ancestors was also done in the present study. So the origin of the alleles linked to the Fec^B gene could be followed up to the Fec^B/Fec^+-father or -grandfather of the ewes. Therefore only 1.9 % of the animals could not be genotyped clearly concerning their Fec^B status.
The genetic distance (sex averaged) between BM1329 and OarAE101 calculated in this study was 13 cM. LORD et al. (1998) described the region between BM1329 and OarAE101 with a genetic distance of 10 cM in a male map.
The two microsatellites BM1329 and OarAE101 represent an efficient and robust genotyping system, but the alleles of both markers linked to the Fec^B allele occur also

in other sheep breeds (Romanov, Tyrolean Mountain Sheep, East Friesian Milksheep) than the booroola merinos but with different frequencies. Because the concerning alleles are rare in the Merinoland sheep population, this system can be used for identifying the introgression of the Fec^B gene when the parents and if need the grandparents are genotyped too.

Until a direct gene test is available at the Fec^B gene locus the two microsatellites BM1329 and OarAE101 can be used for an indirect genetest to identify Fec^B gene carriers.

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Authors' addresses
Dr. CHRISTINA WEIMANN, Dr. BIRGIT LEYHE-HORN, Dr. Dr. MATTHIAS GAULY,
Prof. Dr. GEORG ERHARDT
Institut für Tierzucht und Haustiergenetik
Ludwigstr. 21b
D-35390 Gießen
Germany

E-Mail: Christina.Weimann@agrar.uni-giessen.de