Animal Science Papers and Reports vol. 23 (2005) no. 3, 207-213
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

SHORT REPORT

Single strand conformation polymorphism in exon 2 of the *Ovar-DRB1* gene in two Polish breeds of sheep*

Joanna Gruszczyńska[1], Karolina Brokowska, Krystyna M. Charon, Wiesław P. Świderek

Department of Animal Genetics and Breeding,
Warsaw Agricultural University, Ciszewskiego 8, 02-786 Warsaw, Poland

(Received July 27, 2005; accepted September 19, 2005)

Authors' earlier study on Polish Heath Sheep (PHS) and Polish Lowland Sheep variety Żelazna (PLS) showed 67 and 57 animals, respectively, to be genetically identical as concerns RFLP in exon 2 of the *Ovar-DRB1* (MHC class II) gene. The aim of the present study was to search for the conformation polymorphism in exon 2 of *Ovar-DRB1* of the same animals with, however, the single strand conformation polymorphism (SSCP) analysis.
A total of 33 SSCP patterns were identified, 27 in PHS and 13 in PLS. Among 16 RFLP haplotypes identified earlier with PCR-RFLP, as many as 15 appeared to have two different variants of SSCP bands, whereas the RFLP haplotype 11 12 68 (digestion with *Bst*YI, *Bsu*RI, *Rsa*I, respectively) was present even in three different SSCP variants. It is concluded that PCR-RFLP method when used alone does not supply the complete information on gene polymorphism, while PCR-SSCP approach leads to identify more patterns. Therefore, the use of PCR-SSCP simultaneously with PCR-RFLP is recommended.

KEY WORDS: gene polymorphism / *Ovar-DRB1* / PCR-RFLP / PCR-SSCP

---

*Supported by the State Commitee for Scientific Research (KBN) within the project P06 R0 1414.
[1]e-mail: gruszczyn_j@hotmail.com

Major histocompatibility complex (MHC) plays the key role in initiating immune response in all mammals. MHC molecules are glycoproteins found on cell surface and presenting different antigens against T lymphocytes. MHC class I molecules are found on all nucleated cells and, in much smaller proportion, on erythrocytes. These molecules present antigens, e.g. viral antigens to $CD8^+$ T lymphocytes [Ballingall *et al.* 1992]. MHC class II molecules are found on the surface of antigen-presenting cells (APC), mainly on B lymphocytes, macrophages and dendritic cells, and are responsible for presenting antigens to $CD4^+$ T lymphocytes. These molecules include bacterial and parasitic antigens, but also animal's own proteins, including MHC molecule fragments [Klein *et al.* 1991]. Moreover, there are also MHC class III molecules that are part of the complement complex. These proteins are present in serum and are regarded as components of non-specific pathway of immune response, aiding antibodies functions.

Sheep MHC gene complex is located in chromosome 20, and MHC proteins are known as ovine leukocyte antigens (Ovar). *Ovar class* I is represented by two *loci*: *Ovar-A* and *Ovar-B*, whereas class II by 12 *loci*: *Ovar-DRA, -DRB, -DQA, -DQB, -DMA, -DMB, -DYA, -DYB, -DIB, -DNA, -DOB,* and *-DPB* [Deverson *et al*. 1991, Klein *et al*. 1991, Scott *et al.* 1991, Ballingall *et al*. 1992 and 1995]. Exon 2 of *locus DRB* is highly polymorphic, and encodes the outer domain of MHC molecule (β chains) which is the binding area for an antigen presented [Andersson 1996, Escayg *et al*. 1996]. The high polymorphism in exon 2 sequence gives possibilities for immune response towards the wide variety of pathogens.

In our earlier study [Gruszczyńska *et al*. 2005] an attempt was made at identification of the conformation polymorphism in exon 2 of *Ovar-DRB1* gene in Polish Heath Sheep (PHS) and Polish Lowland Sheep (PLS) – Żelazna variety, with the PCR-RFLP method. In 67 PHS and 57 PLS ewes no polymorphism was found and the animals were considered as genetically identical. The present report shows the results of a search for conformation polymorphism in exon 2 of the *Ovar-DRB1* gene of the same animals, with PCR-SSCP method.

Material and methods

Animals

The study was carried out on 67 ewes of Polish Heath Sheep (PHS) and 57 ewes of Polish Lowland Sheep – Żelazna variety (PLS) kept on the Żelazna Farm of the Warsaw Agricultural University, and described in the authors' earlier paper [Gruszczyńska *et al*. 2005]. The mean relationship coefficient was 0.01745 and 0.01549 in PHS and PLS, respectively.

Polymerase chain reaction (PCR)

The analysis of exon 2 of *Ovar-DRB1* gene polymorphism was performed with the PCR-SSCP method. Genomic DNA was isolated from the whole blood using the

phenol-chlorophorm extraction. In PCR the primers used were those described by Ammer *et al*. [1992].

A single PCR sample volume was 50 µl and contained 5 µl 10 × buffer (FINNZYMES), 200 µM dNTPs (TerPol), 40 pMol of each primer (TIB MOLBIOL), 50 ng of genomic ovine DNA and 2 U of Taq-DNA-polymerase (FINNZYMES). The resulting mixture was made up to 50 µl with sterile, deionised water. PCR reaction was run under the conditions described by Gruszczyńska *et al*. [2005]. The amplified fragment of 279 bp consisted of 9 bp of intron 1 and the entire exon 2, 270 bp in length.

Single strand conformation polymorphism (SSCP)

SSCP analysis was applied to 67 PHS and 57 PLS ewes, *i.e.* those which according to RFLP method were earlier found genetically identical in exon 2 of *Ovar-DRB1* [Gruszczyńska *et al*. 2005]. Amplification products of exon 2 were, after denaturation, separated in 12% non-denaturating polyacryloamide gel with vertical electrophoresis set (DCode TM Universal Mutation Detection System, BioRad) in 1×TBE buffer at 300V, 40W and temperature 10°C for 2.5 h. When the separation had completed, gel was stained with silver. The length of restriction fragments was determined with ScanPack 3.0 programme (BIOMETRA).

Results and discussion

SSCP detects single-base sequence changes by abnormal electrophoretic migration of one or both single strands on a nondenaturing polyacryloamide gel. DNA strands fold differently if they differ by a single base, and it is believed that mutation-induced changes of tertiary structure of the DNA result in a different mobility. Mutations are identified as new bands on autoradiograms (radioactive detection), of silver-stained bands, or of fluorescent PCR products detected by an automated DNA sequencer (non-radioactive detection). In the present study we used the PCR-SSCP method and silver staining to identify polymorphism in exon of 2 *Ovar-DRB1* gene in sheep of two breeds – PHS and PLS.

Table 1 presents haplotypes for exon 2 of *Ovar-DRB1* gene identified in both breeds with PCR-RFLP [Gruszczyńska *et al*. 2005] and SSCP (this study). The former report [Gruszczyńska *et al*. 2005] dealt with the patterns of PCR products that were digested with *Bsu*RI, *Rsa*I and *Bst*YI restriction enzymes. In PHS and PLS eight restriction patterns (nos. 1-8) were found when the *Rsa*I enzyme was used, while six (nos. 1-6) and two (nos. 1-2) when digestion was performed with *Bsu*RI and *Bst*YI, respectively. Sixty-five RFLP haplotypes were found in PHS and 68 in PLS. The combination of restriction patterns was found in the following order: *Bst*YI, *Bsu*RI, *Rsa*I [Gruszczyńska *et al*. 2005].

In the present study a total of 33 SSCP patterns were identified, 27 in PHS and 13 in PLS. Their frequencies varied from 0.02 to 0.03 in PHS and from 0.02 to 0.07 in PLS (Tab. 1).

Table 1. Haplotypes in the exon 2 of *Ovar-DRB1* gene as identified with PCR-RFLP (Gruszczyńska et al. 2005) and PCR-SSCP methods (this study) in two Polish breeds of sheep

PCR-RFLP haplotype[1]	PCR-SSCP genotype	Haplotype frequency[1,2]	
		Polish Mountain Sheep (n = 67)	Polish Lowland Sheep Żelazowa variety (n = 57)
11 11 12	a	0.02	0.03
	a'	0.03	-
11 11 88	b	0.03	0.02
	b'	0.02	-
11 12 13	c	0.02	0.07
	c'	0.02	-
11 12 68	d	0.02	-
	d'	0.02	-
	d''	0.02	-
11 22 13	e	0.02	-
	e'	0.03	-
11 26 12	f	-	0.02
	f'	0.03	-
11 26 13	g	0.02	-
	g'	0.02	-
12 11 11	h	0.03	-
	h'	0.02	-
12 11 15	i	0.02	-
	i'	0.02	-
12 12 11	j	0.02	-
	j'	0.02	-
12 12 12	k	-	0.02
	k'	0.04	0.07
12 12 13	l	0.02	0.07
	l'	-	0.02
12 12 15	m	0.02	-
	m'	0.02	0.02
12 22 12	n	-	0.03
	n'	0.02	-
12 26 12	o	0.04	0.05
	o'	-	0.02
22 12 11	p	0.03	-
	p'	-	0.03

[1] Restriction enzymes in order: *Bst*YI, *Bsu*RI, *Rsa*I (Gruszczyńska et al. 2005)
[2] This study
n – number of animals

Out of 16 haplotypes identified with PCR-RFLP as many as 15 identified with PCR-SSCP showed two different variants of bands, and the 11 12 68 RFLP haplotype (*Bst*YI, *Bsu*RI, *Rsa*I enzymes, respectively) was present even as three different SSCP variants. Moreover, the 11 11 88 RFLP haplotype, originally considered as homozygous [Gruszczyńska *et al*. 2005], in this study presented two variants. The examples of the SSCP polymorphism in the *Ovar-DRB1* gene exon 2 are shown on Photo 1.

Outteridge *et al*. [1996] when applying tl PCR-SSCP method, found exon 2 gene in Meri sheep to be polymorphic. They also demonstrat that SSCP patterns are inherited as a simple qua tative trait.

Gelhaus *et al*. [1995] in their studies on exon of *BoLA-DRB3* with the use of restriction enzym *Bst*YI, *Hae*III and *Rsa*I concluded that PCR-RFI method did not provide the complete information gene polymorphism. They showed that 12 out of restriction patterns corresponded to more than o allele sequence. Moreover, only 22 of 52 sequenc *DRB3* patterns could be identified with RLFP.

Kostia *et al*. 1998] in their study on identific tion of exon 2 gene alleles in 31 Finnsheep a Russian Romanoff and Karelian sheep, showed th SSCP makes it possible to differentiate betwe sequences in that exon. They amplified the exon with PCR, and the products were eventually an lysed with SSCP and sequenced. Nineteen allel were identified, including seven previously n published sequences of exon 2 *Ovar-DRB1* gen One of the newly discovered alleles – *DRB1*01* – appeared breed-specific for Romanoff sheep. Tl same authors observed that all animals with gr wool were highly uniform as far as MHC genes a concerned. And indeed, all grey sheep (n=6) we found to possess only *DRB1*0201* allele.

Exon 2 of the *Ovar-DRB1* gene was also studi by Jugo *et al*. [1996] in 72 Latxa sheep. Using PCI SSCP method the authors were able to identify variants of bands, suggesting wide variation with the *locus*. They claim the method to be simple a relatively sensitive [Jugo *et al.* 1996, Jugo a Vicario 2000]. Provided that each allele has tl established band pattern, typing of individuals quite fast.

Due to employing PCR-SSCP method in anal sis of exon 2 of the *Ovar-DRB1* gene polymorphis we were able to find its intra-gene differentiatio that could not be identified earlier with PCR–RFI method. These results suggest that when studyi

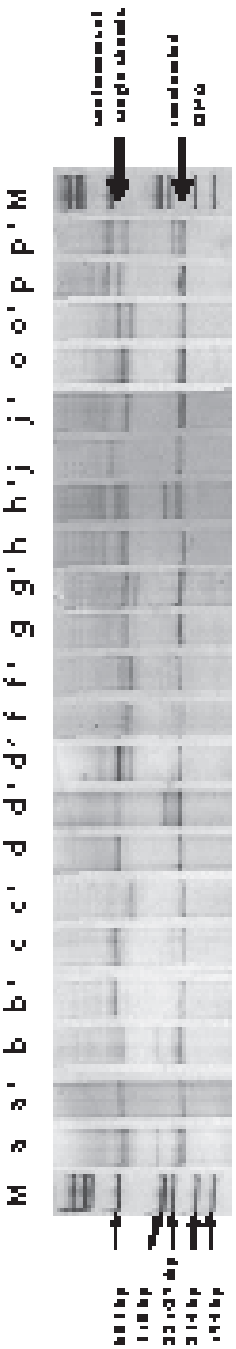

Photo 1. Electrophoretic separation of single conformers of the studied fragment in 12% polyacryloamide gel. M – Marker ΦX174 HaeIII, a and a' etc. – SSCP patern.

polymorphism of exon 2 *Ovar-DRB1* gene, it is advisable to employ both methods, *i.e*. PCR-RFLP and PCR-SSCP, as the results obtained with the latter lead to increase in the number of identified patterns.

Acknowledgement*. The authors are grateful to Ms. Dorota Witkowska from the Department of Animal Genetics and Breeding, Warsaw Agricultural University, for her skilful laboratory assistance.*

REFERENCES

1. AMMER H., SCHWAIGER F-W., KAMMERBAUER C., GOMOLKA M., ARRIENS A., LAZARY S., EPPLEN J.T., 1992 – Exonic polymorphism vs intronic simple repeat hypervariability in *MHC-DRB* genes. ***Immunogenetics*** 35, 332-340.
2. ANDERSSON L., 1996 – The Major Histocompatibility Complex Region of Domestic Animal Species (L.B. Schook and S.J. Lamont Eds.). CRC Press 1-15.
3. BALLINGALL K.T., DUTIA B.M., HOPKINS J., WRIGHT H, 1995 – Analysis of the fine specificities of sheep major histocompatibility complex class II - specific monoclonal antibodies using mouse L-cell transfectants. ***Animal Genetics*** 26, 79-84.
4. BALLINGALL K.T., WRIGHT H., REDMOND J., DUTIA B.M., HOPKINS J., LANG J., DEVERSON E.V., HOWARD, J.C., PURI N., HAIG D., 1992 – Expression and characterization of ovine major histocompatibility complex class II (*Ovar-DR*) genes. ***Animal Genetics*** 23, 347-359
5. DEVERSON E.V., WRIGHT H., WATSON S., BALLINGALL K., HUSKINSSON N., DIAMOND A.G., HOWARD J.C, 1991 – Class II MHC genes of the sheep. ***Animal Genetics*** 22, 211-225.
6. ESCAYG A.P., HICKFORD J.G.H., MONTGOMERY G.W., DODDS K.G., BULLOCK D.W., 1996 – Polymorphism at the ovine major histocompatibility complex class II loci. ***Animal Genetics*** 27, 305-312.
7. GELHAUS A., SCHNITTGER L., MEHLITZ D., HORSTMANN R.D., MEYER C.G., 1995 – Sequence and PCR-RFLP analysis of 14 novel BoLA-DRB3 alleles. ***Animal Genetics*** 26, 147-153.
8. GRUSZCZYŃSKA J., BROKOWSKA K, CHARON K.M., ŚWIDEREK W.P., 2005 – Restriction fragment length polymorphism of exon 2 *Ovar-DRB1* gene in Polish Heath Sheep and Polish Lowland Sheep, ***Journal of Applied Genetics*** 46, 311-314.
9. JUGO B., MARTINEZ N., ESTOMBA A., VICARIO A., 1996 – Single-strand conformation polymorphism analysis of the second exon of MHC class II *DRB* gene in sheep. ***Animal Genetics*** Suppl. 2, 27, 53-54.
10. JUGO B., VICARIO A., 2000 – Single-strand conformation polymorphism and sequence polymorphism of *Mhc-DRB* in Latxa and Karrantzar sheep: implications for Caprinae phylogeny**.** ***Immunogenetics*** 51, 887-897.
11. KLEIN J., BONTROP R. E., DAWKINS R. L., ERLICH H. A., GYLLENSTEN U. B., HEISE E. R., JONES P. P., PARNAM P., WAKELAND E. K., WATKINS D. I., 1991 – Nomenclature for the major histocompatibility comlexes of different species: a proposal. ***Immunogenetics*** 31, 217-219.
12. KOSTIA S., KANTANEN J., KOLKKALA M., VARVIO S-L., 1998 - Applicability of SSCP analysis for MHC genotyping: fingerprinting of *Ovar-DRB1* exon 2 alleles from Finnish and Russian breeds. ***Animal Genetics*** 29, 453-455.
13. OUTTERIDGE P.M., ANDERSSON L., DOYCH P.G.C., GREEN R.S., GWAKISA, P.S., HOHENHAUS M. A., MIKKO S., 1996 – The PCR typing of *MHC-DRB* genes in the sheep using primers for intronic microsatellite: Application to nematode parasite resistance. ***Immunology and Cell Biology*** 74, 330-336.

14. SCOTT P.C., GOGOLON-EWENS K. J., ADAMS T. E., BRANDON M. R, 1991 – Nucleotide sequence, polymorphism and evolution of ovine MHC class II *DQA* genes. ***Immunogenetics*** 34, 69-79.

Joanna Gruszczyńska, Karolina Brokowska,
Krystyna M. Charon, Wiesław P. Świderek

Polimorfizm konformacyjny PCR-SSCP w eksonie 2 genu *Ovar-DRB1* owiec dwóch ras polskich

S t r e s z c z e n i e

Celem pracy było poszukiwanie metodą PCR-SSCP polimorfizmu w eksonie 2 genu *Ovar-DRB1*, należącego do klasy II głównego układu zgodności tkankowej (MHC), i którego nie udało się stwierdzić we wcześniejszych badaniach przeprowadzonych metodą PCR-RFLP. Analizami objęto 67 maciorek rasy wrzosówka (PHS) i 57 maciorek polskiej owcy nizinnej odmiany żelaźnieńskiej (PLS), u których poprzednio nie wykryto polimorfizmu restrykcyjnego w eksonie 2 metodą PCR-RFLP. Stwierdzono 33 wzory SSCP, z czego 27 u owiec PHS i 13 u PLS. Spośród 16 haplotypów stwierdzonych poprzednio metodą RFLP, metoda SCCP ujawniła 15 wykazujących po dwa konformery. Haplotyp RFLP 11 12 68 (kolejność kombinacji wzorów restrykcyjnych odpowiednio: *Bst*YI, *Bsu*RI, *Rsa*I) wykazał nawet trzy konformery.

Uzyskane wyniki wskazują, że metoda PCR-SSCP umożliwia identyfikację polimorfizmu bardziej rozległego niż stwierdzany metodą PCR-RFLP i sugerują łączne stosowanie obu metod do identyfikacji polimorfizmu badanych sekwencji.