Università di Pisa

Polymorphism detection in bovine Stearoyl-CoA Desaturase (SCD) *locus* by means of microarray analysis

G. Conte[1], B. Castiglioni[2] , M. Mele[1] , A. Serra[1] , S. Chessa[3], G. Pagnacco[3] and P. Secchiari[1]

1DAGA, Sezione Scienze Zootecniche, Università di Pisa, via del Borghetto 80, 56124 Pisa, Italy
2IBBA-CNR, via Bassini 15, 20133 Milano, Italy
3VSA, Università di Milano, via Trentacoste 2, 20134 Milano, Italy

INTRODUCTION

SCD is a key enzyme in metabolism of some lipids [Ntambi et al. 2004; Miyazaki et al. 2003]. It catalysed the cisΔ-9 desaturation in long chain fatty acids. Variability in the bovine SCD genotype is one of the major sources of genetic variation in milk fatty acid composition. Two types of this gene have been observed, in which three SNPs in the ORF (Open Reading Frame) region were predicted to cause an amino acid replacement from valine (V) to alanine (A) (Only the third SNP causes the aminoacid substitution) (Fig. 1) [Medrano et al. 2003].

The goal of this work was to check an efficient tool to study the SCD polymorphism. This protocol will be used to check a relationship between SCD polymorphism and fatty acids composition, particularly for conjugated linoleic acids (CLA) present in milk.

Haplotype V:

```
gtactacaaacctggtgtcctgttgttgtgcttcatcctgcccacactcgtgccatggtatctgtgggatga
aacgtttcaaaacagcctgtttttttgccaccttattccgttatgcccttgggctcaacgtcacctggctggt
gaatagtgctgcccatatgtatggataccgcccttatgacaagaccatcaacccccgagagaatattctggt
ttccctgggagctgcgg
```

Haplotype A:

```
gtactacaaacctggtgtcctgttgttgtgcttcatcctgcccacactcgtgccgtggtatctgtgggatga
aacgtttcaaaacagcctgtttttttgccaccttattccgttacgcccttgggctcaacgtcacctggctggt
gaatagtgctgcccatatgtatggataccgcccttatgacaagaccatcaacccccgagagaatattctggt
ttccctgggagctgtgg
```

Fig. 1 SCD polymorphism in bovine genotype

RESULTS

We found 5 samples homozygous for the allele V (red signal) and 6 heterozygous (AV) (yellow signal), but no samples were detected homozygous for the allele A (green signal) (Fig. 4). Microarray analysis and SSCP technique gave the same results (Fig 5), which were confirmed by direct sequencing. This work shows that such microarray assay is an efficient method to the SNPs identification at the SCD locus and can therefore be included in an array considering many other mutations point.

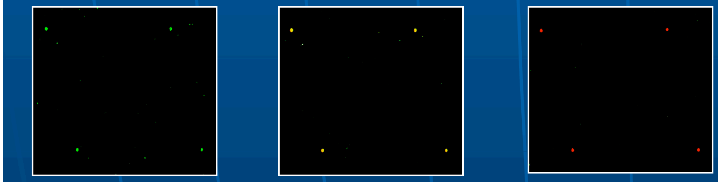

Fig. 4: microarray results

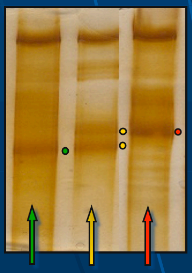

Fig. 5: SSCP results. Homozygous sample (VV) is marked with red arrow, heterozygous (AV) with yellow arrow. The second homozygous (AA marked with green arrow) was checked in the Holstein breed (we are going to organize a work about the relationships between SCD polymorphism and milk fatty acids composition in the Holstein).

This work was supported by PRIN 2003 (MIUR). Coordinator: PIERLORENZO SECCHIARI

MATERIALS AND METHODS

We describe an application of the microarray technology for the polymorphism identification at the SCD gene. This method, based on the discriminative properties of DNA ligation detection reaction (LDR), combines enzymatic processing with "tag sequences" hybridisation, in Universal array (Fig. 2). Genomic DNA was extracted from 11 Somba (African breed) cows blood samples and the 5th exon of the SCD gene was amplified by PCR using locus-specific primers (Fig. 3). The resulting PCR products were subjected to LDR and then hybridised onto Universal Array [Consolandi et al. 2004]. In the second step of this work microarry results were compared with those obtained using another method such as SSCP (Single Strend Conformation Polymorphism analysis). This tool is based on two steps: first the DNA sequence of interest is PCR-amplified, and second , the amplified DNA is heat-denatured and size-fractionated by native polyacrilamide gel electrophoresis (PAGE). After heat denaturation the mobility of single-stranded DNA fragments is size- and sequence-dependent with single-strand DNA molecules adopting secondary structure conformation (Fig. 5).

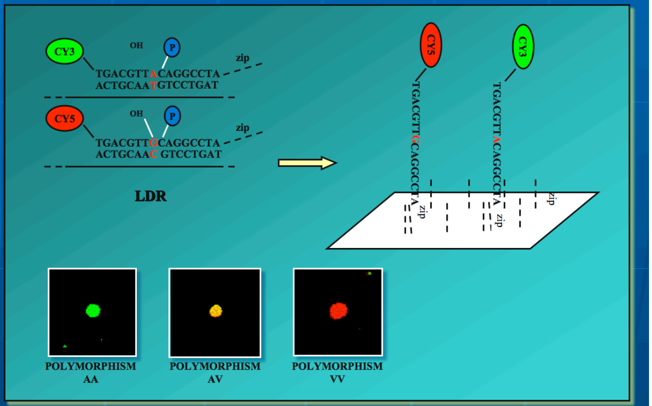

Fig. 2 LDR detection and enzymatic processing combines with Universal array

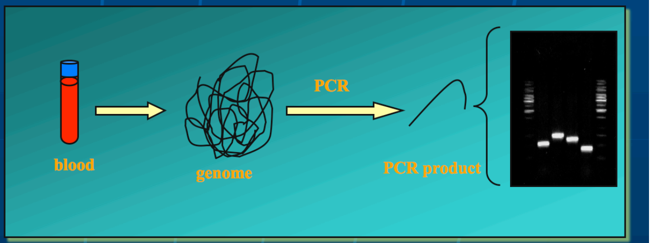

Fig. 3 DNA extraction and amplification of SCD 5th exon. The PCR products was four obtained using four specific primer

Bibliography

- Consolandi C, Frosini A, Pera C, Ferrara GB, Bordoni R, Castiglioni B, Rizzi E, Mezzelani A, Bernardi LR, De Bellis G, Battaglia C: POLYMORPHISM ANALYSIS WITHIN THE HLA-A LOCUS BY UNIVERSAL OLIGONUCLEOTIDE ARRAY. *Human Mutation* 2004 24 pp. 428-434
- Medrano JF, Islas-Trejo AD, Johnson AM : data bank NCBI access code AY241932 (2003)
- Miyazaki M, Ntambi JM : ROLE OF STEAROYL-COENZIME A DESATURASE IN LIPID METABOLISM. *Prostaglandins, Leukotrienes and Essential Fatty Acids.* 2003 68 pp. 113-121
- Ntambi JM, Miyazaki M : REGULATION OF STEAROYL-CoA DESATURASES AND ROLE IN METABOLISM. *Progress in Lipid Research* 2004 43 pp. 91-104