

---

## Molecular basis and diagnostication of SCID in Arabian Horses

**S. E. GEORGESCU, E. CONDAC, ANCA DINISCHIOTU, MARIETA COSTACHE\***

*\*University of Bucharest, Department of Biochemistry and Molecular Biology, Molecular Biology Center, 91-95 Spl. Independentei, 050095 Bucharest, Romania*

### Abstract

*McGUIRE and POPPIE [3], Australia first reported SCID (severe combined immunodeficiency) in Arabian foals in 1973. SHIN et al. [6] discovered the molecular basis of SCID in horses in 1997. They showed that SCID in horses is due to a frameshift mutation in the gene for DNA-dependent protein kinase catalytic subunit (DNA-PK), resulting in the lack of full-length kinase, and absence of kinase activity. Our objective was to develop an easy and efficient method that can be used to correctly identify the normal and the carrier horses for SCID trait and to sequencing a fragment from the gene that contain the deletion. We analyzed one population of Arabian horses from Romania using a set of primers who amplify a fragment from the SCID gene containing the 5-basepair deletion. Conditions for PCR were selected so that the two primers could amplify the DNA from normal, carriers and SCID affected horses. The number of allele peaks depends on whether the horse tested is a heterozygote (carrier) or homozygote (normal or SCID affected). Results suggest that the genetic test will be useful in identifying Arabian horses heterozygous for the SCID trait and foals with SCID.*

Keywords: Arabian, SCID, carrier, sequencing, diagnostication.

### Introduction

McGUIRE and POPPIE [3], Australia first reported SCID in Arabian foals in 1973. In 1980, PERRYMAN and TORBECK [5] showed that SCID in Arabian horses was inherited as an autosomal recessive condition, which means that one copy of the disease gene is inherited from a carrier stallion and another from a carrier mare. The foal, which inherits two copies of the disease gene, is affected with a lethal inability to fight infections, and dies within the first few months of life. POPPIE *et al.* [4], STUDDERT *et al.* [9] and BERNOCO *et al.* [2] placed the SCID carrier frequency at about 20 - 28%.

After many years of frustration for many researchers, SHIN *et al.* discovered the molecular basis of SCID in horses [6]. They showed that SCID in horses is due to a frameshift mutation in the gene for DNA-dependent protein kinase catalytic subunit (DNA-PK), resulting in the lack of full-length kinase, and absence of kinase activity. BAILEY *et al.* [1] showed that the gene for DNA-PK is part of Kentucky synteny group 3. By FISH analysis, they also showed that this group physically maps to horse chromosome ECA9p12.

The specific defect in SCID lies in a DNA-dependent protein kinase (DNA-PK), which is an essential enzyme in gene segment rearrangement during the synthesis of B- and T-cell receptors (BCR and TCR). During this process, large segments of DNA are excised so that V (variable), D (diversity), and J (joining) gene segments can be joined together. The DNA-PK, which rejoins the cut ends, consists of 3 linked peptides called KU80, KU70, and p350. In SCID foals, the p350 component is totally absent. As a result, neither T nor B cells can form

functional V regions in their TCR or BCR, so they are unable to respond to any antigen [7; 10].

## Material and methods

### *DNA extration*

In our research we analyzed 60 Arabian horses. Blood samples were obtained from the Mangalia stud. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

### *PCR Amplification and Sequencing*

We used one set of unlabelled primers who amplify a fragment from the SCID gene containing the 5-basepairs deletion. Efficiency and specificity of PCR were optimized by varying the annealing temperature (50–60°C) on a gradient thermocycler IQCycler (BioRad).

The amplified fragments were purified with the Wizard PCR Preps DNA Purification System Kit (Promega). The purified fragments were sequenced by ABI Prism 310 Genetic Analyzer, using the ABI Prism® BigDye Terminator Cycle Sequencing Ready Reaction Kit. The sequences were processed with DNA Sequencing Analysis 5.1 Software (AppliedBiosystems). The nucleotide sequences were aligned with the Clustal X multiple alignment program and refined manually.

### *PCR Amplification and SCID diagnostication*

For SCID diagnostication we used one set of primers who amplify a fragment from the SCID gene containing the 5-basepairs deletion. The forward primer was labelled with 6-FAM dye. PCR was performed in a GeneAmp 9700 PCR System (Applied Biosystems). The reactions were carried out in 25 µl final volume containing PCR Buffer (final concentration: TRIS-HCl 10 mM and KCl 50 mM), MgCl<sub>2</sub> (final concentration 1.5 mM), 200 µM of each dNTP, 0.5 µM of each primer, 0.5 units of AmpliTaq Gold DNA Polymerase, diluted DNA (50 ng per reaction) and nuclease-free water. PCR amplifications were performed in 0.2 mL tubes using 30 cycles with denaturation at 95°C (30s), annealing at 53°C (30s) and extension at 72°C (60s). The first denaturation step was performed at 95°C (10min) and the last extension was to 15 min at 72°C.

PCR products were loaded with the GeneScan-500 ROX Internal Size Standard (Applied Biosystems) into one of the ABI Prism 310 DNA Sequencer (Applied Biosystems). As the samples migrate past the fluorescence detector, the GeneScan 3.1.2. Software (Applied Biosystems) collects the signal and assigns a base pair size for each one. GeneScan data can then be exported directly to Genotyper 2.5.2. Software (AppliedBiosystems) for automated genotyping.

## Results and discussion

The genetic defect responsible for this disease was identified as a 5-basepair deletion in the gene encoding DNA-protein kinase catalytic subunit. Horses with one copy of the gene appear normal, while horses with two copies of the gene manifest the disease. Our objective was to develop an easy and efficient method that can be used to correctly identify the normal and the carriers horses for SCID trait and to sequencing a fragment from the gene that contain the deletion.

In Figure 1 the profile of the region from the PCR product that may contain the deletion (the sequence of deletion is TCTCA) is shown. In Figure 2 Clustal X fragment alignment of a region from SCID gene [6] and our PCR product is shown.

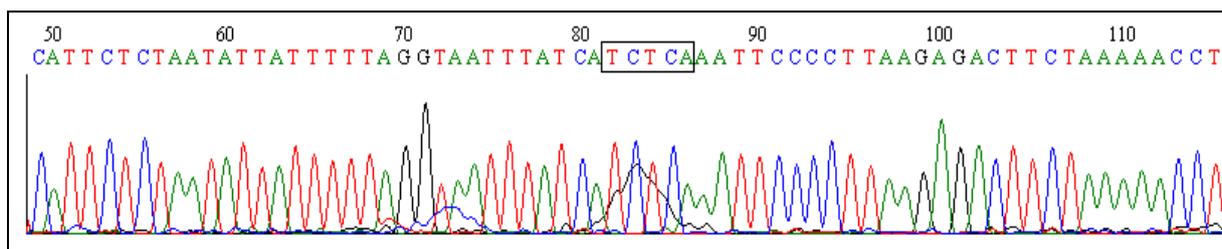


Figure 1. The sequence of the region from the PCR product that may contain the 5-basepair deletion.

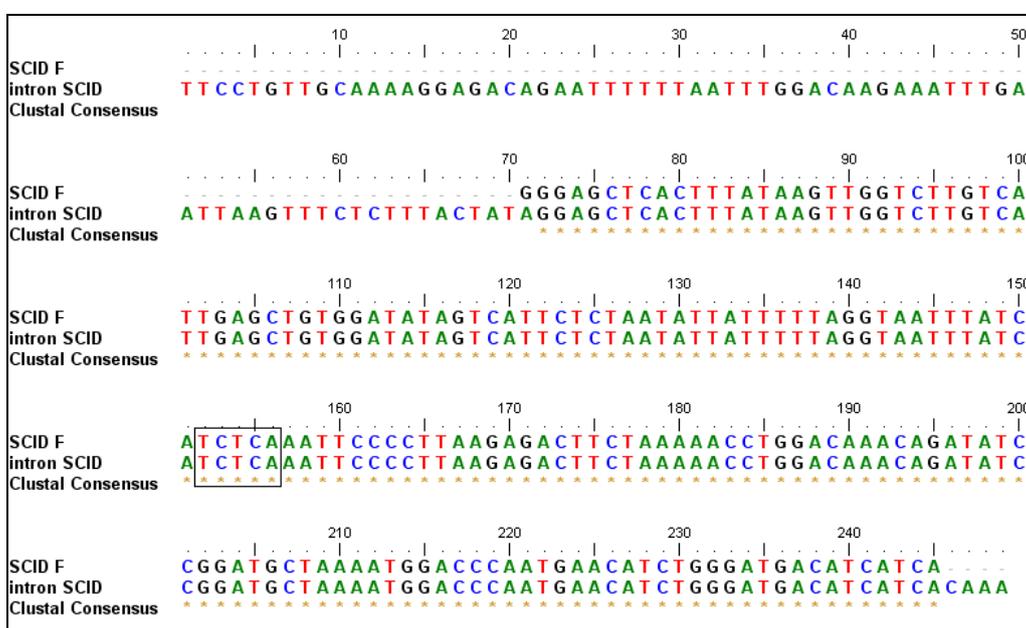


Figure 2. Clustal X fragment alignment of a fragment from SCID gene and our PCR product.

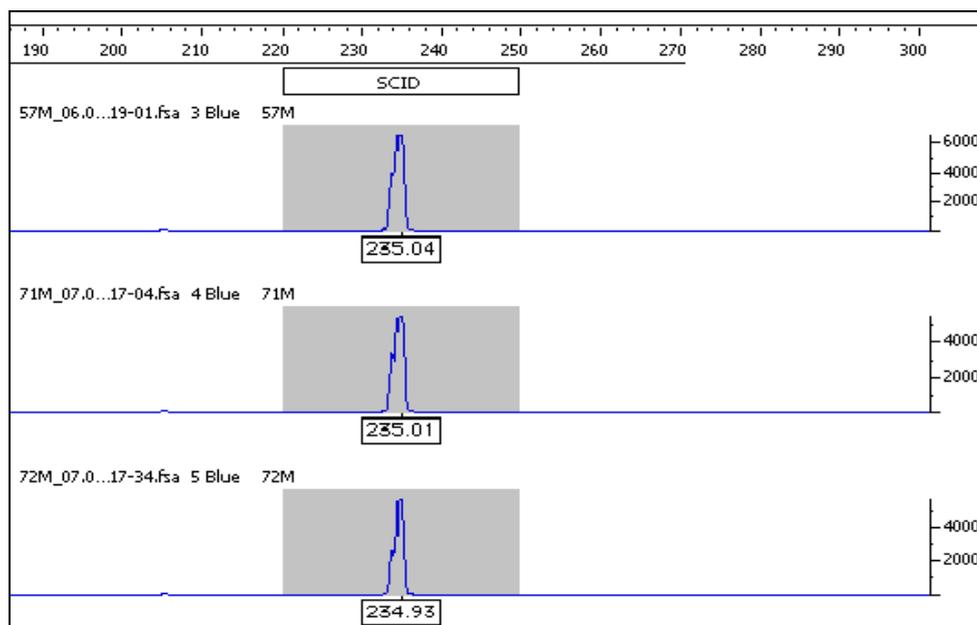


Figure 3. Genotyper Software analysis of PCR amplification product for three normal homozygous horses.

The primers were designed to amplify only a fragment from the gene for DNA-dependent protein kinase catalytic subunit containing or not the 5-basepair deletion. The amplified fragment for a normal horse had 235 bp. Conditions for PCR were selected so that the two primers could amplify the DNA from normal carriers and SCID affected horses.

In our experiment successful amplification yields one or two allele peaks with an expected size of 230 and (or) 235 bp. The number of allele peaks depends on whether the individual tested is a heterozygote (carrier) or homozygote (normal or SCID affected). When testing a normal horse we must obtain just one peak at 235 bp. If we analyze a homozygous affected horse we also obtain just one peak, but at 230 bp. In the case of heterozygous carrier we must obtain two peaks at 230 and 235 bp because one allele is normal and the other one contain a 5-basepairs deletion. In Figure 3 the profile for a homozygous normal horse is shown.

In our case all the analyzed horses were normal. Fortunately we did not find any carrier or SCID affected horse in the population studied.

## Conclusions

Results suggest that the genetic test will be useful in identifying Arabian horses heterozygous for the SCID trait and foals with SCID, provided that all Arabian horses with SCID have the same genetic mutation.

Consequently, it will be of interest to use in investigating the possible occurrence of the affected gene among other Arabian horse populations from other countries and among other breeds derived from Arabian horses.

## References

1. BAILEY, E., REID, R.C., SKOW, L.C., MATHIASON, K., LEAR, T.L., MCGUIRE, T.C. (1997) Linkage of the gene for equine combined immunodeficiency disease to microsatellite markers HTG8 and HTG4 - synteny and FISH mapping to ECA9. *Animal Genetics* **28**: 268-273.
2. BERNOCO, D., BAILEY, E. (1998) Frequency of the SCID gene among Arabian horses in the USA. *Animal Genetics* **29**: 41-42.
3. MCGUIRE, T.C., POPPIE, M.J. (1973) Hypogammaglobulinemia and thymic hypoplasia in horses: a primary combined immunodeficiency disorder. *Infectious Immunology* **8**: 272-277.
4. POPPIE, M.J., MCGUIRE, T.C. (1977) Combined immunodeficiency in foals of Arabian breeding: evaluation of mode of inheritance and estimation of prevalence of affected foals and carrier mares and stallions. *J. of the Am. Veterinary Medical Association* **170**: 31-33.
5. PERRYMAN, L.E., TORBECK, R.L. (1980) Combined immunodeficiency of Arabian horses: Confirmation of autosomal recessive mode of inheritance. *Journal of the American Veterinary Medical Association* **176**: 1250-1251.
6. SHIN, E.K., PERRYMAN, L.E., MEEK, K. (1997) A kinase-negative mutation of DNA-PKcs in equine SCID results in defective coding and signal joint formation. *Journal of Immunology* **158**: 3565-3569.
7. SHIN, E.K., PERRYMAN, L.E., MEEK, K. (1997) Evaluation of a test for identification of Arabian horses heterozygous for the severe combined immunodeficiency trait. *Journal of the American Veterinary Medical Association* **211**: 1268 ff.
8. SHIN, E.K., RIJKERS, T., PASTINK, A., MEEK, K. (2000) Analyses of TCRB rearrangements substantiate a profound deficit in recombination signal sequence joining in SCID foals: Implications for the role of DNA-dependent protein kinase in V(D)J recombination. *Journal of Immunology* **164**: 1416-1424.
9. STUDDERT, M.J. (1978) Primary, severe, combined immunodeficiency disease of Arabian foals. *Australian Veterinary Journal* **54**: 411-417.

10. WILER, R., LEBER, R., MOORE, B.B., VANDYK, L.F., PERRYMAN, L.E., MEEK, K. (1995) Equine severe combined immunodeficiency – a defect in V(D)J recombination and DNA-dependent protein kinase activity. *Proceedings of the National Academy of Sciences of the United States of America* **92**: 11485-11489.