FREQUENCY SAMPLING OF THE SCID ALLELE IN THE BELGIAN POPULATION OF ARABIAN HORSES

Frequentiebepaling van het SCID-allel in de Belgische populatie van Arabische volbloeden

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ABSTRACT

Severe combined immunodeficiency (SCID) of horses is an inherited autosomal recessive defect carried by pure Arabian horses and their crossbreds. SCID foals have a deficiency in the number and function of mature lymphocytes and are very prone to infectious diseases. The defect is caused by a mutation in the *PRKDC* gene coding for the catalytic subunit of the DNA activated protein kinase (DNA-PKcs). Identification of the causative mutation made it possible to design a genotyping test for identifying carriers of the defective allele and to use this information in selective breeding against SCID. Here, the first frequency sampling of the SCID allele in the Belgian population of Arabian horses is presented. Four hundred and thirteen animals were genotyped, 25 or 6.05% of which were found to carry the SCID allele. Calculations based on these results showed the SCID allele frequency to be around 3.1% and the occurrence of SCID to be 0.096%.

SAMENVATTING

Severe combined immunodeficiency (SCID) bij paarden is een autosomaal recessief overervende ziekte voorkomend bij Arabische volbloeden en bij de nakomelingen van hun kruising. Veulens met SCID vertonen een duidelijk tekort aan functionele, mature lymfocyten en zijn bijgevolg heel gevoelig voor infecties. De deficiëntie wordt veroorzaakt door een mutatie in het *PRKDC*-gen coderend voor de catalytische subeenheid van het DNA geactiveerde proteïnekinase (DNA-PKcs). De identificatie van de oorzakelijke mutatie maakte het mogelijk om een genotyperingstest te ontwikkelen waarmee dragers kunnen opgespoord worden en waarvan de informatie kan gebruikt worden bij de selectie om SCID tegen te gaan. Hier worden de resultaten beschreven van een eerste frequentiebepaling van het SCID-allel bij de Belgische populatie van Arabische volbloeden. Vierhonderddertien dieren werden gegenotypeerd via een zelfontwikkelde DNA-test. Vijfentwintig dieren of 6,05% van de getypeerde paarden bleken drager te zijn van het SCID-allel. Gebaseerd op deze resultaten werd de SCID-allelfrequentie berekend op 3,1% en het voorkomen van SCID op 0,096%.

INTRODUCTION

Severe combined immunodeficiency (SCID) was first described in horses in 1973 (McGuire and Poppie, 1973). A similar disorder has also been described in man, mice and dogs. SCID in horses is limited to the Arabian horse and its derived breeds and is inherited as an autosomal recessive disorder caused by a 5 base pair (bp) deletion in the *PRKDC* gene coding for the catalytic subunit of the DNA dependent protein kinase (DNA-PKcs) (Shin *et al*., 1997). Affected horses are incapable of generating antigen-specific immune responses due to impaired V(D)J recombination during maturation of T- and B lymphocytes. This defective gene rearrangement leads to the elimination of lymphocyte precursors and near absence of functional T- and B lymphocytes. Affected foals produce no antibodies after infection or immunization and frequently die before the age of 5 months due to infections. First signs of disease often appear between two days and eight weeks of age (Perryman *et al*., 1978; Bjorneby *et al*., 1991). The immunologic defects in SCID foals can be corrected through bone marrow transplantation (Bue *et al.,* 1986).

Horses with one SCID allele (heterozygotes or carriers) appear normal but will transmit it, statistically, to half of their offspring. Hence, the defective SCID allele can spread (unseen) in the population unless counter measures are taken. The best way to prevent this spread is to identify carriers by means of a DNA test (genotyping) and to prevent the breeding of these animals. A second reason for identifying carriers is their higher risk of developing neoplasia. The DNA-PK enzyme is expressed in all cell types and is believed to play an important role in DNA repair (Anderson and Carter, 1996). Arabian horses that develop sarcoids, the most frequent tumor in domestic horses, show a statistically higher frequency of the SCID allele (18% carriers in animals with tumors compared to 8% carriers in normal animals)(Ding *et al*., 2002). Interestingly, the frequency of sarcoids is also higher in Arabians compared to other horse breeds (Mohammed *et al*., 1992). These findings suggest that DNA-PK can act as a tumor suppressor through its DNA repair function.

Here we report the genotyping results of a sample of the Arabian horse population in Belgium.

MATERIAL AND METHODS

Animals and sample collection

Blood samples were taken on EDTA or heparin to prevent coagulation. Samples from a total of 413 Arabian horses from all over Belgium were collected. Of these, 369 were stallions and 44 were mares. Preferentially stallions were sampled since they produce more offspring than mares and thus have a higher chance of spreading the mutant allele in the population

DNA purification

100 µl of the blood samples was washed three times with 10 mM Tris-HCl (pH 7.5)/1 mM EDTA (pH 8). Lysis of the white blood cells was performed at 56 °C for 45 minutes using 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5% Tween 30 and proteinase K (100 µg/ml). The proteinase K, which selectively digests proteins, was subsequently inactivated by incubation at 96 °C for 15 minutes. The genomic DNA thus obtained was used in the following PCR (polymerase chain reaction) amplifications.

DNA amplification

The PCR amplifications were performed in a total volume of 10 µl using a T3 Thermocycler (Biometra, Göttingen, Germany). The reaction mixes contained 20 – 200 ng genomic DNA (2.4 µl), 1.5 mM MgCl2, 200 nM dNTPs, 70 ng of each primer, 1x PCR buffer and 0.5 U BioTaq polymerase (Gentaur, Brussels).

The primers, which were designed on the basis of the equine PRKDC (DNA-PKcs) sequence published by Shin et al. (1997), surround the position of the 5 base pair deletion.

The primers used are:

EcabPRKDC+2 *CATTGAGCTGTGGATATAGTCATTC
EcabPRKDC-2 TCATTGGGTCCATTTTAGCA

With * indicating the position of the anchored fluorochrome Cy5.

This primer pair amplifies a 126 bp fragment of the normal (wild type) *PRKDC* allele and a 121 bp fragment of the *PRKDC* SCID allele.

The following thermal cycling profile was followed after 5' of initial heating at 94 °C: 30" at 94 °C, 30" at 60 °C and 1' at 72 °C. This cycle was run 35 times and afterwards followed by 10' finishing at 72 °C.

A positive (known carrier) and negative control (including no genomic DNA) were taken along with each run.

Allele scoring (genotyping)

PCR products were loaded on a 4% polyacrylamide gel and separated using an ALFexpress (Pharmacia, Uppsala, Sweden) sequencer. An example of a gel reading is given in Figure 1.

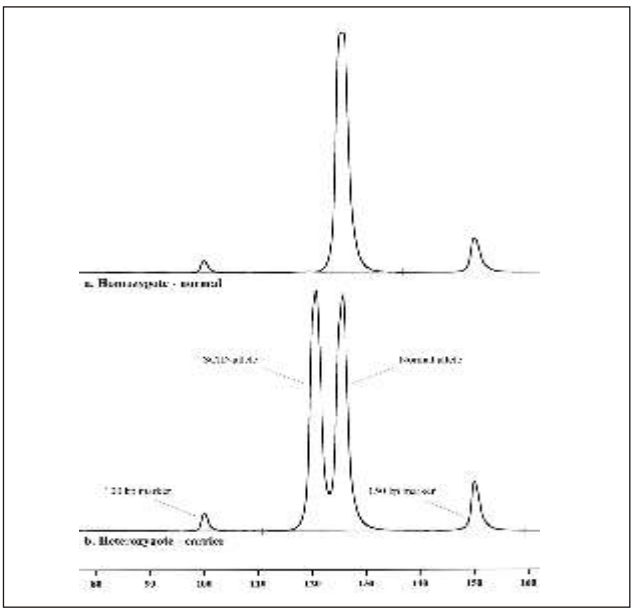

Figure 1. Example of a genotyping result of a homozygote normal horse and a carrier (heterozygote). Fragment lengths of the alleles (normal = 121 base pairs; SCID allele is 126 base pairs (bp)) are calculated by calibration to an external DNA ladder (ruler at the bottom of the figure) and two internal size markers (100 and 150 bp).

RESULTS

Of the 413 horses tested, 388 were homozygous for the normal allele and 25 or 6.05% were identified as carriers. Since SCID horses die before the age of 5 months and all animals tested were adults, no homozygotes for the SCID allele were detected, as expected. On the basis of these facts, the prevalence of the SCID allele in the Belgian population of Arabian horses can be estimated at around 3.1% and the occurrence of SCID at 0.096% (calculated assuming Hardy-Weinberg equilibrium).

DISCUSSION

Severe combined immunodeficiency was first described by McGuire and Poppie (1973) in two foals in the state of Washington (USA) and later also in other countries. The same authors estimated the frequency of carriers in the USA at 25.7% and the frequency of affected horses at 2-3% (Poppie and McGuire, 1977), considerably higher than what was found here. Studdert (1978) estimated the frequency in the Australian population of Arab horses at close to 30%. Before the identification of the gene mutation causing SCID and the subsequent development of a genotyping test estimation of the frequency of SCID in Arabian horses was complicated by difficulties in diagnosis. SCID foals were recognized by low blood lymphocyte counts and low immunoglobulin levels, but since the major physical characteristic of SCID is hypoplasia of the lymphoid tissues, a definite diagnosis could only be made postmortem (Felsberg *et al*., 1992). Incorrect diagnosis was probably not the only reason for the high incidence reported by these researchers. It is possible that the samples analyzed by them were not taken ad random in the population but had a bias introduced by selection carried out by breeders who had experienced problems in their breeding stock. A third possible reason for the big difference is that selection against the SCID allele has reduced its frequency dramatically, but this seems unlikely given the relatively short time period involved.

The frequency estimates described here are more in line with those presented in three other studies using a genotyping test to detect carriers. Bernoco and Bailey (1998) found a carrier frequency of 8.4% in the Arabian horses in the USA and calculated the SCID allele frequency at 4.2%. In a second independent study in the USA the carrier frequency was found to be 8.9% (Ding *et al*., 2002). A study performed on UK Arabian horses found a carrier frequency of 2.8% (Swinburne *et al*., 1999). The results of the four genotyping studies are summarized in Table 1.

Unlike in dogs, where two types of SCID inheritance have been described, one autosomal recessive (Jack Russell Terriers and Welsh Corgi) (Bell *et al*., 2002) and one X chromosome linked (Basset Hound) (Somberg *et al*., 1995), only one type has been described in horses. All SCID foals genetically tested so far are homozygous for the 5 bp deletion in the *PRKDC* (DNA-PKcs) gene. So the same gene mutation seems responsible for all SCID cases in Arabian horses and breeds genetically influenced by or derived from the Arabian breed. This finding points to a common origin of SCID in the Arabian breed. Preliminary analysis of the pedigrees of carriers indicates that the mutation was present in a stallion from the early 20th century.

Table 1. Summary of 4 SCID genotyping studies in Arabian horses.

Population	Total N tested	N homozygous normal	N carriers (and %)	Reference
USA	250	229	21 (8.4)	Bernoco & Bailey, 1998
USA	258	235	23 (8.9)	Ding *et al*., 2002
UK	106	103	3 (2.8)	Swinburne *et al*., 1999
Belgium	413	388	25 (6.05)	This study

N = number of animals.

This particular stallion sired several studs that were used world wide and hence could have spread the SCID allele (Bernoco and Bailey, 1998).

Practically, this means that SCID carriers can be identified by a single genotyping test, as described here. The results of the genotyping test can be used in breeding schemes to reduce the frequency of the SCID allele and the incidence of SCID cases. Since this DNA test can be performed at any age, foals can be tested early on. The most radical way of reducing the SCID frequency would be to avoid breeding with carriers. However, this would mean that the genes of carriers with a high breeding value would be lost. For this reason one can argue for allowing breeding with these valuable animals as long as they are only crossed with homozygous normal animals. Nevertheless, one should keep in mind that the mutant SCID allele will thus remain in the population because half of its offspring will have inherited it and will also be carriers, in turn transmitting the SCID allele to half of their foals. For this reason, systematic genotyping of these offspring for the SCID allele will also be necessary in order to avoid carrier x carrier crosses in the following generation, because 25% of such carrier x carrier offspring would have SCID.

Another argument against using carriers in breeding schemes is the putative association of the SCID allele with neoplasia, as explained in the introduction. The frequency of SCID carriers in animals with sarcoids was 18.6% (19 animals of 102 tested), which is significantly higher than observed in non-tumor bearing animals (8.9%) in the same study (Ding *et al*., 2002). However, more studies should be performed in order to get more conclusive results concerning the association between the SCID allele and tumor development.

In conclusion, breeding with carriers can be justified in some cases but should be avoided whenever possible.

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