



Equine Severe Combined Immunodeficiency

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Sammanfattning

Severe Combined Immunodeficiency (SCID) är en immunbristsjukdom med genetisk bakgrund som förekommer hos Arabiska fullblod och korsningar med dessa. Sjukdomen har varit känd och dokumenterad sedan 1970-talet och tidigt föreslagen som en autosomal recessiv sjukdom.

Föl som är drabbade av sjukdomen producerar varken B eller T lymfocyter och dör efter en kort tid i följsjukdomar som uppstått p.g.a. bristande immunförsvar. Den vanligaste dödsorsaken är lunginflammation orsakat av adenovirus. Det är svårt att hålla föl som är drabbade av SCID vid liv. Man har dock lyckats förlänga deras liv genom att ge injektioner av plasma innehållande antikroppar. Även en benmärgstransplantation har utförts där fölet överlevde.

Under 1990-talet utvecklades ett gentest för kontroll av bärare av sjukdomen vilket har gjort det möjligt att bestämma om en häst är bärare av genen utan att använda sig av testparningar. De uppskattningar av frekvensen heterozygoter som utfördes innan testet utvecklades var baserade på kliniska undersökningar av de föl som misstänktes vara drabbade av sjukdomen. Dessa uppskattningar visade sig senare ha överskattat frekvensen anlagsbärare.

I Sverige är frekvensen bärare av SCID låg och avel på dessa är förbjuden. Förbudet följs dock inte upp i praktiken då hästar endast testas för SCID då de misstänks vara bärare. Om heterozygoter bör få ingå i avel borde avgöras av populationens storlek och av frekvensen anlagsbärare. Den totala populationen Arabiska fullblod i Sverige är så stor att bärare bör uteslutas från avel.

Abstract

Severe Combined Immunodeficiency (SCID) is a disease with a genetic background in Arabian horses and crossbreeds with Arabians. The disease has been known and documented since the 1970's and was early suggested to have an autosomal recessive mode of inheritance.

Foals that are affected with SCID lack production of both B and T lymphocytes and consequently die from secondary diseases within a short period of time. The most common cause of death is infections in the respiratory tract caused by adenovirus. Maintaining foals affected with SCID has been proven difficult, but their lives can be prolonged with injections of hyperimmune plasma. A successful bone marrow transplant has also been performed.

A DNA-test for detection of carriers of the defective allele was developed during the 1990's. The test made it possible to detect whether a horse was a carrier or a non-carrier of SCID, without the use of test mating. Previous estimations of the frequency of the defective allele were based on clinical diagnosis on foals suspected to be affected with SCID. These studies were later found to have overestimated the frequency of carriers.

The frequency of SCID carriers in Sweden is low and carriers are not allowed in breeding. The present legislation is however not acted upon since horses are only tested for SCID if suspected to be carriers. The question whether heterozygotes should be allowed in breeding or not should be decided while considering the population size and the frequency of carriers. The total population of Arabian horses in Sweden is large enough to exclude carriers from breeding.

Introduction

Severe Combined Immunodeficiency (SCID) is a genetic disease that has been observed in humans, dogs, mice and horses (McGuire & Poppie, 1973; McGuire et al., 1975; Bosma et al., 1983; Felsburg et al., 1999). Horse breeds affected with SCID are Arabian horses and Arabian crossbreeds (Swinburne et al., 1999). Foals that are born with the genetic defect are unable to produce functioning B and T lymphocytes and consequently die within a few months from infections due to their insufficient immune defence. SCID is a recessive disease which means that in order to express it, the individual has to inherit the gene from both parents. Therefore, mating carriers with non-carriers will never produce an affected foal.

A reliable DNA-test to determine whether an individual is a carrier of the defective gene or not was developed in 1997 (Shin et al., 1997a). This test makes it possible to exclude carriers from breeding without using test mating to determine if they are carriers or not.

The frequency of the defective gene differs from population to population, and different countries might not even have the same view on breeding with carriers of recessive alleles. This literature study aims to summarise what is known about the disease at present time, the gene frequency in Sweden and in the rest of the world, and to conclude whether carriers should be accepted in breeding or not.

Background

SCID was first termed combined immunodeficiency (CID), the term severe was added later to standardise the nomenclature (Studdert, 1978). SCID was first described in 1973 by McGuire and Poppie who studied two Arabian foals and tissues from other deceased foals. Both foals had pneumonia and did not respond to antibiotic therapy. Blood samples showed remarkably low concentration of lymphocytes. When lymphoid tissues of the foals were studied, lack of lymphocytes was found as well. The authors came to the conclusion that the foals had a defective B-lymphocyte system leading to a lack of immunoglobulin synthesis. Evidence that the disease was of genetic origin was found, such as the presence of SCID in full siblings and several reported cases in Arabian foals whereas no reported cases in any other breeds were found.

Foals affected with SCID lack functioning B and T lymphocytes (McGuire and Poppie, 1973). Lymphocytes are white blood cells that carry out the adaptive immune responses and are found in large numbers in the blood, lymph and lymphoid organs such as the spleen and thymus (Alberts et al, 2002). B and T lymphocytes constitute the two main classes. B lymphocytes produce antibodies, which are proteins called immunoglobulins. T lymphocytes kill cells that are infected with viruses and regulate the activities of other white blood cells.

SCID has probably been around for a long period of time, only never considered to be a hereditary disease. Poppie and McGuire confirmed the mode of inheritance through test mating in 1977. They showed that a simple recessive mutation was the cause of the disease.

Studies of pedigrees have indicated that a stallion that was active in breeding in the early parts of the twentieth century in the USA, might be the source of the mutation (Bernoco and Bailey, 1998; Swinburne et al., 1999).

Pathological picture

Symptom and clinical diagnosis

Foals affected with SCID are susceptible to microbial infections due to their lack of a functioning immune system and usually die before 5 months of age (Poppie and McGuire, 1977; Perryman and McGuire, 1978; Swinburne et al., 1999). Symptoms are fever, pneumonia, coughs, nasal discharges, diarrhoea and infections (McGuire and Poppie, 1973; Swinburne et al., 1999). In the first 1-3 months of life, foals are protected by immunoglobulins received from their dams via colostrum, but these are eventually eliminated by the catabolism, causing a lack of resistance against infections (Perryman, 2000). There are some conditions that could be mistaken for SCID, such as failure in the passive transfer of immunoglobulins (FPT) from dam to foal via colostrum (Poppie and McGuire, 1976). Foals with FPT have immunoglobulin M (IgM) in their serum from birth whereas foals affected with SCID have no IgM within 2 to 3 weeks after birth.

Perryman and McGuire (1978) found in a study of 66 foals affected with SCID that infections in the respiratory tract were the predominant disorder. The infections were mainly caused by adenovirus which was the main cause of death, but several kinds of bacteria and the protozoan *Pneumocystis carinii* caused infections as well. Significant bacterial lesions were found in liver, pancreas, intestines, heart and kidneys.

Before the development of a DNA-test for SCID, foals were diagnosed clinically. Perryman and Torbeck (1980) established the criteria for determining if a foal had in fact died from SCID or not. The criteria foals had to meet to be diagnosed with SCID were (1) to have lymphopenia, (2) a lack of immunoglobulin M, (3) to have lymphocytes that respond little or not at all to stimulation of the antigen phytolectin in vitro and (4) to have hypoplastic alterations of spleen, thymus and lymph nodes.

Treatment

The main obstacle in maintaining foals with SCID alive for experimental reasons is to be able to control the respiratory tract infections (Perryman and McGuire, 1978). Perryman and McGuire (1978) placed affected foals in semi-isolation with their dams in their study of 66 foals affected with SCID. The foals were given hyperimmune plasma intravenously twice a week and antibiotics when bacterial infections developed. The four foals that were treated with plasma injections were maintained for 3-11 months without any signs of adenovirus-infection. The plasma also contained antibodies against other microbial agents. Seven foals that were given insufficient amounts of plasma or none at all died between 2 and 4 months of age due to severe adenovirus infections.

Bue et al. (1986) reported of a 32-day-old horse with SCID that had a successful bone marrow transplant from a sex-matched full sibling donor. The recipient foal reached normal B and T lymphocyte values 170 days after the transplant and when immunised with bacteriophage, the foal showed an antibody response which had not been observed in untreated SCID foals. The foal survived to five years of age when it died from causes unrelated to SCID and the transplant (Perryman, 2000).

Genetic background

Mode of inheritance

Poppie and McGuire suggested as early as 1973 that SCID was inherited as an autosomal recessive trait (Figure 1). This was supported with the fact that the dam and sire of the foals in the study had no history of immunodeficiency and that both a female and a male foal that were affected were found.

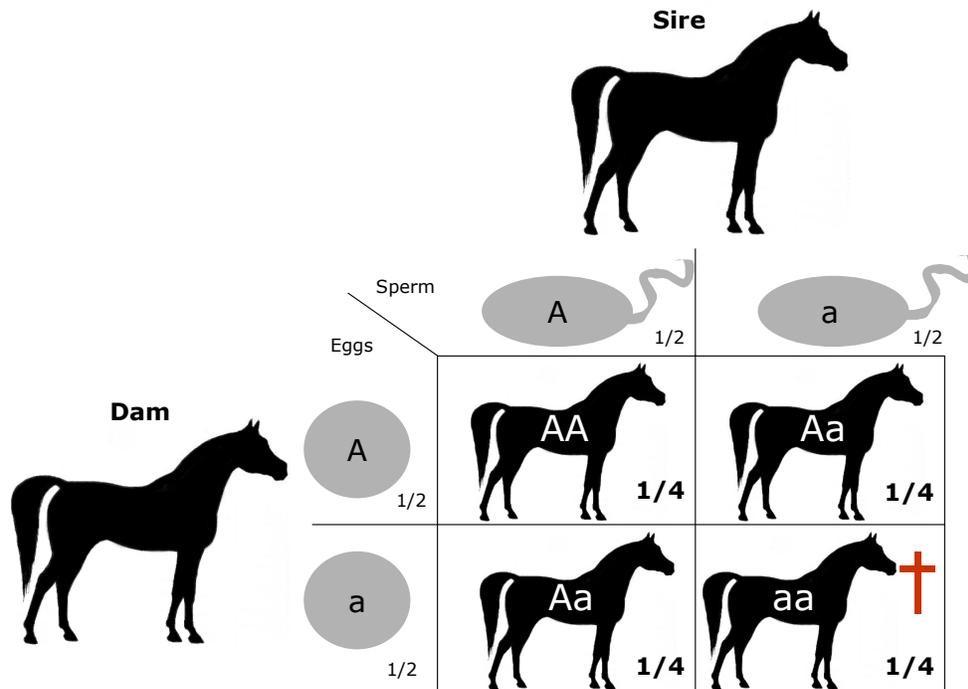


Figure 1. Inheritance of an autosomal recessive trait. The diagram shows the outcome of mating between two horses that are heterozygous for the SCID trait. 50% of the offspring will be heterozygotes i.e. carriers, 25% will be homozygous for the dominant allele and 25% will be homozygous recessive i.e. affected with SCID.

Poppie and McGuire (1977) later determined the mode of inheritance through test mating of 17 mares to 1 of 10 stallions for a total of 22 matings. All mares and stallions had previously produced offspring affected with SCID. The matings resulted in 22 foals out of which 7 had SCID, which is slightly higher than the expected outcome of 25% (5.5/22) but still supports an autosomal recessive mode of inheritance. The difference in expected to actual outcome of affected foals could be explained by the small numbers of horses studied.

The mode of inheritance was confirmed in a larger study performed by Perryman and Torbeck (1980) who test mated 26 mares that had previously produced offspring affected with SCID to a stallion that also had offspring affected with SCID. The study was performed over a time period of three years and gave a total of 50 living foals, two aborted fetuses and one stillborn foal. The stillborn foal and the two aborted fetuses were determined to be nonaffected as they had IgM and well-developed lymphoid tissues. Of the 50 other foals, 15 were affected with SCID which gave a percentage of 28.3% (15/53) affected. With a larger group of tested individuals it is reasonable to assume that this number would come closer to 25%. The distribution was 25:28 between male and female foals and between foals affected with SCID 7:8. The even distribution between males and females supports an autosomal mode of inheritance and also supports previous results by Poppie and McGuire (1977), where 4 males

and 3 females where affected. Precise criteria, described by Perryman and Torbeck (1980), were used to classify the foals as affected or nonaffected. They could exclude that the disease would be inherited by a sex-linked recessive pattern as there would then have been more affected males than females. A dominant pattern of inheritance could also be excluded since both sire and dam were unaffected.

Molecular mechanism

The genetic defect responsible for SCID is a five base pair deletion (Figure 2) in the gene encoding for the catalytic subunit (DNA-PKcs) of DNA-protein kinase (DNA-PK) (Shin et al., 1997a). The base pair deletion causes a frame-shift mutation at codon 3155 of the transcript, resulting in deletion of the 967 following amino acids which causes a non-functional enzyme. The DNA-PK enzyme is usually involved in the mechanisms of V(D)J recombination during early lymphoid differentiation where it is required for the repair of DNA double-strand breaks (Wiler et al., 1995; Shin et al., 1997a; Alberts et al., 2002). The V(D)J recombination reaction generates diverse T-cell receptors and immunoglobulin molecules that are necessary for recognition of foreign antigens. Because the lack of DNA-PK leads to an inactivation of V(D)J recombination, SCID affected foals have a block in the B and T lymphocyte development (Shin et al., 1997a; Shin et al., 1997b).

Normal GeneAATTTATCATCTCAAATTCCCCTTAA.....
SCID GeneAATTTATCA AATTCCCCTTAA.....

Figure 2. Partial sequence of the DNA-PKcs gene showing the 5 base pair deletion in the mutated SCID gene (Shin et al., 1997a). The mutation results in a frame-shift which causes a premature stop.

Bailey et al. (1997) demonstrated with fluorescence in situ hybridisation (FISH) that the mutated gene responsible for SCID is located on equine chromosome ECA9p12. Shin et al. (1997b) found in their study of 17 carriers, 4 affected foals and 1 healthy foal (whose dam and sire were carriers), that all horses had the same DNA-PKcs mutation, which supports previous results by Shin et al. (1997a). Since all tested horses had the same mutation it is likely that the DNA-PKcs allele has a common origin that results from a genetic “founder” effect.

DNA-test for identification of carriers

The discovery of a five base pair deletion responsible for SCID led to the development of a Polymerase Chain Reaction (PCR) based test for detection of SCID carriers (Shin et al., 1997a). The normal and SCID equine DNA-PKcs transcripts were cloned and sequenced with PCR based technology. Two oligonucleotide hybridisation probes, scanning the region of deletion, were synthesised representing the normal (N probe) and SCID (S probe) sequences. The S probe, specific for the mutated allele, hybridises in the foals affected with SCID whereas the N probe, specific for the normal allele, does not. In horses that do not carry SCID, only the N probe hybridises. In animals that are heterozygotes, both the N and S probes hybridise.

The test is an important asset in the development of breeding programs and makes it possible to avoid ever letting a SCID foal be born (Perryman, 2000). It also makes it possible to determine if a foal should be treated or not since there is little point in treating a foal affected with SCID except for study purposes. With the test it is possible to determine that a horse is free of the defective allele whereas with progeny testing, one can never be 100% sure that the

horse is actually free of the mutation as the allele is recessive. In Sweden, SCID tests are carried out by 'Husdjursgenetiska laboratoriet' SLU, Uppsala. The cost of the test was in April 2007, 650 SEK per horse (SLU's homepage, 2007).

The whole horse genome was sequenced by February 2007 which presents a wide range of new opportunities to study the origin of genetic defects in horses (National Institutes of Health, 2007). All nucleotides in the equine DNA-PKcs allele were however already sequenced, but the entire sequencing of the horse genome could provide material for future studies.

Frequency

Before the development of a genetic test for carriers of the defective allele, estimations of the gene frequency was based on clinical identification of foals affected with SCID (Swinburne et al., 1999). This method of estimation is not as accurate as a gene test and gave an overestimation of the prevalence of the defect. The overestimation could be explained by a non random selection of horses. It is likely that populations where SCID was suspected to be present were selected and that breeders who had experienced a problem with the defect were interested in having their horses being a part of the research. Another explanation is that foals were wrongly suspected to have died due to SCID when they in fact did not (Bernoco & Bailey, 1998; Swinburne et al., 1999). Poppie and McGuire (1977) expressed that the high frequency of SCID suggested that the defect had been selected for unknowingly, when selecting for another desirable trait. The frequency of carriers i.e. heterozygotes were calculated using the law of Hardy-Weinberg equilibrium; $A/A: p^2$, $A/a: 2pq$, $a/a: q^2$, where p is the frequency of allele A , q is the frequency of allele a and $p + q = 1$ (Griffiths et al., 2005). A/a are heterozygous horses, while foals affected with SCID are homozygous for the defective allele and hence a/a . Hardy-Weinberg equilibrium is the stable frequency distribution of the different genotypes and is achieved when mating is random and no mutation, migration, natural selection or random drift occurs.

Poppie and McGuire (1977) estimated the frequency of carriers in the USA to be 25.7% in 1977, based on 2-3% affected foals. The method used to determine if a foal was affected was clinical identification, which was performed on 257 foals from 19 different states. In the late 1990's, Bernoco and Bailey (1998) estimated the frequency of carriers in the USA by using the new DNA test (Shin et al. 1997a) on 250 randomly selected Arabian horses. The frequency of carriers was estimated to be 8.4% which was far less than Poppie and McGuire's (1977) result. The likely occurrence of affected foals would then be 0.18%, which is 11 to 16-fold less compared to Poppie and McGuire's result.

Another study to determine the number of carriers by DNA-testing was carried out in the UK in 1999 by Swinburne et al. They randomly selected and tested 106 blood samples which were taken previously during 1997 and 1998. They found that 2.8% of the tested horses were carriers. The frequency of carriers could be estimated to be between 1-5% in the UK at 95% confidence level using that sample size.

Poland has a total population at about 1500 Arabian horses and has been an important exporter of Arabian horses to several countries (Terry et al., 1999). In a test performed in 1999, 271 Polish Arabian horses were selected and tested for the mutated allele. These horses were selected to represent the major blood lines of Arabian horses in Poland. None of the 271

horses were carriers of the mutated allele which indicates that the allele is rare if present in the Polish population.

The Swedish population

Arabian horses in Sweden are only tested for SCID when evaluated for breeding if suspected to be heterozygous (the Swedish Horse Board's homepage, 2007). Stallions and mares that are found to be carriers are not allowed in breeding.

In a small study performed in Sweden during 2000 – 2001, 122 Arabian horses were tested for the SCID gene (Sandberg K., personal communication). The horses selected for the study were not closely related. Only two horses were found to be carriers which give a carrier frequency of 1.64%. This material is however not sufficient to draw definite conclusions from.

The effective population size (N_e) can be calculated using the equation: $N_e \approx (4N_m \times N_f)/(N_m + N_f)$, where N_m and N_f are the number of males and females used in breeding (Falconer, 1996). This is however not true if there is variation in family size, and a more correct definition of N_e is calculated as $N_e = 1/2\Delta F$, where ΔF is the rate of inbreeding. As the rate of inbreeding in the Swedish Arabian horse population was not known, a rough estimation of N_e was calculated with use of the former equation. Using available data from 2005 when 70 stallions and 278 mares were used in breeding, the effective population size was estimated to 224 individuals (Swedish Horse Board, 2005). The total Arabian horse population in Sweden has been estimated to approximately 7,000 horses (World Arabian Horse Organisation's homepage, 2007).

Compared with horses of other breeds in Sweden, the Swedish Arabian horse population is average in size (Swedish Horse Board, 2005). The effective population size seem to be about the same size as for Swedish Ardennes horses and New Forest ponies ($N_e = 237$ and 192 , respectively). The Swedish Arabian horse population is small in comparison to the Swedish Warmblood who has an effective population of 833 individuals.

Laws and recommendations

As for breeding, Perryman (2000) points out that two heterozygotes never should be mated to each other. If it is of interest to preserve a certain blood line through a horse found to be heterozygous, it should only be mated to a horse that is determined to be homozygous normal. The offspring from such mating should be tested and only if found to be homozygous normal, be allowed to be used in breeding. Jones (1997) expressed that not only should stallions be tested for SCID, but mares should be tested as well before being used for breeding.

In the Swedish government's agricultural act 1999:106 on animal welfare demands regarding breeding, issues that have to be considered are regulated (Swedish department of agriculture's homepage, 2007a). According to 3 §, breeding with horses that inherit lethal genes to its offspring is prohibited. Breeding with carriers of lethal alleles is not acceptable from an animal welfare perspective (Swedish Animal Welfare Agency's homepage). Breeding with carriers lead to an increased prevalence of the defective gene.

Since December 1st 2005, it is voluntary to evaluate stallions for breeding (Swedish department of agriculture's homepage, 2007b). It is now up to the breeding organisation for each breed to decide whether this is a demand or not. Breeders are not obligated to evaluate

mares either. Even when evaluated, horses are only tested for SCID if suspected to be carriers. This means that carriers could be used in breeding in Sweden even though the law prohibits it. The Swedish Arabian Horse Association (SAHF) recommends stallions to be evaluated for breeding. They can however not deny registration of Arabian horses that are not evaluated as they are obligated to register horses that are registered in the World Arabian Horse Organisation (SAHF's homepage, 2007).

Discussion

It is not likely that the frequency of the SCID gene in the USA has been reduced so drastically since first estimated just by selection (Bernoco and Bailey, 1998). The frequency was most likely overestimated due to non-random selection.

Even though Bue et al. successfully cured a SCID affected foal through bone marrow transplant, it has little value in practice (Perryman, 2000). Such a foal still has the same genetic makeup and would transfer the defective gene to its offspring and should for that reason never be mated.

A larger study with DNA-testing would give a reliable determination of the frequency of SCID carriers in the Swedish Arabian horse population. Those few horses that are carriers and are used in breeding in Sweden, could easily be replaced by non carriers from the total population. Arabian horses that are free from the mutated allele could also be imported from other parts of the world. In this way the effective population size will remain the same, not increasing inbreeding, when excluding carriers from breeding. The breeding progress could however to some extent be slowed down if some of the best horses are excluded on the basis that they are carriers. If testing all horses active in breeding and only allowing SCID free horses, the need for testing will only be temporary as no new carriers will be produced. Horses that are imported will need to be tested before being part of breeding. The cost of the DNA test is just a fraction of the cost in money and time that it takes to produce a foal.

Complete elimination of the SCID gene from the world population would be the ultimate aim for the future. If carriers are allowed in breeding in other countries, they should only be mated with non carriers. The method in which to eliminate carriers in these countries should be immediate or possibly gradual withdrawal of carriers from breeding. Carrier to carrier mating should not be allowed due to the high risk of the outcome of an affected foal. With reliable DNA testing, it is possible to assure this will never happen.

Conclusions

The aim should be to eliminate the carriers of SCID in Sweden through breeding by following present legislation. In other countries where mating with carriers of lethal genes is allowed, carriers should only be mated to non-carriers and this type of mating should be reduced and ultimately terminated.

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