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LATEST DEVELOPMENT IN GENETIC RELATED
DISEASES OF ARAB HORSES

Review of current literature and genomic studies

Diploma work

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1. Introduction

The elegance and temperament of the Arab horse has been fascinating people for decades and turned the Arabian into a very popular breed. The value of these horses is not only of economic but also emotional importance since these horses are outstanding companions for every horse owner.

This work will give an insight into the genetic disorders that endanger the sound breeding of Arabian purebreds and will show the most recent developments in managing, and hopefully eradicating, genetic related diseases of the Arab horse.

2. Genetic disorders and pathways of inheritance

2.1 Definition of genetic diseases

Improved technology and ongoing research lead to a greater understanding of the horse's genetics as well as drew attention to diseases that find their origin in the horse genome. The success of mapping the horse genome is a major breakthrough in managing genetic diseases, by allowing scientists to perform and generate DNA-tests that are readily available for every horse owner. But in order to target a genetic disease, the biological and molecular mechanisms in which the condition finds its origin need to be understood.

A genetic or breed-related disorder can result in a clinical disease that caused by a defective gene. The disorder commonly appears multiple times within a pedigree and is passed on from the parent generation to its offspring. Genetic diseases have to be distinguished from congenital diseases. A disorder that is present at birth and may or may not be the result of an abnormality in the genome is considered to be a congenital disease. In contrast, a genetic disease is always a result of an abnormality in the genetic material of the individual. Genetic diseases might not be expressed in the phenotype of the individual at birth.¹

Table 1; Terms and Definitions²

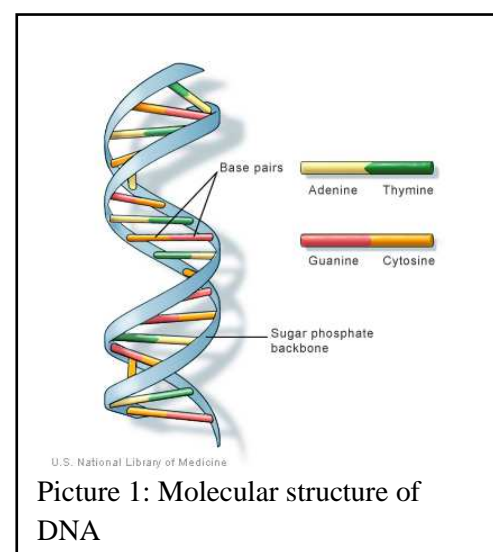
Genetic Terms	Definitions
DNA	Deoxyribonucleic acid is the molecule carrying genetic information in all living organisms
Base pair	Binding of adenine with thymine and guanine with cytosine forming the double helix shape of the two DNA strands
DNA-sequence	Order in the arrangement of base pairs at a specific location on the DNA
RNA	Ribonucleic acid is found in the nucleus and in the cytoplasm and is important for protein biosynthesis and other cell functions; the base thymine is replaced by uracil
Gene	Section of a chromosome that encodes a specific trait; Gene locus: gene location on a chromosome
Allele	Form of a gene that can be present in different forms and determines whether a trait is expressed or not
Polymorphism	Variations in a gene that are seen frequently in a population and do not have a negative impact
Autosomal	A gene located on a non-sex chromosome
Gonosomal	A gene located on a sex chromosome
Exon	Part of the gene that codes for a protein
Intron	Non-coding part of the gene
Promotor	DNA sequence that can be found at the beginning of a gene and initiates transcription

Transcription	Formation of a DNA sequence copy in form of RNA
Translation	Transformation of the encoded information from RNA into a amino acid chain that forms the protein
Genetic code	Information encoded on a gene for cell functions
Genetic marker	Part of the DNA that can be identified and whose inheritance can be traced
Gene family	Closely related genes that result in similar expression of products
Gene product	Biochemical material produced after the code of the gene
Gene mapping	Determination of the positions of genes on a DNA molecule
Carrier	An individual that does not express the genetic disease but possesses the genetic mutation in its genome and is capable of passing it on to its offspring
Gamete	Sperm or ovum containing an haploid set of chromosomes (32 in horse) formed by meiosis; fertilization results in an embryo with the full chromosome set
Genotype	Inheritable genetic information of an individual
Phenotype	Physical manifestation of an individual's genetic information
Conserved sequence	DNA sequence that has not changed throughout evolution
Mutation	Alteration in a DNA-sequence
Frame-shift mutations	Alteration of the DNA-sequence resulting in a different gene product
Point mutation	Alteration of one base pair that can result in a different gene product
Segregation analysis	The listing of offspring according to distinct and mutually exclusive phenotypes; used as a test from a putative pattern of inheritance ³
Linkage analysis	Study performed to locate a causative gene mutation by identifying traits co-inherited with it ⁴

2.2 Structure of the DNA

The horse's karyotype consists of 64 chromosomes which can be grouped into autosomes and the xx or xy sex-chromosomes for mares and stallions, respectively. Chromosomes are condensed DNA double helix strands which carry the genetic information for the physical appearance of each individual, defining for example the color of the hair coat and the specific body frame of every breed. Even breed specific character traits that distinguish the hot-blooded Arab from the steady and stoic coldblooded breeds are encoded in the DNA.⁵

Deoxyribonucleic acid (DNA) is the molecular hereditary material found in every cell of an individual and is located mostly in the nucleus. Mitochondria, the cell organelle that is responsible for energy production through cellular respiration, also carry a small portion of the DNA. The DNA structure can be described as a "twisted ladder", with sugar and phosphate building the side pieces that are connected through base pairs forming the rugs. The four bases are adenine, thymine, guanine and cytosine which are held together by weak bonds. Bases are arranged in specific pairs. Adenine binds to thymine and guanine to cytosine.⁶



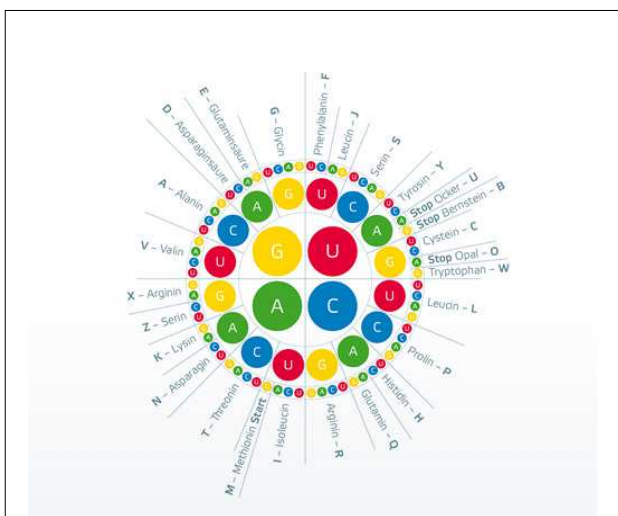
2.3 Protein synthesis

The process during which encoded information on a DNA sequence is transformed into a protein that expresses the information of the coding gene is called protein synthesis and consists of two separate steps. The following section will demonstrate the protein synthesis in a very simplified manner, which is sufficient to gain an understanding for the background of genetic related diseases.

First, the coding gene of the DNA is used as a template to generate a homologous messenger RNA strand (mRNA) which carries the complementary base arrangement of the coding DNA-sequence, with the exception of the base thymine which is substituted by the base uracil on the mRNA strand. This process is called transcription and takes place in the cell nucleus. The enzyme RNA-polymerase binds to the promoter on the DNA sequence and assembles nucleotides to produce the mRNA strand according to the DNA template. The DNA sequence that is used to assemble an mRNA strand is called operon. The operon is enframed by a promoter region, which initiates the transcription process⁷, and a terminating region consisting of a sequence of several adenine bases resulting in an uracile base sequence on the mRNA strand. This base arrangement causes the transcription complex to be rather unstable, resulting in the termination of the transcription process and the release of RNA polymerase.⁸ The end product of the transcription is the pre-mRNA strand that undergoes several modification processes preparing the m-RNA for the next step of the protein biosynthesis, the translation.

The translation is the second step of the protein synthesis and results in the end product consisting of an amino acid chain forming the protein according to the instructions of the coding gene. This process takes place in the cytoplasm. The mRNA is transported from the nucleus to the cytoplasm of the cell where ribosomes can attach to the mRNA strand. After amino acids are activated and bound to transfer RNA (tRNA) by the enzyme amino acid synthetase, the ribosomal subunits bind to the mRNA strand. The translation process begins at the initiating codon with the support of initiating factors. During the assembly of a peptide chain, the tRNA loaded with a specific amino acid binds to the complementary base triplet on the mRNA strand.⁹ The first binding tRNA molecule is released after its amino acid load forms a bond with the amino acid of the subsequent tRNA that matches for the next base triplet of the mRNA strand. This generation of a polypeptide chain is continued until all amino acids are assembled to form the encoded protein.

Translation displays the core mechanism that each base tripled encodes for an amino acid according to the “code sun”. This deciphering system of the genetic code is read from the inside to the outside of the circle, with each base triplet arrangement resulting in the amino acid named at the outside of the circle. The base triplet UAG is coding for the amino acid methionine and also serves as the “start codon” which



Picture 2: The “code sun”

initiates the protein biosynthesis.¹⁰ The so called “stop codons” UAA, UAG and UGA lead to the termination of the translation process, resulting in the release of the peptide chain and the disassembly of the ribosomal subunits.¹¹

2.4 Classification of genetic diseases and mutations

The knowledge of the pathway from the information encoded in a gene to the production of proteins, that are necessary for various essential metabolic mechanisms of the individual, leads to greater understanding of the impact of an alteration in a DNA-sequence, also known as a mutation. Hence, a mutation is changing the genetic information for the protein production and can result in change of the protein and its function. A harmful mutation that occurs spontaneously and can be passed on from the parent to the offspring is the foundation of a genetic disease.¹²

Inheriting a genetic disease is possible if the mutation appears in the germ cells, the egg or sperm cell of the parent generation, through which the genetic defect is passed on to the offspring.¹³ All cells of the offspring contain the DNA with the defective gene causing either a disease or carriage of the mutation in the genome without showing clinical signs.¹⁴

Classification of genetic diseases

Genetic disorders can be grouped into three categories. The first is described as the Mendelian or simple genetic disorder involving a single abnormal gene that is passed on from the parent generation to the offspring. The second category includes chromosome number aberrations and is considered rather spontaneous than inherited. An example for this would be a lack of one x-chromosome in a mare leading to failure of developing a reproductive tract. The third category counts for diseases that involve more than one gene, a multifactorial inherited disease. Several abnormal genes and sometimes even environmental factors need to be present to cause the disorder. Detection of this group of genetic disorders is a major accomplishment of the horse genome mapping. Finding a genetic link to diseases like osteochondrosis is subject of ongoing research that can only be done due to the success of the horse genome project.¹⁵

The impact of a change in the genome can vary greatly depending how the alteration affects the function of the individual's cell mechanisms. An alteration in a gene can be beneficial. For example it can result in an improvement in the phenotypic display of breed specific traits, which is then enhanced by selective breeding with exactly those mares and stallions that express the desired traits of the breed. On the other hand, mutations can also cause a pathological presentation resulting in a disease by altering the cell mechanism and function of proteins. If the mutation is affecting the viability of a fetus it can even lead to the abortion of the fetus.

Focusing on a mutation's effect on the function of the affected protein different outcomes can be described. A loss of function is considered either a complete loss of the protein's initial function or decreased function ability. Gain of function is associated with an increase in the protein's desired function ability. Another outcome is the so called dominant negative mutation. Here, the protein derived from the abnormal gene interferes with the wild type protein. A mutation that involves essential mechanisms can lead to the death of the affected individual and are called lethal mutations. The manifestation of these outcomes in the

phenotype can be either on a morphological level, for example coat color, or on a biochemical level regarding the alteration of the protein function and the metabolic process it affects.

Classification of mutations

The classification of mutations can be done on the basis of inheritance or structure.¹⁶ During the mitotic cell divisions, the diploid chromosome set is duplicated and divided equally among each daughter cell. This results in a cell multiplication ensuring that all daughter cells carry the exact same genetic information. In contrast, the process of producing gametes for sexual reproduction, called meiotic cell division of the sperm and egg cell production, results in a cell containing only half of the chromosome set. These cells are considered to be haploid gametes that will form the diploid zygote after fusion of the sperm and egg cells of the parent generation. This mechanism equips the offspring with a diploid chromosome set consisting of half of the maternal and half of the paternal chromosomes. Therefore, if we want to classify mutations from an inheritance point of view, the offspring inheriting a mutation can be either considered as heterozygous or homozygous. The term heterozygous implements that the allele with the defective gene was passed on through the haploid chromosome set of either the mother or the father. Only one of the gametes carries the mutation. In contrast, homozygous inheritance is the result of a zygote formed by an egg and sperm that both carry the mutation. The altered DNA sequence is inherited through both, the maternal and paternal gametes.¹⁷

Focusing on the alteration in the DNA structure caused by a mutation, they can be grouped into small scale mutations and large scale mutations. Small scale mutations include point mutations, insertions and deletions.

A point mutation occurs if a single nucleotide is exchanged with a different one. Commonly a purine base is substituted by a different purine base or a pyrimidine base by a different pyrimidine base. Bases belonging to the purines are adenine and guanine. Pyrimidines include uracil, thymine and cytosine. If a purine is replaced by a pyrimidine base the process of alteration is called a transversion. Changing a single nucleotide can alter the triple nature of the genetic code. A change in the base triplet of a DNA sequence coding for a specific amino acid can have different impacts on the outcome of the protein production. If the point mutation results in a base triplet coding for an amino acid different from the wild type, it is considered to be a mis-sense mutation. This alteration can have a major impact on the protein function. A non-sense mutation refers to a nucleotide substitution that transforms a base triplet coding for an amino acid into a base triplet coding for the stop codon. The translation process is terminated at a random stage of the peptide chain resulting in an unfinished production of the protein. Having the deciphering system of the code sun in mind, it is known that different base triplets can code for the same amino acid. This mechanism can also apply for a point mutation. The substituted nucleotide changes the base triplet but not the amino acid it is coding for. This phenomenon is called a silent mutation and has no impact on the natural occurring amino acid order of the peptide chain.¹⁸ However, a silent mutation may have an effect on the phenotype by for example accelerating or slowing down the speed of the protein biosynthesis.¹⁹

Mutations that result in adding or removing a single or even several nucleotides of the DNA sequence are called insertions or deletions. This alteration is also called a frameshift mutation

as the reading of the base triplets is shifted in comparison to the natural frame by altering the arrangement of the amino acid chain of the original protein.

A change in structure or rearrangement of the gene on a chromosome level is grouped to the class of large scale mutation. This class of mutations includes amplifications, translocations, inversions and large scale deletions. An amplification of a gene expression can be the result of a duplication of a gene on the same chromosome. A chromosome fragment exchange between two non-homologous chromosomes resulting in “swapping” a segment between those chromosomes is called a translocation. The term inversion stands for a change in the orientation of a DNA sequence, in other words, a segment of the DNA rotates 180° in comparison to the initial orientation. The impact of a deletion on a large scale level can lead to a loss of a whole DNA section and subsequently the loss of the gene.²⁰

Having gained an insight in molecular and biological mechanisms of an individual’s genetic makeup and the impact of an alteration of the genetic information, there is still one question that needs to be answered. What causes a mutation?

The triggering act of a mutation to occur can be either spontaneous, induced or due to a base damage. If a mutation appears due to natural chemical processes, it is considered to be spontaneous. DNA-replication errors fall into this category, as well as spontaneous lesions for example caused by superoxide radicals which lead to mispairing of the bases. Mobile gene elements called “wandering” DNA sequences and the abnormal pairing of bases, called tautomerization are also considered to be spontaneous mutations.

Furthermore, mutations can be induced by chemicals that are analogues to certain bases and can therefore be incorporated into the DNA strand instead of the base. An example for this is the substance 5-bromouracil which is analogue to the base thymine but can form a base pair with both adenine and guanine. Oxidation, chemicals, high energy radiation and UV light can lead to base damage.²¹

2.5 Pathways of inheritance

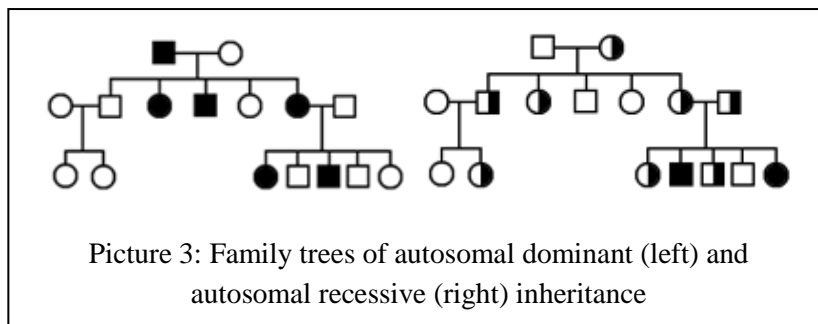
Now that the causative background of a genetic disease has been demonstrated, it is important to focus on the mechanisms how the different diseases are passed on from the parent generation to their progeny.

A gene can be present in several versions, which are called alleles. In terms of the previously mentioned homozygous and heterozygous genotypes, an individual is considered homozygous if it inherits the same allele variant from both parents. A heterozygote, in contrast, inherits different alleles of the same gene for its parents. One allele can differ according to its dominance in respect to a different allele of the same gene. The same principle counts for a recessive allele. This differentiation is only possible by comparing the different alleles with each other, as the dominant allele will be expressed over a recessive allele. Genetic diseases can be inherited through both dominant and recessive alleles that incorporate the mutation. It is important to point out that the terms dominant and recessive only refer to the comparison of the alleles of the same gene. They do not determine the likelihood of inheriting a dominant or recessive allele, since both have the same probability to be passed on to the offspring.

Autosomal dominant inheritance

An autosomal dominant genetic disorder can cause a clinical picture in the individual with the involvement of only one defective dominant allele. The affected horse can either be heterozygous or homozygous but is potentially affected by the disease in both cases. Examples for dominant inheritance are Juvenile Idiopathic Epilepsy in Arab Horses or Hyperkalemic Periodic Paralysis (HYPP) in Quarter Horses.²² Diseases with this kind of inheritance pattern are rare, cause a clinical picture in every offspring generation²³ and are easy to trace back through the bloodline and to eliminate from the breeding system.

A family tree can be used as a tool to follow the path of autosomal dominant inheritance, which is also referred to as a pedigree analysis. In this schematic follow up of a related population, the female individuals are demonstrated as circles and the males as squares. A line is drawn between those individuals that produce the offspring population. The individuals of the progeny are linked with a connecting line to the parent generation and displayed below the parent generation. Individuals expressing the disease in their phenotype are demonstrated with a black coloration of the circles or squares.



If a horse expressing the clinical signs of a dominant genetic disorder is used for breeding, the probability, meaning the likelihood of the offspring inheriting the defective dominant allele, can be calculated as the following:

Table 2; Probability of inheriting dominant genetic disorders at each mating in %²⁴

A: dominant allele a: recessive allele	Clear stallion (aa) Not affected	Homozygous stallion (AA) Affected	Heterozygous stallion (Aa) Affected
Clear mare (aa) Not affected	100% Clear offspring	100% Affected heterozygous offspring	50% Affected heterozygous offspring 50% Clear offspring
Homozygous mare (AA) Affected	100% Affected heterozygous offspring	100% Affected homozygous offspring	100% Affected offspring (50% homozygous and 50% heterozygous)
Heterozygous mare (Aa) Affected	50% Affected heterozygous offspring 50% Clear offspring	100% Affected offspring (50% homozygous and 50% heterozygous)	75% Affected offspring (25% homozygous and 50% heterozygous) 25% Clear offspring

Autosomal recessive inheritance

In case of autosomal recessive disorders, both defective alleles must be present to cause a phenotype expression. Therefore, recessive inheritance can also result in carriers that possess only one defective allele. These individuals do not show any clinical signs of the disease, but are capable of passing the defective gene on to their offspring. Carrier individuals cannot be found in case of autosomal dominant diseases, as in this case only one defective allele is sufficient to cause a clinical disease. Recessive genetic disorder can stay hidden and be passed on as a “silent passenger” over generations. Using a family tree analysis, the carrier stage is demonstrated through the half-colored symbols in picture 3. This kind of genetic disorder is more commonly found but due to the occurrence of carrier individuals much more difficult to trace in the bloodline and to eliminate from the breeding system.²⁵ Exactly these difficulties are a major problem in targeting and eliminating genetic disorders in the Arabian breed, since most of the genetic diseases affecting Arabs are of autosomal recessive nature.

Since carrier individuals cannot be distinguished from a healthy horse by their phenotype, heterozygotes might be accidentally used for breeding. The probability of inheriting recessive genetic disorders can be demonstrated as the following with a being the recessive defective allele and A being the natural dominant allele:

Table 3; Probability of inheriting dominant genetic disorders at each mating in %²⁶

		Clear stallion (AA)	Carrier stallion (Aa)
Not affected	Clear mare (AA)	100% Clear	50% Clear 50% Carrier
	Carrier mare (Aa)	50% Clear 50% Carrier	25% Clear 50% Carrier 25% Affected
Affected	Affected (aa)	Carrier (Aa)	Clear (AA)
	100% Affected	50% Affected 50% Carrier	100% Carrier

Table 3 demonstrates that an affected foal can only be the result of mating two carriers.

Other pathways of inheritance

Autosomal dominant and recessive diseases are the most commonly encountered forms of inheriting genetic disorders. Other forms of inheritance include x-chromosome linked dominant or recessive inheritance, codominant inheritance and mitochondrial inheritance.

A defective allele which is located on the x-chromosome can be inherited from both the mare and the stallion in case of fillies, but only from the mare in case of colts. In this form on inheritance there is no male to male transmission as the colt always inherits the y-chromosome from the stallion and the x-chromosome from the mare.²⁷ Dominant x-linked genetic diseases affect females more frequently than males and are expressed in every generation like autosomal dominant disorders. X-linked recessive disorders affect male individuals more frequently than females as males only possess one x-chromosome. Again,

male to male transmission is not possible. Codominant inheritance occurs when two different alleles of a gene are expressed at the same time. Consequently, the characteristics of the genetic condition are determined by both alleles, each leading to a slight variation of the protein.²⁸ Mitochondrial or maternal inheritance refers to the dam's ability to pass on mitochondrial DNA to the foal, as only the egg cells contribute mitochondria to the fetus. Both fillies and colts can be affected by an alteration of the dam's mitochondrial DNA.

3. The Horse Genome Project

3.1 The Horse Genome Project and genome mapping

The Horse Genome Project was established in 1995 when 70 scientists from 20 countries met in Lexington, Kentucky. Achievements of the Human Genome Project initiated the idea to map the genome of the horse. At this point selective breeding was the only way to amplify or abolish a phenotype trait. Researchers hoped that the information gained through the Horse Genome Project would help to identify the genetic background of various individual traits ranging from physical appearance to susceptibility to diseases.

Since the size and organization of the genome shows comparable features in all mammals, including humans, the methods and tools of the Human Genome Project were applicable for establishing a genome map of the horse. Furthermore, information about the human genome could be used as a template to make projections about the arrangement of equine genome sequences.²⁹ There are over 80 genetic related diseases known to show phenotypic similarities in both, horses and humans.³⁰

The primary goal of the research initially was to create a genetic map of the horse genome by identifying all 32 chromosomes and their genetic landmarks as reference points to the human genome. The comparison to the human genome was first anticipated to avoid the great effort and expense to sequence the whole equine genome. This strategy changed in 2005 when only 20,000 genes could be identified which made up only 2% of the genetic information. Researchers came to the conclusion that, since genes are similar in all mammalian species, the turning point to understand genetic variations would be to analyze the 98% “junk DNA” which is unique for each individual. A whole genome sequencing of the horse would be necessary to gain this insight. Fortunately, the National Human Genome Research Institute was trying to decide which species out of the order Perissodactyla, which also includes the domestic horse, was suitable to be involved in the genome mapping process next. The members of the Horse Genome Project successfully demonstrated the benefits of mapping the genome of the domestic horse and gained a strong partner for their work.³¹

By 2006 the Horse Genome Project succeeded in sequencing the 2.7 billion base pairs of the genome belonging to the Thoroughbred mare called “Twilight”. The assembly of the genome sequencing was based on a 6.8 fold coverage, thus each base pair had been sequenced almost seven times.³²



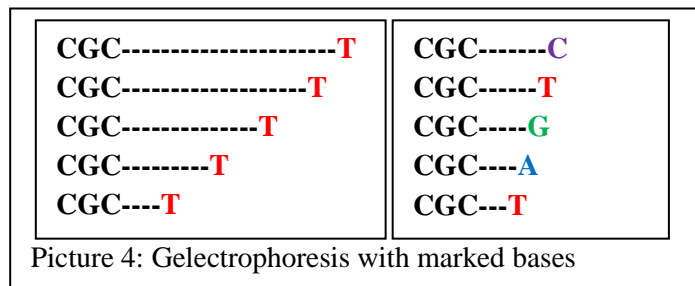
Picture 3: Twilight

The sequences were assigned to their specific location on the chromosomes creating a map of the horse genome.³³ This genome map displays the order of genes or DNA markers along the chromosomes. Containing about 4000 markers the equine genome map is the densest among all studies species.³⁴ Comparing DNA samples from a variety of different breeds, 1 million areas of genetic variations called single nucleotide polymorphisms (SNPs) could be identified in the horse species. The results of this project enable researchers to gain a genome wide insight in the genetic variability in horses.³⁵

3.2 The DNA sequencing process

The DNA sequencing process resembles a polymerase chain reaction (PCR) used for replicating DNA. A mixture of DNA template, free nucleotides, primers and the enzyme Taq polymerase is heated to separate the DNA double strands. The primer binds to single strand DNA and initiates the nucleotide assembly of a complementary strand by the polymerase enzyme.

A small amount of the nucleotides are modified dideoxynucleotides. Adding a modified nucleotide with the base thymidine (T) will stop the replication process randomly whenever the dideoxy-T is included in the synthesized strand resulting in a fraction of the original template. This occurs only in 5% of the occasions where a thymidine nucleotide is required since the modified nucleotides just make up a small fraction of the overall available nucleotides for replication. Although, repeating this process continuously will eventually lead to copies that uniformly end with a modified thymidine nucleotide. The results are homologous strands that all have the same start but stop at every possible thymidine base



along the strand. These fragments can be sorted by their size through gelelectrophoresis using a fluorescence dye to make the modified thymidine base visible. As the end base of each fragment is thymidine, the positions of all thymidine bases along the replicated

strand can be identified. Using this technique including all four bases with different colors for each base, the complete base sequence of the complimentary and therefore the base sequence of the template strand, according to the specific base pairing, can be identified.

This process is explained very simplified but basically resembles the sequencing process performed by machines called automated DNA sequencers.³⁶

3.3 Significance of the Horse Genome Project's achievements and the Equine SNP50 Bead Chip

The map of the horse genome allows performing a genetic analysis ranging from equine phenotype traits to the genetic basis of physiological and disease related mechanisms. Today eleven mutations that cause ten clinical diseases have been identified and genetic tests were developed for nine of those disorders. This is an exceptional step towards a sound breeding system that can eliminate the appearance of affected foals, by testing the sire and dam for mutations causing a disease.³⁷

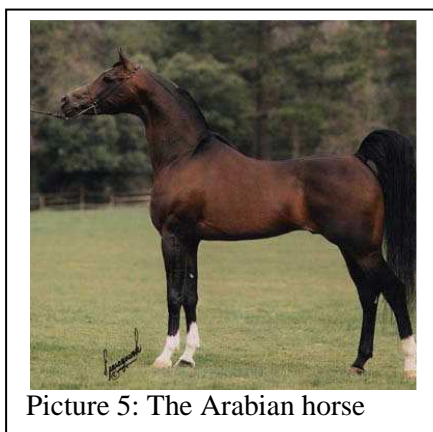
Continuing the research on identification the genetic background of disease also has the potential to refine treatment options, create new diagnostic tools and help veterinarians to apply the newly gained knowledge in the field.³⁸ First steps towards this direction have already been made by establishing “daughter projects” based on the information gained through the Horse Genome Project. Current research includes investigation of diseases caused by simple genetic traits, multiple genes and even non inherited diseases where the knowledge of the genetic background of the affected functions is a valuable tool. Examples for these studies are projects currently working on the genetic investigation of laminitis, immunity to *Rhodococcus equi* in foals and muscle response to exercise in Thoroughbred.³⁹ A recent study published in 2014 used whole genome sequencing to characterize breed associated and non-breed horses by identifying individual mutations involving the genetic information for several vital processes in breed and non-breed horses. Genetic variants were identified which is considered as a first approach towards a possibility to differentiate breed and non-breed horses.⁴⁰

A new diagnostic tool created after the results of the Horse Genome Project is the EquineSNP50 genotyping BeadChip generated by the company Illumina. This device includes over 54,000 SNPs that were identified through the horse genome assembly and is the first genome wide genotyping tool for horses. The SNP chip can help to identify mutations and genes in all major horse breeds. It was designed after sequencing seven breeds including the Arabian horse which lead to creating probes that target homogenously distributed SNPs over the whole genome. The genetic basis of phenotype diversities can therefore be located by identifying and mapping the gene location coding for the trait in question.⁴¹

This demonstrates that the Horse Genome Project can be considered as a milestone in the history of equine genetic research and that the information gained from this project is applied to various current research projects.

4. The Arabian horse breed and the importance of testing for genetic related diseases in the Arabian

4.1 The Arabian horse breed



Picture 5: The Arabian horse

The Arabian is the oldest horse breed and originates from the Arabian peninsula where it was bred by the Bedouin desert tribes suggestively at 2500 BC. Already at this early development of the breed, the tribes established and maintained the purebred status of the Arab horse.⁴² Their endurance and excellent performance as war horses was proudly cherished. Admirers introduced the Arabian to other continents including Europe and North America in the 1800’s. The popular characteristic traits of the Arabian horse were soon used to develop new breeds like the Thoroughbred and other light breeds.⁴³ Today, there are

six bloodlines of the Arabian horse, including Domestic, Crabbet, Russian, Egyptian, Polish and Spanish Arabians. A unique feature of the Egyptian bloodline is that only carefully

selected breeding animals, descendant from strict Egyptian purebreds are used to continue the bloodline.⁴⁴ Classical features of the Arabian are the small and fine head with a dished face, the high carried tail, the long arched neck and the light but well muscled body frame.⁴⁵

4.2 Importance of testing for genetic related diseases of Arab horses

“Nature and time formed the perfect Arabian; it is man’s responsibility not to spoil it.”⁴⁶
(Author unknown)

The highest concern of every Arabian horse owner and breeder is to maintain a healthy population of the breed. Genetic disorders of the Arabian are often fatal and cause a significant financial loss for the breeder. Its fine body structure, endurance and energetic character make the Arabian a highly popular breed. These outstanding traits have been carefully preserved through selective breeding of the most desirable individuals within a closed population of Arabian bloodlines.

But the purebred status also has its downsides. The Arabian breed is particularly affected by the negative impact of inbreeding. Being one of the oldest breeds, the Arabian horse population has a very narrow gene pool due to early inline breeding. The result of this closed gene pool is a higher frequency of genes that are associated with the desired phenotype traits. Intensive breeding of animals that display a similar phenotype are likely to have the same ancestors. This breeding system increases the chance of identical gene combination which, in turn, is leading to foals affected by genetic diseases, especially in case of autosomal recessive disorders.⁴⁷ An example for this is the frequent appearance of several genetic diseases associated with the Arabian horse in the AlKhamsa bloodline whose population makes up less than 5% of the registered Arab population in the USA and Canada.⁴⁸

Since autosomal dominant genetic diseases are readily expressed in the phenotype of the affected individual, they are easy to trace and eliminate from the breeding program. Thus, the main focus is placed on finding solutions to manage and prevent autosomal recessive disorders, especially through identifying carrier animals.

Performing a pedigree analysis to define the probability of non-carrier parents is a useful tool to manage recessive disorders, as well as carrying out test matings of horses that are known to be carriers with individuals of which the genetic status is questionable. Test matings are of a great financial expense and are very time consuming, considering the long gestation period of the horse and the low number of offspring.

The importance of using genetic methods to define carriers are of increasing importance, as ongoing research detects more and more causative genes for the genetic diseases in question. Abnormalities in the number and structure of chromosomes can be revealed through a chromosomal analysis. Direct gene analysis using polymerase chain reaction (PCR) and restrictive fragment length polymorphism (RFLP) or indirect gene analysis with DNA-markers are used to define the section of the altered gene that causes the phenotype appearance of the disease. Those methods are used to produce test kits for currently ten disorders like severe combined immunodeficiency (SCID) and are available to every horse owner.⁴⁹

Table 4; Genetic Conditions by Body System⁵⁰

Body system	Condition	Breed(s) affected	Available test?
Neurologic	Cerebellar abiotrophy (CA)	Arabians	Yes
	Juvenile epilepsy syndrome (JIE)	Arabians	No
	Lavender foal syndrome (LFS)	Arabians	Yes
	Neuroaxonal dystrophy	Various	No
	Wobbler syndrome*	Various	No
Musculoskeletal	Polysaccharide storage myopathy type 1 (PSSM 1)	Quarter horse, Drafts, Warmblood	Yes
	Polysaccharide storage myopathy type 2 (PSSM 2)	Various	No
	Glycogen-branching enzyme deficiency	Quarter horse and related breeds	Yes
	Hyperkalemic periodic paralysis (HYPP)	Quarter Horse and related breeds	Yes
	Myotonia	New Forrest Ponies	Yes
	Osteochondrosis/osteochondritis dissecans (OC/OCD)*	French Trotters, Dutch Warmbloods	No
	Recurrent exertional rhabdomyolysis*	Thoroughbreds	No
	Distal limb fractures, superficial flexor tendon injury*	Thoroughbreds	No
	Lordiosis	American Saddlebreds	No
	Lateral (sub)luxation of the patella*	Shetland ponies	No
Malignant hyperthermia	Quarter Horse and related breeds	Yes	
Occipito-Atlantoaxial Malformation (OAAM)	Arabian	No	
Dermatologic	Heritable equine regional dermal asthenia (HERDA)	Quarter Horse	Yes
	Junctional epidermolysis bullosa (JEB)	American Saddlebreds, Belgians, Drafts	Yes
	Gray horse melanoma	Gray horses	No
Ocular	Equine multiple congenital ocular anomalies (MCOA)	Silver-coated horses	Yes
	Corneal dystrophy	Friesians	No
	Equine recurrent uveitis (ERU)	Appaloosas, German Warmblood	No
	Congenital stationary night blindness	Appaloosas, Thoroughbreds, Paso Finos	Yes
Respiratory	Guttural pouch tympany* (GPT)	Arabians, German Warmblood	No
	Recurrent laryngeal neuropathy (RLN)*	Thoroughbreds, Drafts	No
	Recurrent airway obstruction (heaves)*	Various	No
	Exercise-induced pulmonary hemorrhage*	Various	No
Miscellaneous	Severe combined immunodeficiency (SCID)	Arabians	Yes
	Foal immunodeficiency syndrome	Fell and Dales ponies	Yes
	Chronic progressive lymphadenopathy	Draft breeds	No
	Overo lethal white foal syndrome	Paints, Quarter Horse	Yes
	Dwarfism	Miniature Horses	Yes
	Equine metabolic syndrome*	Various	No
Hoof wall separation diseases	Connemara Ponies	Yes	

* Suspected polygenetic condition

But testing for the defective gene carriage alone does not prevent the appearance of affected foals. A well coordinated breeding strategy that prevents mating two carriers of the disease must be established. Banning carriers of a specific genetic disease from the breeding system would decrease the already limited gene pool of the Arabian breed even more and might lead to the loss of important bloodlines. Furthermore, by decreasing the gene pool, the frequency of the appearance of other genetic diseases might increase. The overall goals of a sound breeding strategy should therefore be to avoid affected foals and to decrease the presence of the abnormal allele in the gene pool by breeding a known carrier and only use offspring for further breeding that do not carry the mutant allele. Attention must be paid not to limit the genetic diversity that is necessary to sustain the welfare level in the breed.⁵¹

The Arabian Horse Association defined these goals through the "Code of Ethics" that requires members to reveal the genetic status of their registered horses and to act according to the guidelines described in the amended section. These guidelines were first established for SCID carriers in 1984 and now include the disclosure of cerebellar abiotrophy (CA) and lavender foal syndrome (LFS) carrier status. In 2010 the affected status of hyperkalemic periodic paralysis (HYPP), a significant disease for breeders of Arabian-Quarterhorse crossbreeds and Quarterhorse related breeds, was added to the list. The amendments require the member not to breed, sell or lease a horse known to be a carrier of the listed diseases or affected by HYPP without informing all involved parties about the genetic status of the horse in question. Owners of mares that give birth to an affected foal are requested to notify the owner of the parent stallion in order to investigate the genetic status of the breeding animals in a joined effort.⁵²

The "Code of Ethics" is an excellent role model for targeting the eradication of genetic diseases in the Arabian breed. The overall significance of genetic disorders in the horse species is demonstrated in table 2. Not only the Arabian breed, but many others are affected by the often fatal disorders. DNA-tests are only available for a few of those diseases but hopefully future research will allow to develop more tests of the increasing list of genetic related disorders.

5. Genetic related diseases in Arab horses

5.1 Guttural pouch tympany

Guttural pouch tympany (GPT) is a disease of young horses characterized by a pathological enlargement of the guttural pouch due to air accumulation which is macroscopically visibly as an enlargement of the Viborg's triangle.

The Viborg's triangle marks the topical outlines of the guttural pouch. It is cranially enframed by the caudal border of the mandible, dorsally by the insertion tendon of the musculus sternomandibularis and ventrally by the lingofacial vein. The guttural pouches are bilateral air filled structures that hold a volume of 300-500 ml. Both pouches expand from the neck region to the nasopharynx and join ventrally to the musculus longus capitis. They do not communicate and are separated by a median membrane. Due to their anatomical location, they either embed or are adjacent to vital blood vessels and nerves. The internal and external carotid artery, retropharyngeal lymph nodes and several nerves including n.

glossopharyngeus, n. vagus, n. hypoglossus, n. facialis, n. accessories and the sympathetic trunk are associated with the guttural pouch. Furthermore, it includes the pharyngeal opening of the auditory tube, also called the Eustachian tube.⁵³ Hence, the guttural pouch is part of the upper respiratory tract and is considered to be the mucosal diverticulum of the Eustachian tube.⁵⁴

The function of the guttural pouch has not been definitely revealed yet, but it is suggested, that it serves as an air space cooling the blood flow to the brain by surrounding the internal carotid artery with air.⁵⁵

GPT can be seen in foals up to one year of age. The disease seems to have a link to the sex of the affected Arab foal, as fillies are affected two to four times more frequently than colts.⁵⁶ In Arab and German Warmblood horses and several other breeds, GPT is thought to be a genetic disorder. But the disease can also be acquired due to an upper respiratory tract inflammation,⁵⁷ which is commonly seen in older horses.⁵⁸

The mechanism which causes the non-acquired air accumulation in the guttural pouch is not fully understood but two hypotheses exist. One theory states that the mucosal flap, the plica salpingopharyngeus, at the pharyngeal opening of the auditory tube is enlarged. Another explanation is a functional abnormality of the pharyngeal orifice of the Eustachian tube. Both hypotheses would explain why the plica salpingopharyngeus acts like a one-way valve trapping air in the guttural pouch of affected animals.⁵⁹



Picture 6: GPT in a foal

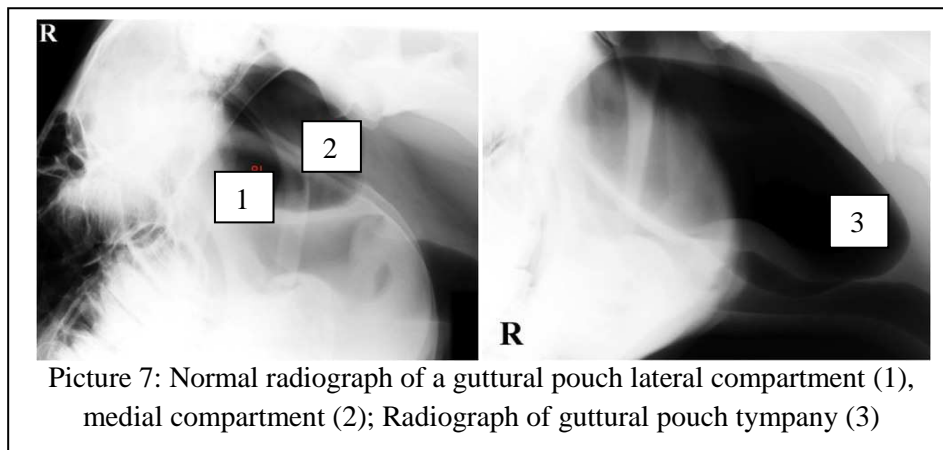
Clinical signs of GPT include a uni- or bilateral non-painful swelling at the parotid area that can be manually compressed.⁶⁰ Applying appropriate manual pressure on the superficial swelling can lead to an expulsion of the accumulated air and reduction of the swelling.⁶¹ During percussion of the Viborg's triangle a tympanic sound is audible.⁶² The degree of the swelling can worsen progressively and lead to difficulties in breathing and swallowing due to the compression of larynx and pharynx. Respiratory stridor and the appearance of milk or feed in the nostrils are the clinical manifestations of this compression. Consequently, the most common accompanying disease is aspiration pneumonia secondary due to impaired swallowing and aspiration of feed particles. Aspiration pneumonia presents with

clear to purulent nasal discharge, fever and coughing. This can be a life-threatening complication of GPT.⁶³

Diagnosis is based on the typical clinical signs, the age of the affected horse and diagnostic examinations including endoscopic examination of the pharynx, gutturocentesis and radiographs of the head.⁶⁴ An enlarged radiolucent area on the x-ray film at the location of the guttural pouches indicates GPT tympany.

Both endoscopic examination and gutturocentesis release the trapped air from the guttural pouch and can help to distinguish unilateral from bilateral GPT. Unilateral air accumulation is indicated if the visible swelling resolves completely after one guttural pouch is punctured or entered by the endoscope. Occasionally, secondary pharyngeal collapse⁶⁵ or milk droplets can

be visible during endoscope examination. Generally no pathological abnormalities that cause the tympanic enlargement of the guttural pouch can be detected during examination⁶⁶



Picture 7: Normal radiograph of a guttural pouch lateral compartment (1), medial compartment (2); Radiograph of guttural pouch tympany (3)

Treatment options are surgery and placement of a Foley catheter accompanied by antibiotic and non-steroid anti-inflammatory drug application to counteract secondary aspiration pneumonia.⁶⁷ In rare cases GPT resolves without treatment as the foal grows.⁶⁸

Surgery involves the resection of the mucosal flap or a fenestration of the median septum or both in bilateral cases to allow air flow out of the guttural pouch.⁶⁹ Surgery is a treatment option that ensures the resolution of air accumulation in the guttural pouch⁷⁰ but requires special instruments and adequate skills. Post op complications include dysphagia caused by nerve damage due to surgical manipulation and aspiration pneumonia.⁷¹ Puncture of the guttural pouch and placement of a Foley catheter are generally not considered to be a permanent solution, as the air can re-accumulate.⁷² However, in 2010 a method using the Foley catheter was published that would not allow further air accumulation and can therefore be a definite solution to treat the disorder. The key point is to insert the catheter into the pharyngeal opening of the auditory tube and fix it by inflating the balloon of the Foley catheter for four to six weeks. The intentionally caused pressure necrosis at the orifice permanently cures the affected foal and prevents re-accumulation by widening the pharyngeal ostium. This method is less time consuming and invasive than the surgical approach.⁷³ Successful interventions that are a permanent resolution of the airflow obstruction allow the affected foal to develop and grow without any restrictions or defects.⁷⁴

The genetic background of this disease has not been fully determined yet, but research points towards a polygenic, sex-linked inheritance.⁷⁵ The first study demonstrating the genetic background of GPT was published in 2004. To examine the pathway of inheritance, pedigree analysis of 27 purebred Arabians that were affected by the disease was performed. The analysis revealed that the cases originated from a total of four different families including 276 individuals. The fact that some of these horses shared common ancestors or were bred at the same stud farm supported the hypothesis of a genetic basis and allowed a genetic analysis. Complex segregation analysis was applied to determine the inheritance of the trait within the pedigree and significance tests for non-genetic, monogenetic, polygenetic and mixed monogenetic-polygenetic inheritance were performed. The results of these tests strongly indicated a polygenic or mixed-monogenic-polygenic mode of inheritance. Additionally the achievements of this study allow using breeding values of the parent generation to estimate

the incidence of an affected offspring and giving advice to breeders in order to avoid such matings that can result in a foal with GPT.⁷⁶

In 2009 a study about the whole genome scan for guttural pouch tympany in Arabian and German warmblood horses was published. The genome of 143 horses from five Arabian and five German warmblood families was scanned and 257 microsatellites were genotyped. The results revealed a chromosome-wide significant linkage on the chromosomes ECA2 for fillies and ECA15 for colts. Quantitative trait loci including two to four microsatellites on ECA2 in fillies and ECA15 in colts showed significant association with the disorder occurring in both Arabian and German warmblood horses. In conclusion, this study confirmed that a sex-linked inheritance can be strongly suspected. This article was the first description of a quantitative trait locus for GPT in horses and an important approach towards identifying the genetic background of the diseases.⁷⁷

A more recent study focusing on the genetic background of GPT was published in 2012 and revealed the identification of major gene loci for traits associated with GPT in the Arab and German warmblood horse. The objective of this study was to perform a genome-wide linkage and association analysis to identify target genomic regions and trait-associated SNPs of the GPT causing gene. A possible involvement of one major gene for GPT could be suspected after carrying out complex segregation analysis in both breeds. In order to locate the region of the potential major gene, genome-wide linkage and association analysis in combination with single nucleotide polymorphisms (SNPs) markers were used. The Illumina equine SNP50 beadchip was applied to genotype samples of 85 Arab and 373 German warmblood horses. A genome-wide significance was demonstrated on ECA3 for German warmblood horses and on ECA15 at 64 Mb for Arabians.

Linkage analysis with the Illumina equine SNP50 beadchip revealed a genome-wide significant peak on ECA15 and on ECA3 as the second highest peak in Arabians. Increasing the marker density to repeat the test on the two chromosomes could confirm the previous results for ECA15 but not for ECA3. The highest peak on ECA15 was observed at 64-65Mb, which also represented the most common haplotypes for affected foals, although it was not possible to identify a common homozygote haplotype among the affected foals.

The identification of breed-specific quantitative trait loci on ECA15 led to the further detection of two possible candidate genes. One is TTC27, tetratricopeptide repeat domain 27 which facilitates protein interactions. Commonly, Tetratricopeptid repeat motif (TPR) – containing proteins can be found in connection with multi-protein complexes. TPR motifs therefore may play an important role in the function of mechanisms involving cell-cycle, transcription or protein transport. The second candidate gene is BIRC6. Its expression is vital for the cell and inhibits apoptosis.

Focusing on the hypothesis that GPT is caused by a functional abnormality of the plica salpingopharyngeus, other candidate genes were taken into consideration, too. The chromosome ECA3 involves the candidate gene IBSP, integrin-binding sialoprotein, which influences cartilage formation, an important substance in the structure of the auditory tube and the medial aspect of the mucosal flap. Other candidate genes code for proteins which share similarities to zinc transporter proteins. Zinc is a co-factor for numerous enzymes that are involved in various mechanisms including carbohydrate and lipid metabolism, transcription,

growth and development and many more. Genes that are associated with these proteins are located on ECA3, SLC39A8 and on ECA15, SLC30A6.

This study can not only be considered as a great step towards the genetic understanding of GPT, but also demonstrates the facilitation of analyzing complex inheritance pathways by the equine SNP bead chip.⁷⁸

Future research will hopefully reveal the genetic basis of GPT to a degree that allows developing a DNA test for this disease and may even reveal the still not fully understood function of the guttural pouch in horses.

5.2 Occipito-Atlanto-Axial Malformation

Occipito-Atlanto-Axial Malformation (OAAM) is a genetic disorder causing neurological symptoms in Arab foals due to a malformation of the cervical vertebrae of the neck which compress the spinal cord. The malformation involves the occipital bone of the skull, the atlas and the axis⁷⁹ and is described as a familial occipitalization of the atlas and an atlantalization of the axis. The occipital bone and the atlas are fused at the base of the skull and the axis is not articulating with the atlas.⁸⁰ A study about the examination of 16 horses affected by OAAM combined with the results of three reported cases in 1986 revealed that all affected foals presented with a congenital atlantooccipital fusion, hypoplasia of atlas, malformation of the axis with hypoplasia of its dens and wings and a modification of the atlantoaxial joint frequently presenting as a subluxation.⁸¹ An uncommon variation is the duplication of the atlas. A case report published in 1989 describes a two week old part Arabian foal presenting with tetraparesis in connection with the presence of two atlases. The first atlas was fused with the occipital bone and the following duplicate articulated with the first atlas and axis. The axis had an elongated dens that was compressing the spinal cord. A further examination of the ossification center of the axis even raised suspicion of a partial duplication of the axis.⁸²

Clinical signs of the disorder vary greatly in severity and time of appearance. Foals can be stillborn, show signs at or a few weeks after birth or at several years of age if the malformation is only present in a mild form.⁸³ The mean age is considered to be below one month, which is a significant fact since OAAM is the only spinal cord disorder of the neck that occurs in horses at this age.⁸⁴

Foals can present with symptoms ranging from mild incoordination up to full tetraparalysis or even tetraparesis, hence they are unable to rise and nurse.⁸⁵ An unusual position of the head and neck, with the neck being extended and signs of neck twisting is associated with OAAM.⁸⁶ Affected foals also show symmetric ataxia and hypometria. Clinical signs are usually progressive due to the spinal cord compression and myelopathy caused by the malformation of the cervical vertebrae.⁸⁷ If the malformation affects the function of the brainstem, the foal presents with hypoventilation leading to an increased carbondioxid level in the arterial blood.⁸⁸

Examining a young Arab horse that presents with neurological symptoms as described should raise suspicion of the disease. A definite diagnosis is based on a throughout clinical examination and radiographs of the cervical vertebrae. Clicking sounds might be audible when moving the head and neck to examine motion of the joints and the flexion ability of the atlanto-occipital joint is limited. The malformation of the atlas and axis can also be detected

by palpating the cervical spine. It is important take radiographs of the neck region to rule out atlantoaxial fractures, as they can present like OAAM.⁸⁹

Once the diseased foal is diagnosed with OAAM euthanasia is usually the only option. In mild cases they can be kept as a pasture pet but breeding these individuals should be strongly discouraged.⁹⁰ Attempts have been made to stabilize the cervical vertebrae by fusing the axis to the atlas but the prognosis remains poor.⁹¹

Unfortunately, limited research has made only little progress in revealing the genetic background of this disease. Due to the lack of breeding trials, the mode of inheritance is not defined at present but with the given information an autosomal recessive inheritance is highly suggestive.⁹²

Interestingly, familiar OAAM is comparable to a syndrome in mice causing occipitalization of the atlas and atlantalization of the axis. These mice are homozygous for a mutation in the Hox-3 gene and that causes loss of its function. It is assumed that the Hox-3 gene influences the mesenchymal proliferation rates and therefore may be involved in the development of vertebral cartilage. The parallels between the presentation of the diseases in Arabians and mice indicate the Hox-3 gene as a candidate gene for OAAM. Hopefully future research will reveal more information about the genetic background of this genetic disorder.

5.3 Juvenile Idiopathic Epilepsy

Epilepsy is defined as a disorder of the brain that causes recurrent seizures, precisely more than two seizure incidences⁹³, for which no other underlying cause can be identified.⁹⁴

There is only little information in the literature that describes epilepsy in horses. In comparison to humans or companion animals affected by this disease, epilepsy in horses is not explained in a way that allows clinicians to use uniformly systematic terms for their diagnosis.

Therefore Dr. Lacombe performed a study at Ohio State University where 104 horses that showed clinical signs of epilepsy were examined between 1988 till 2009. The goal was to establish a classification system for seizures in horses which supports veterinarians to make a more precise diagnosis and prognosis for the case and helps to adjust their treatment plan. Only horses that had suffered from more than two seizures before presenting at the clinic were used for this study and 70% of the 104 horses were diagnosed with epilepsy.⁹⁵ Methods used for diagnosis included the history of the animal, observation of clinical signs, diagnostic examinations with electroencephalography (EEG) and computer tomography (CT), cerebrospinal fluid (CSF) analysis and post mortem examinations. Findings that indicated epilepsy were a normal neurological exam, occurrence of seizures for which no trigger could be identified and a paroxysmal epileptiform activity on the EEG.

35.6% of the horses diagnosed with epilepsy showed a pathological brain lesion and were classified into the category called symptomatic epilepsy. For 54.8% of the cases no underlying cause could be identified. Those were grouped into the category called cryptogenic epilepsy. If the background of the epilepsy was likely to be a genetic predisposition, the case was classified as idiopathic epilepsy which made up 2.7% of the studied horses.⁹⁶

This study also revealed that horses suffering from full seizures were less likely diagnosed with epilepsy than cases presenting with partial seizures which are triggered by a localized

area in the brain. Furthermore, a classification called ‘reactive seizures’ was established that includes seizures which are caused by an underlying systemic disease, for example hepatic encephalopathy, in the presence of normal brain function.⁹⁷

Juvenile Idiopathic Epilepsy (JIE), also called Idiopathic Epilepsy (IE) or Juvenile Epilepsy Syndrome (JES), is a non fatal neurological genetic disorder in Arabian foals⁹⁸ and can predominantly be seen in the Egyptian bloodline⁹⁹, although cases have been reported in all important bloodlines.¹⁰⁰ The disease is self-limiting and signs disappear at the age of one to two years. The genetic abnormality causes an alteration in the electrical activity of the brain leading to seizures that start to occur in a time interval ranging from several days after birth up to six months. Affected foals are born normal and also show a normal mental status between the reoccurring seizure episodes.¹⁰¹

Seizures are divided into three different phases, the aura which precedes the seizure, the ictus which is the time span of the seizure and the postictal phase after the seizure.¹⁰²

Foals or young horses suffering from JIE often show signs of injuries which they acquired during a seizure as a consequence of the loss of control over body movements and sometimes consciousness. These animals are also a safety hazard to their surroundings depending on the severity of the seizures. In mild cases the owner often does not notice the seizure activity itself but detects only the secondary injuries or the individually consistent signs of the aura and postictal phases.¹⁰³ Postictal clinical signs can include depression, prolonged blindness, confusion, head pressing and an absent suckling reflex. Consequently, aspiration pneumonia and head trauma¹⁰⁴ are commonly seen as a complication of JIE and it is advised to avoid suckling till the foal has recovered from the postictal phase.¹⁰⁵

During seizures general clinical signs like urination, defecation, sweating, clamping of the jaw or eye rolling can be observed. Generalized seizures are characterized by loss of consciousness, falling to the ground and a typical involuntary muscle activity presenting as a tonic phase involving rigid muscles which is succeeded by rapid muscle contractions and relaxations called the clonic phase. Partial seizures can include a wide range of clinical signs including limb twitching, facial or eye lid twitching and chewing motions.¹⁰⁶

Foals over three months affected by JIE commonly suffer from “cluster seizures”, which is a term for several seizures occurring over a period of three days. They manifest in clinical signs associated with partial seizures.¹⁰⁷

Diagnosis of JIE is done by exclusion of any other underlying cause. An Egyptian Arab foal suffering from recurrent seizures strongly hints towards JIE but it is crucial to rule out any conditions that trigger seizures for a definite diagnosis. A list of diseases that should be included in the differential diagnosis can be found in table 5.¹⁰⁸

Especially if the foal does not present with generalized seizures, it is difficult to establish a definite diagnosis. Using an EEG can be a helpful tool, although there is little information about normal EEG findings in horses. The EEG allows displaying and recording the electrical activity of the brain, mainly of the cerebral cortex. Abnormal findings that indicate a disruption of the electrical activity in the brain present as spikes, sharp waves or spike and wave discharges of the three measured brain waves which include alpha, beta and delta waves.¹⁰⁹ In contrast, performing a CT or taking radiographs of the skull do not show

abnormalities in case of JIE. As head trauma is a common secondary finding due to JIE and can be identified through these imaging tools. Further diagnostic approaches need to target the question if the trauma cause or result from the seizures.¹¹⁰

Table 5; Differential diagnosis for JIE¹¹¹

Disorders causing seizures	Alteration in brain function	Diagnostic methods
Head trauma	Swelling of the brain	x-ray, recent trauma history
Brain tumor	Pressure on the brain	x-ray, CT, MRI
Electrolyte disturbance	Disturbance of chemical signals	Blood chemistry
Equine protozoal myeloencephalitis	Mechanical trauma due to migrating parasites	Positive CSF test and/or response to treatment
Drugs: intracarotid injection of Xylazin, Procain Penicillin injection	High drug concentration in the brain	Seizures appear instantly after injection
Metabolic disorders e.g. liver disease	Toxins are not metabolized by the liver and enter brain	Blood tests and liver biopsy
Viral diseases e.g. Rabies, West Nile virus	Virus attacks brain	Blood or CSF tests
Toxins	Disrupt chemical signals in the brain	Access to e.g. brackenfern, organophosphates, lead etc.
Meningitis, Meningoencephalitis	Inflammation within the CNS	CSF analysis
Strangles	Brain abscess	Culture of abscess swap
Neonatal maladjustment syndrome	Lack of oxygen during birth	Typical appearance of the foal
Heat stroke or fever due to an infection not located in the CNS	Increased temperature	Measuring rectal temperature, blood tests

JIE can usually be managed with administration of individually adjusted anti-seizure medication.¹¹² The most commonly used drugs are diazepam for short term treatment and phenobarbital for long term treatment.¹¹³ Caution should be taken in establishing the right treatment plan and to apply medication as soon as possible, as a lack of those actions can lead to the death of neurons and permanent seizure foci. Addressing any events that could trigger a seizure is also crucial.¹¹⁴

The genetic background of JIE is not fully understood but studies conducted at the University of California, Davis indicate an autosomal dominant pathway of inheritance. Research projects performed in 2010 analyzing the genetic basis of Lavender Foal Syndrome (LFS) discovered a possible genetic link between these two genetic diseases.¹¹⁵ As both disorders frequently appear within the same families, JIE could be a non-fatal phenotype variation of Lavender Foal Syndrome carriers. This hypothesis is supported by observing a comparable syndrome of JIE in mice with the same mutation that has been identified to cause LFS in horses.¹¹⁶

A more recent study published in 2012 discovered similarities between JIE and benign-familial neonatal convulsion syndrome (BFNC) in humans, an idiopathic form of epilepsy in newborns. As it is suggested that the origin of JIE is of a genetic nature, and significant phenotypic analogies between both disorders could be observed, the hypothesis that JIE is caused by the same mutations as BFNC was established.

BFNC occurs due to mutations in the KCNO2 and KCNO3 genes coding for voltage-gated potassium channel subunit. In horses, these genes can be found on chromosome 22 (KCNO2) and 9 (KCNO3). These locations are quite similar to the one found in humans. Gene trees for the two genes in question were drawn between horses, mice and humans and compared to approved phylogenetic trees of mammals. The result showed that the calculated distance values between horses and humans were lower than between horses and mice. The KNCO2 gene tree in particular revealed a close relation between horses and humans. This was a significant finding, as the majority of the mutations associated with BFNC can be found on the KNOC2 gene and would justify the hypothesis suggesting a common genetic abnormality.¹¹⁷

Proving this assumption is still subject to current research projects, but now that the genetic cause of LFS is fully identified the potential connection to this disease might reveal more information about JIE.¹¹⁸

5.4 Severe Combined Immunodeficiency

Severe Combined Immunodeficiency (SCID) is a lethal genetic disorder of Arabian foals causing a lack of humoral and cell-mediated immune response. SCID can be seen in all Arabian bloodlines and can also affect Arabian cross-breeds.¹¹⁹ SCID can also be seen in humans, dogs and mice and one case was reported in an Appaloosa horse.¹²⁰ The disease was first described by McGuire and Poppie in 1973 and in 1980 the autosomal recessive pathway of inheritance of this disease was discovered by performing test breeding at Washington State University.¹²¹

The genetic abnormality that accompanies SCID prohibits a functional B and T-lymphocyte maturation in the affected horse and is therefore classified as a primary immunodeficiency. Foals affected by SCID are born normal.¹²² The onset of clinical signs depends on the maternal immunity, hence the colostrums supply and quality and the foal's maternal antibody elimination rate. Symptoms of a compromised immune system can be seen from approximately two weeks of age, but the onset can be as late as two months post partum. SCID affected foals are highly susceptible to opportunistic infections commonly involving the respiratory tract and are even less resistant to intracellular bacteria and protozoa infection while still under the protection of the maternal antibodies due to their lack of T-cell function.¹²³

Opportunistic pathogens that can cause a disease in an affected foal include parasites like *Cryptosporidium parvum* and *Pneumocystis carinii*, which invade the gastrointestinal and respiratory tract, respectively. The bacterium *Rhodococcus equi* colonizes in the lungs and is one of the causative agents of equine bronchopneumonia, which is frequently observed in SCID patients. Adenovirus infection is the most strongly equine SCID associated pathogen resulting in bronchopneumonia, often with involvement of the gastrointestinal and urogenital tract and subsequent pancreatitis which leads to retarded growth and weight loss due to the impaired endocrine and exocrine function of the pancreas. These disorders are frequently observed in SCID affected foals among others like arthritis and omphalophlebitis.

On physical examination nasal discharge, cough, dyspnea, diarrhea and fever can be observed. Diagnostic examination of affected foals includes a complete blood cell count (CBC) and serum immunoglobulin measurement. The CBC shows an absolute lymphopenia with levels below 1000 lymphocytes/ μ L blood and occasional neutrophilia due to bacterial

infections. If the colostrums supply was adequate, the total serum globulin and serum IgG concentration can be normal in affected foals, but the level of these parameters will steadily decline. Blood samples of presuckling and two to four weeks old SCID foals don't have measurable IgM levels. It is important to use blood samples obtained during these time intervals only, as the foal's serum will contain maternal IgM after receiving colostrum. Blood samples of healthy presuckling foals will contain IgM as they are produced by the unborn foal from day 180 of gestation.

Diagnosis is based on clinical signs, lymphopenia, the lack of IgM and the age and breed of the affected foal.¹²⁴ Necropsy also reveals some characteristic features which were already described in the first publication of the SCID syndrome. McGuire and Poppie performed a post mortem examination on two full sibling Arabian foals affected by SCID and could detect macro- and microscopic evidence of the impairment of both B and T-lymphocyte systems. Hypogammaglobulinemia, lymphopenia and the absence of germinal centers in the lymph node cortex indicated a malfunction of the B-lymphocytes. The findings pointing towards a T-lymphocyte impairment included the complete absence of thymic tissue in one foal and the lack of thymic dependent lymphocytes in the spleens of both horses. Signs of opportunistic infections like bronchopneumonia were noted, too.¹²⁵ Hence, necropsy findings that are associated with SCID are hypoplasia of spleen, thymus and lymph nodes with the lack of normal lymphoid structure.¹²⁶

All attempts to treat SCID affected foals have not been successful and they usually succumb to opportunistic infections by the age of five months. One reported case of a 32 days old foal that was treated with a bone marrow transplant from a full sibling reached the age of five years and died due to a disorder that was not in relation with SCID. Out of ethical and humane reasons, the diagnosis of SCID in a foal indicates euthanization of the affected individual.¹²⁷

Before the DNA test for SCID was developed in 1997¹²⁸ the only way to identify carriers of the mutant allele was to perform progeny testing during which for example a stallion with an unknown status was bred to a known carrier mare. This method is not only very time consuming, but also involves great financial expenses and results in affected unviable foals. Therefore the DNA test is a great improvement in identifying carrier animals compared to this impractical method.¹²⁹

The genetic abnormality responsible for the inability of a SCID affected horse to mount an immune response is a mutation on chromosome 9. The location of the mutation affects the coding sequence for the protein DNA-protein kinase catalytic subunit (DNA-PKCS), which is part of the enzyme DNA-protein kinase (DNA-PK). This enzyme is necessary for the formation of immune molecules.¹³⁰

The biochemical background of the importance of this enzyme is the arrangement of variable (V), diversity (D) and joining (J) genes during early lymphoid differentiation. The V(D)J rearrangement is crucial to form coding sequences for the variable regions of immunoglobulin antigen binding sites and T-cell receptors. Random arrangement of those genes results in mature lymphocytes that are able to mount a specific immune response to individual pathogens. The V(D)J rearrangement includes two double-stranded DNA cuts followed by religation to form new DNA joints and is dependent on recombinase activating gene products and a DNA double strand break repair mechanism (DSBR) which is carried out by the DNA-

PK. This information led researchers to the suggestion that SCID affected foals suffer from a defect that impairs the process of V(D)J rearrangement¹³¹ and therefore inhibits lymphocyte maturation and subsequent degradation of immature cells.¹³²

A study published in 1995 identified DNA-PKCS expression deficiency as the causative mechanism of SCID in horses by comparing cases of equine and murine SCID. Parallels found between SCID in Arab foals and mice with the SCID mutation were the decreased number of B and T-lymphocytes with normal cell count of natural killer cells in both species. Further investigation included analysis of V(D)J recombination, radiation sensitivity, DNA end-binding activity, DNA-PK activity and DNA-PKCS expression. Tissue from SCID affected and SCID clear horses was used. The most significant results showed that the defective factor is necessary for the V(D)J recombination process and that the DNA-PK enzyme activity was absent in samples of affected foals. This led to the conclusion that the defect seemed to involve the DNA-PKCS expression. As the deficiency in the expression of this protein is more severe in horses than in mice, researchers implemented that SCID in horses and mice are caused by different mutations.¹³³

The study that led to the identification of the equine SCID mutation and generation of a DNA test continued the comparison of SCID in horses and mice. Previous studies had already demonstrated that foals affected by SCID are incapable of forming both, coding and signal joints. In contrast, SCID affected mice showed a normal signal joint formation. As the protein DNA-PKCS, which had been identified as the defective factor in both species, was still measurable to some degree in mice and was completely absent in affected horses, scientists analyzed the involvement of DNA-PKCS in each of the two steps of the V(D)J rearrangement individually and came to the conclusion that the enzyme is involved in both processes. This explained the phenotypic difference between these two species, as the defect in DNA-PKCS affected both the lymphocyte-specific component of the recombination and the DSBR factor and therefore caused a more severe disease in horses which completely lacked a DNA-PKCS expression.

In order to identify the location of the mutation that causes SCID in Arabian foals, researchers hypothesized that the genetic defect causing the disease is localized in a single gene. The facts that led to this conclusion are that the mutation causes a defect in the V(D)J recombination process and that cells derived from a SCID affected foal are hypersensitive to radiation causing DNA damage, as the absence of DNA-PKCS impairs DNA repair.

Subsequently cell lines derived from affected foals were analyzed. Cloning and sequencing of DNA-PKCS transcripts demonstrated that the cells were hypersensitive to ionized radiation, had no measurable DNA-PK enzyme activity or DNA-PKCS levels, and could not carry out artificially induced V(D)J recombination. The next step was to compare conserved protein kinase motifs by using human and equine amino acid sequences of DNA-PKCS transcripts. This comparison revealed a five nucleotide deletion at amino acid 3155, which equals the assumptive location of the DNA-PK gene.

1: AG.....TCATCTCA AATTCCCCTTAA...
2: AG.....TCA AATTCCCCTTAA

Picture 8: DNA-sequence of a healthy (1) and SCID affected horse showing the 5 nucleotide deletion and premature stop codon in red (2).

To confirm this finding RT-PCR and genome sequencing of this DNA-sequence was performed and the same five nucleotide deletion could be detected. This mutation leads to a shift in the base-triplet reading frame of the translation process, forming a premature stop codon that terminates the protein synthesis.

The same alteration of the genetic material could be detected in all examined samples of SCID affected Arabian foals. The absence of the mutant DNA-PKCS protein in Western Blot analysis led to the suggestion that the alteration of the protein forms a very unstable product, which was supported by the detection of degradation products of the DNA-PKCS protein.

In conclusion this research confirmed the hypothesis that the equine and murine SCID is caused by different mutations and revealed the specific alteration in the equine genome, although the scientists involved in this study expressed, that other possible mutations within the non-sequenced sections of the DNA-PCKS allele could not be excluded.¹³⁴

The results of this study were the basis for the development of the DNA-test at the University of Texas in 1997.¹³⁵

5.5 Cerebellar Abiotrophy

Cerebellar Abiotrophy (CA) or Cerebellar Cortical Abiotrophy is an autosomal recessive genetic disease involving the nervous system and can be also seen in dogs, cats and ruminants.¹³⁶ Affected horse breeds besides Arabians of all bloodlines¹³⁷ and Arabian crossbreeds are Oldenburger, Miniature ponies, Gotland and Eriskay ponies.¹³⁸ This genetic disease is the most common disorder of the cerebellum in horses and Arabian horses in North America are most affected. In contrast to the Oldenburger, where the disease is progressive and has a fatal outcome, CA is described as a syndrome that stabilizes by reaching adulthood in Arab horses.¹³⁹ Test breeding performed at the genetics laboratory of the University of California, Davis revealed the autosomal recessive inheritance pattern of this disorder in the 1980s. This discovery was the breakthrough for further investigation of this disease that used to be considered a cerebellar hypoplasia up to this point.¹⁴⁰ A more recent study on the mode of inheritance of CA was published in 2011 and confirmed the previous finding, that this disease is inherited through a single gene Mendelian autosomal recessive pathway.¹⁴¹

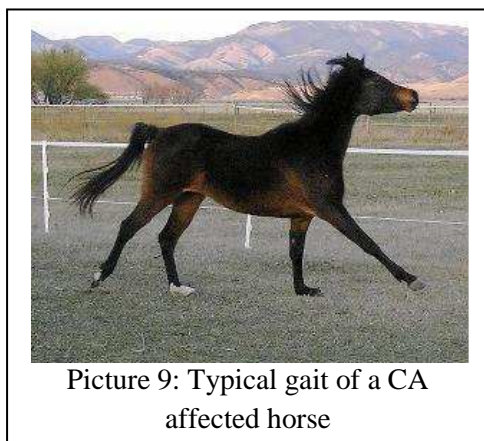
The alteration of the genome associated with CA leads to a premature degeneration and apoptosis of the Purkinje cells in the cerebellum after birth.¹⁴² In contrast, cerebellar hypoplasia is due to loss of Purkinje cells in utero.¹⁴³ The function of the cerebellum is to coordinate the motor system and is connected to ipsilateral body functions. The cortex of the cerebellum contains thin transverse folds, called folia, and is made up by three layers. The superficial or molecular layer contains granular cells. The deeper Purkinje layer is connected to other parts of the cortex through the axons of the Purkinje cells. The third innermost layer is called granular layer where basket and stellate cells can be seen.¹⁴⁴

Purkinje cells are involved in the afferent pathway of signal transmission. A signal input stimulates the mossy fibers and the climbing fibers of the cerebellum, which conduct the signal to deep cerebellar nucleus neurons provoking an excitatory effect. The second

conduction pathway of the mossy fibers is a signal transmission through fibers that branch as they enter the cortex and synapse with granular cells. The axons of granular cells then stimulate the Purkinje cells by crossing them at the location of the folia. Here, millions of granular cell axons come together to form parallel fibers that transmit the signal to the Purkinje cells. The axons of the Purkinje cells then transmit the signal to deep cerebellar and vestibular neurons, provoking an inhibitory effect. Therefore a stimulation of the mossy fibers provokes both an excitatory and, through stimulation of the Purkinje cells, an inhibitory stimulus. This ensures that the excitatory stimulation of the motor activity results in an appropriate output regulated by the inhibitory function of the Purkinje cells.

Climbing fibers receive stimuli from the inferior olivary nucleus, an area that detects faulty movements, and synapse with the Purkinje cells. This results in an inhibitory effect on the deep nucleus neurons in order to stop the unwanted action detected by the olivary nucleus.

Consequently if Purkinje cells are damaged the individual shows abnormal sensation of space and distance and has difficulties in balance and coordination.¹⁴⁵



Picture 9: Typical gait of a CA affected horse

Arabian foals affected by CA appear normal at birth and start to show signs from the age of one to six months on.¹⁴⁶ Clinical signs include an intentional head tremor that does not include the neck and fitful head movements during motion. Affected foals lack proper proprioception, balance and depth perception. They have difficulties in correctly estimate speed and distances, and show poor control over the correct placement of their feet. In rest they present with a characteristic wide-legged stance¹⁴⁷ and show ataxia during movement with hypermetric activity of the

front limbs¹⁴⁸, which can also be described as “goose-stepping”. Noticing a decreased or absent menance reflex¹⁴⁹ in CA foals needs to be assessed with caution as this reflex is only fully developed in foals reaching 7-10 days of age.¹⁵⁰ A good approach would be to compare the foal suspected to suffer from CA to normally developing foals of the same age.¹⁵¹ The neurological abnormalities are symmetric and worsen when the horse is blindfolded and asked to step over an object. In contrast, they present with normal spinal reflexes and show no muscle weakness.¹⁵²

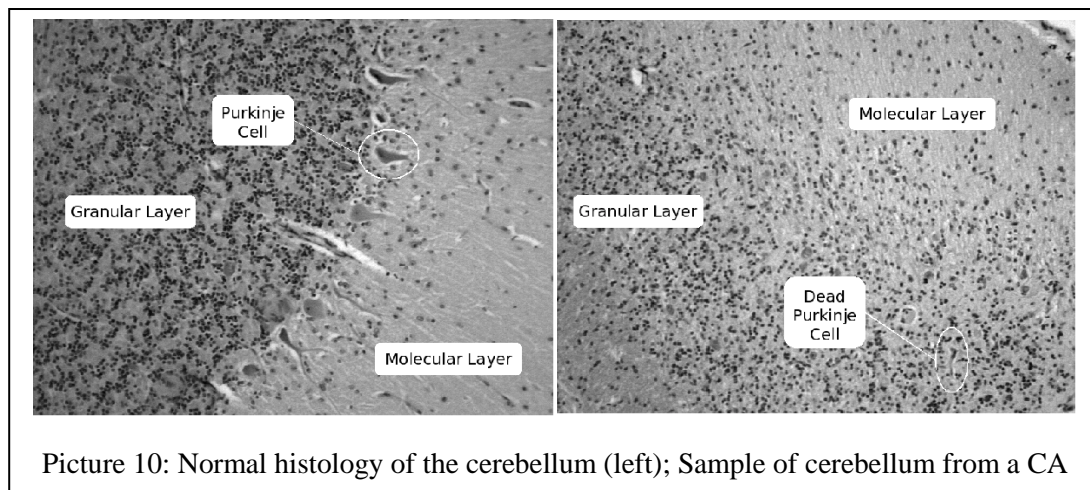
Affected foals are easily startled and tend to injure themselves and others by falling and uncoordinated movements.¹⁵³ As a consequence they are often euthanized before reaching adulthood although the disease itself is not fatal and affected animals can be kept as a pasture pet. Their condition sometimes even improves after stabilizing¹⁵⁴ and older horses orient themselves on another horse they have a close relationship with.¹⁵⁵

It is important to mention that the severity and onset of clinical signs varies greatly among affected animals. One reported case involved an affected mare that started to display symptoms at the age of five years.¹⁵⁶ Furthermore, with the development of the DNA-test for CA, researchers at UC Davis Veterinary Genetics Laboratory were confronted with a new discovery in the CA expression that needed to be addressed. A small group of horses that tested positive for the CA homozygote phenotype did not show any clinical signs of the

disease. This phenomenon is subject to current research, which will hopefully disclose the full genetic background of CA.¹⁵⁷

Diagnosis of the disease is based on exclusion of other neurological disorders like Wobbler syndrome, which is caused by a malformation of the cervical vertebrae, or head trauma.¹⁵⁸ An exposure to organophosphates in utero¹⁵⁹, cranial malformations, congenital spinal malformations and inflammation of the cerebellum also need to be ruled out. Diagnostic analysis of the CSF can show increased levels of total protein and the enzyme creatinine kinase, which is associated with neuronal degeneration. Both of these parameters are no diagnostic indicators for CA and often normal values are found during analysis of the CSF of affected patients. Radiographic imaging can be used to rule out head trauma or Wobbler syndrome, but is not a useful diagnostic tool to diagnose CA.

The clinical signs, the age and the breed of the affected animal can direct the diagnosis towards CA, but a definite diagnosis needs confirmation through performing a histopathological examination of the cerebellum. The histological picture of CA is characterized by a generalized loss of Purkinje cells with degenerative neuronal changes including shrunken, angular and hyperchromatic cells. Dispersed Nissl's substance and an overall thin granular layer with a decreased cell count can be observed.¹⁶⁰



Picture 10: Normal histology of the cerebellum (left); Sample of cerebellum from a CA

The genetic background of CA has been the subject of several research projects and is now understood to a degree that allows the development of genetic tests for CA. A DNA test replacing the indirect marker based screening test could be developed 2010 as a result of the successful mapping of the candidate mutation to a microsatellite marker at the location of two overlapping genes on ECA2.¹⁶¹

The study that led to these achievements was performed at University of California, Davis. A whole genome scan of four Arabian horse families affected by CA allowed mapping the disease to the p-arm of ECA2. This region was narrowed down to 142kb by developing a marker for this region and analyzing the homozygosity in affected foals. Four potential causative genes could be identified at this location which includes MUTH and TOE1. Both genes were further investigated and complete sequencing revealed 22 mutations which could not be associated with CA. Only one SNP located on exon 4 of TOE1 was exclusively seen in

Arabians and confirm with the CA trait. This mutation causes an amino acid substitution and is also close to the MUTYH gene on the opposite strand and next to the transcription binding site for the factor GATA2. This finding was followed by a qPCR analysis of cerebellar RNA samples from normal and affected horses which showed a decreased MUTYH expression in CA affected individuals. The identified SNP possibly affects the binding affinity of GATA2 and therefore the regulatory function of MUTYH.

The detailed process which led to this hypothesis started with the gene sequencing of the CA region of 142kb. This region included four known genes, TESK2, HPDL, TOE1 and MUTYH which made up 46.7kb of the region. Only the last two were considered to be expressed in the brain. The DNA that held those four genes was completely sequenced including exons, introns using samples from affected and healthy Arabian horses in order to search for polymorphisms. Any mutation that could not be detected in the control sample and was homozygous in CA affected samples was subject to additional genotyping. A total of 22 mutations were identified out of which only one could not be detected in all control samples. This SNP called ECA2:13074277 could be located on exon 4 of TOE1 and causes a substitution of histidine for arginine through a base change from guanine to adenine. The significance of this genetic alteration on the protein structure was questionable as both amino acids carry a positive charge. However, further investigation reveal that exactly this region shows a high level of evolutionary conservation and that a mutation in this area is likely to have harmful effects. An additional interesting finding was that the 5'-end of TOE1 gene overlaps with the 5'-end of MUTYH gene which allows transcription of both genes from alternate strands of partially overlapping DNA sequences. Also, the mutation followed the location of the GATA-2 site which could explain the possible influence of on the regulatory function from MUTYH.

Analysis of RNA samples from affected and control animals showed that the MUTYH expression not only shows different values in affected and control samples but also differs according to the age of the animal. A general increase with age could be measured in both sample groups but the levels of the affected horses were overall lower than the control samples.

The results of these investigations left researchers with two possible explanations. One possible consequence of the mutation could be the harmful effect on the protein function through the base exchange on the TOE1 gene, which is a gene target of Early Growth Response 1, a regulator for transcription. The protein encoded by TOE1 takes part in the regulation of the cell cycle regulation.

The second possible conclusion includes the assumptive regulatory effect of the mutation on the MUTYH gene expression which codes for an enzyme called DNA glycosylase. This enzyme removes incorrectly added adenine residues located opposite of 8-oxo guanine which is a waste product of oxidative damage. The expression of MUTYH was significant in the cerebellum and its Purkinje cells with high expression in cell nuclei of embryos and mitochondria in adults whose neuron proliferative processes and mitochondrial maturation had been finalized. The functions of MUTYH involve DNA-repair in nuclei of rapidly dividing Purkinje cells of growing individuals and repair of oxidative damage of mitochondrial DNA in adults. The proximity of ECA2:13074277 to the suggestive binding

site for the transcription factor GAT-2 supports this hypothesis of an influence on the regulatory effect on the MUTYH gene. GAT-2 is predominantly expressed in cells localized in a proliferative area of the brain and is therefore associated with neuron formation.

The final conclusion left the team with the hypothesis that close location of the mutation to GATA-2 causes a lower level of MUTYH. This would also explain the variations in the clinical presentation of the disease as the impact of oxidative damage differs in individuals. Another explanation for the great differences in the phenotype suggests the presence of a suppressor mutation, which might compensate the defect caused by the CA mutation.¹⁶²

This hypothesis is subject to current research conducted at UC Davis, where scientists work on the genetic background for asymptomatic affected horses. Objective of this study is to perform a whole genome scan using the Equine SNP chip on affected, clear and the so called “reportedly asymptomatic” horses. First results confirmed the previous findings on the designated location of CA within the genome but could not identify specific variations in the genome of affected horses without clinical signs. Based on this result, the current investigation focuses on identifying new genome regions that might influence the clinical picture of CA.¹⁶³

Not only the phenomenon of “reportedly asymptomatic” horses is under current investigation, but researchers also currently analyze the precise function of the candidate genes MUTYH and TOE1 with respect to the impact of the identified mutation for CA by conducting expression studies. This research project might allow a deeper understanding of the triggering event for the degeneration of Purkinje cells and will hopefully reveal a definite confirmation of the mutation causing CA.¹⁶⁴

Regardless of the precise mechanism, the identified mutation on ECA2 made the development of a direct DNA-test possible and is therefore an outstanding success in managing Cerebellar Abiotrophy.

5.6 Lavender Foal Syndrome

Lavender Foal Syndrome (LFS), also called Coat Color Dilution Lethal (CCDL), is a fatal autosomal recessive genetic disease that affects the nervous system and presents with a diluted coat color. Arabian foals of the Egyptian bloodline and breeding lines that include a high percentage of the Egyptian bloodline are severely affected by LFS, although the disease also occurs in other major bloodlines of the Arab horse.¹⁶⁵



Picture 11: LFS affected foal

This rare disease¹⁶⁶ was first described by Dr. Bowling in 1996. Affected foals show neurological clinical signs immediately after a commonly difficult birth due to their large size¹⁶⁷. They are unable to rise and remain in lateral recumbency¹⁶⁸ while suffering from tetanic episodes which present as a rigid extension of the legs, neck and back. Although showing similarities to a seizure episode, these episodes cannot be classified as a real seizure

as the foal does not appear normal during the incidences and the mental state is generally not

affected during a tetanic episode. LFS affected foals are often observed in a position called opisthotonus which is characterized by a caudally drawn head and neck and rigid, extended limbs. Nystagmus and paddling movements of the legs, which are suggested to be either due to the efforts of the foal to rise or due to partial seizures, are frequently observed clinical signs, too. Although the viability of a LFS affected foal is severely compromised by this disease, foals still express a strong suckling reflex.

One of the most characteristic features of LFS is the telltale diluted color of the hair coat that can be seen in the majority of affected foals which are often born with a lavender, pale pink or silver hair coat. Attention must be paid in case of grey or roan colored foals where the color dilution might differ. Interestingly not only the color of the hair coat but also the eye color is of a grayish brown or a bluish shade.¹⁶⁹

Diagnosis is based on the typical clinical signs, the breed and the age of the affected foal. Important findings that should point a clinician's diagnosis towards LFS are that these foals do not improve on anticonvulsant medication and have normal values on blood sample analysis.¹⁷⁰ For a definite diagnosis it is necessary to rule out other disorders that present with similar clinical signs, as not all foals are born with the pathognomic diluted hair color. Neonatal maladjustment syndrome or "dummy foal" is caused by a lack of oxygen during a difficult birth. As this disorder shows some common feature to LFS in its clinical pictures and due to the fact that the birth of LFS affected foals is usually accompanied by dystocia, FLS are often misdiagnosed as "dummy foals". Furthermore should spinal cord injury and encephalitis¹⁷¹ be included in the list for differential diagnosis.¹⁷²

There are no treatment options for LFS affected neonates and euthanasia is indicated out of humane reasons since these foals are not viable.¹⁷³

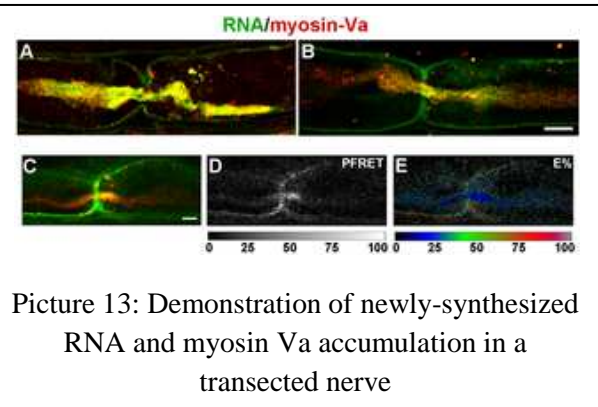
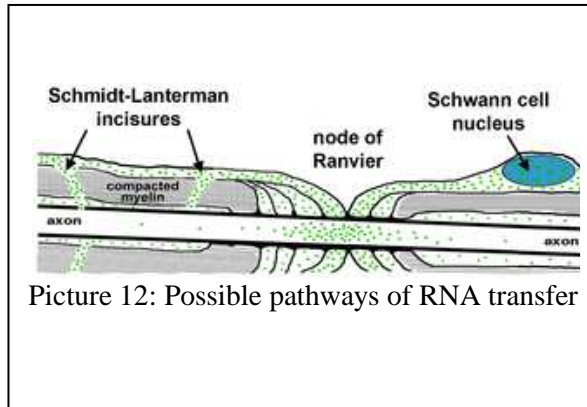
In 2008 scientists from Cornell University and the Arabian Horse Foundation founded a project that focused on the location of the mutation responsible for LFS using the information gained from the Horse Genome Project. This study is an exemplar for genetic research as it represents the first successful whole genome SNP scan for any trait by identifying the causative mutation of LFS as a single base deletion on the gene MYO5A, which is located on chromosome 1 and codes for the protein myosin Va.¹⁷⁴ The achievements of this research led to the development of a DNA-test for LFS at the Cornell University in 2010.¹⁷⁵

The myosin Va protein can be found in neurons and melanocytes and is necessary for several intracellular transport processes. It acts by using long filaments of actin as pathways to transport molecules.¹⁷⁶ The LFS mutation affects of the protein's binding region for transport material and therefore interferes with normal neuron and melanocyte activity.¹⁷⁷

In melanocytes the myosin Va protein transports melanosomes, which are responsible for melanin production, the pigment in skin, hair and eyes. Melanosomes are transported to the cell border and distributed to pigmented areas. For this process myosin Va forms a transport complex with proteins derived from MLPH and RAB27A genes.¹⁷⁸

A study published in 2013 identified the role of myosin Va in neuron function through investigating the protein synthesis in axons. The objective of this research was to identify whether the location of the mRNA transcription takes place in the neuron cell body or in glia cells or if the process involves both cells. The translation process was known to take place in the axon.

Trials on transected neurons showed an accumulation of newly synthesized RNA in the proximal part of the neuron. The origin of this RNA was determined to be from Schwann cells by marking RNA in the absence of neuronal cell bodies. The best route for this RNA, originating from Schwann cell nucleus, to reach the axon was suggested to be through the node of Ranvier or Schmidt-Lantermann incisures and requires the factors F-actin and myosin Va. The involvement of myosin Va was proven by investigating nerves of mice lacking the MYO5A gene.



The result was that the nerves of these MYO5A negative mice showed no newly-synthesized RNA accumulation in their axons. This observation led to the conclusion that not only is myosin Va a crucial component of the RNA transfer in nerve cells, but also that this transfer mechanism might be comparable to the pigment transfer from melanocytes as MYO5A negative mice also had a diluted coat color. The results of this study are outstanding since it not only allows a better understanding of the neuronal dysfunction seen in LFS but also hints towards a possible future treatment option for nerve damage. During this work a cell-to-cell RNA transfer was accidentally induced and the results indicated that gene therapy of damaged nerves can be carried out by application of the therapy to glia cells or implanted stem cells at the site of the injury to support regeneration.¹⁷⁹

The detection of the genetic mutation was published in 2010 and dealt with the investigation of candidate genes which were chosen on the basis of showing similarities of the disease occurring in horses, mice and humans.¹⁸⁰ The syndrome occurring in humans is called Griscelli Syndrome and is characterized by a hypopigmentation of the skin, silvery grey hair, neuronal deficits and seizures.¹⁸¹ The severity of this syndrome depends on which protein of the transport complex encoded by the genes MYO5A, RAB27A and MLPH is affected by the mutation. As patients with a mutation in MYO5A predominantly presented with neuronal clinical signs, it was chosen as the primary candidate gene for LFS followed by RAB27A.

Pedigree analysis was indicated due to the small number of LFS affected samples and to obtain control samples of healthy horses. The pedigree of six affected foals was investigated and revealed one common ancestor in all six test individuals. Whole genome scan of these six foals and 30 family members led to the discovery of one significant region on chromosome 1 which included 14 significant SNPs. Another finding was that chromosome 1 of all six affected foals included unique haplotypes for the region in question with 27 common SNPs. This area included a 1.6Mb region which was homozygous for affected foals and

heterozygous for carrier relatives and included the MYO5A gene but not the RAB27A gene. This finding confirmed the assumption of the MYO5A gene being the primary candidate gene and also demonstrated the effectiveness of the SNP chip which was used for the whole genome scan.

PCR amplification and sequencing of the 39 exons of the MYO5A gene in one sample of an affected foal identified a single base deletion on exon 30 resulting in a premature stop codon. This finding was confirmed by repeating the test on samples from a second foals and its relatives with the same result. Further studies of the myosin Va wild type led to the conclusion that this mutation is likely to cause severe damage, as the protein is highly conserved.

A subsequent PCR-base Restriction Fragment Length Polymorphism (PCR-RFLP) assay with Fau I restriction enzyme was designed to detect the deletion. The presence of the deletion eliminates a Fau I site which changes the normal pattern. This test was used to perform frequency analysis of the mutant gene. Results showed a uniform homozygous test result for affected horses and a high number of heterozygous individuals in their family. Egyptian Arabians which did not originate from the same pedigree showed a significant carrier percentage of 10.3%.¹⁸²

In conclusion, this study revealed the specific mutation responsible for LFS by using the latest genetic tools and might also be the basis on future research on the genetic link between LFS and JIE.

6. Available tests for SCID, CA and LFS

6.1 Testing and sampling methods

DNA-tests for these genetic diseases were developed to identify horses that carry the mutant allele. As all three testable diseases are inherited through and autosomal recessive pathway it is crucial to avoid carrier matings in order to prevent the appearance of affected foals. Tests can be also used to make a definite diagnosis for an affected horse.

Severe Combined Immunodeficiency

The test for SCID was developed in 1997 as the first commercial available DNA-test for genetic diseases in Arabian horses. With the identification of the five nucleotide deletion in the genome of affected horses, a PCR test for SCID was developed.

The first step of the testing process is to extract DNA from fibroblasts and leukocytes of the submitted sample. The obtained DNA is then amplified with PCR and hybridized with synthesized probes that are individually specific for the normal and mutant allele for the DNA-PKCS.¹⁸³ These probes are two synthesized oligonucleotide hybridization probes for the normal (N-probe) and the SCID (S-probe) allele.¹⁸⁴

DNA samples from healthy horses only hybridize to the N-Probe, whereas samples of affected foals hybridize only to the S-Probe. DNA samples of carrier horses which are heterozygous for the mutant allele hybridize with both probes.¹⁸⁵

Cerebellar Abiotrophy

Before the possible causative mutation for CA was identified, the test for this disease was a marker based test developed in 2007.¹⁸⁶ This indirect DNA-test is based on screening for alleles which are co-inherited with the CA mutation. Several genetic markers, which are adjacent to the CA gene on chromosome 2 were used in this diagnostic process.¹⁸⁷ Horses were considered as clear if no markers associated with CA could be detected. Affected horses with both mutant alleles were identified by a positive result for co-inherited markers in the presence of clinical signs of the disease. Horses that did not show any signs for CA but had a positive test result for the markers were considered carriers. The accuracy of this test was questionable as few results showed the presence of only some of the 17 used markers. These inconclusive test results were classified as “undetermined” until the direct DNA-test was developed in 2010 which allowed a definite diagnosis of those questionable samples.

The direct DNA-tests allows specific screening for the identified mutation that is suspected to cause CA. UC Davis therefore used this new method to retest samples that had been analyzed with the indirect marker based test and showed that the old version of the test for CA had a 97% accuracy, since the direct DNA-test revealed a higher number of carrier and affected horses. The direct DNA-test is now a generally accepted tool for an accurate diagnosis of CA affected and carrier horses.¹⁸⁸

Lavender Foal Syndrome

The direct DNA-test developed at Cornell University in 2010 allows the identification of the single nucleotide deletion in exon 30 of the myosin Va gene which is responsible for LFS. The process to identify this mutation involves an amplification of the DNA-sequence which includes the target region with PCR followed by digestion with the restriction enzyme Fau I. Subsequent agrose gel electrophoresis shows the banding pattern that is created by the digestion reaction. This visualizes the mutation, as the enzyme Fau I is abolished in its presence resulting in a 476 base pair product. In contrast, the normal sequence produces two bands of 386 and 90 base pairs.¹⁸⁹

Sampling methods for all available DNA-tests

The institute Veterinary Genetic Services (VetGen) offers tests for all three diseases and requires samples from the horse whose genetic status should be analyzed. The submitted sample can be either a hair, blood or cheek swab sample. Hair samples should contain 30 to 40 pulled out hairs from mane or tail with attached root bulbs. The sample should be bundled and placed in a labeled plastic bag. Blood samples should contain 3ml whole blood in a labeled EDTA blood tube. Cooling or freezing is not necessary during shipment of the sample.¹⁹⁰

For the LFS DNA-test carried out at Cornell University, a 1g tissue sample from heart, kidney, lung, spleen, muscle or skin of the fetus or neonate can be submitted, too.¹⁹¹

6.2 Institutes offering tests

Institutes that offer commercial tests can be found worldwide. Owners interested in the genetic status of their horse can easily find the most conveniently located laboratory via internet research or through contacting a veterinarian. The following table shows an international selection of institutes offering genetic tests for horses.

Table 6; International list of institutes providing genetic testing for horses¹⁹²

Country	Institute	Offered tests	Pricing
USA	Veterinary Genetics Laboratory at UC Davis	CA / LFS	CA/LF: \$40 (results in 4-6 business days)
	VetGen	CA / LFS / SCID individually and combined test; also accepts internationally shipped samples	CA/LFS: \$50 SCID: \$140 Combined: \$216 (discounts are given with increased orders)
	Arabian Foal Association	SCID / CA individually and combined test	SCID: \$99 CA: \$42.50
	Cornell University	LFS	LFS: \$47
	Animal Genetics	CA / LFS / SCID individually and combined test	CA/LFS: \$40 SCID: \$95 Combined: \$125 (results in 1-2 business days)
Germany	Gene Control	CA	CA: 50€
	University of Veterinary Medicine Hannover	CA	CA: 50€
UK	LABOKLIN	CA / LFS / SCID individually and combined test	CA/LFS: £55 (results in 7 days) SCID: £64 (results in 1-2 weeks) Combined: £148 (results in 2 weeks)
	Animal Genetics UK	CA / LFS / SCID	CA: £30 LFS: £35 SCID: £65 (results in 5-7 days)
Australia	University Of Queensland	CA / LFS	CA/LFS: \$70

6.3 Prevalence of the genetic diseases according to test results

Performing DNA-tests for genetic diseases in the Arab horse is a great tool to gain an insight on the extent of the disease in question. Collected data of test results over a period of time can help to determine the frequency of the mutant allele within the tested population and also gives an estimate of the carrier and affected percentage of the population. Monitoring these numbers will show if the offered tests and public education of the disease is sufficient to achieve the main goal which is to completely avoid affected foals.

Severe Combined Immunodeficiency

The institute VetGen published a survey on test results which were received in a time frame from 1997 till 31st of December 2011. It is important to emphasize that the calculated numbers only represent the foals tested at this institute and cannot be taken as reference values for all SCID cases, which are suggested to be higher than the calculated value. Still, this survey is a helpful tool to evaluate the prevalence of this genetic disorder as the number of affected foals is directly proportional to the mating of two carriers. But the percentage of affected foals does not correlate with the probability of receiving an affected foal by mating

two carrier animals. The probability is subject to the autosomal recessive inheritance pattern and is a constant 25% value for each carrier mating.¹⁹³

Table 7; VetGen survey on SCID testing¹⁹⁴

SCID Status	Number	% of Total
Clear	8,974	83.97%
Carrier	1,681	15.73%
Affected	32	0.30%
Total	10,687	100%

Cerebellar Abiotrophy

UC Davis published a summary of test results collected from 2008 till 16th of August 2011. The same restrictions, as mentioned for the SCID data, on interpreting these values apply for this survey.

Table 8; UC Davis survey on CA testing¹⁹⁵

CA Status	Number	% of Total
Clear	6,508	79.62%
Carrier	1,590	19.45%
Affected	76	0.93%
Total	8,174	100%

With the development of the direct DNA-test, UC Davis tested about 4,200 horses and calculated a carrier rate of 19.7% and an affected rate of 1.4%. The evaluation of the carrier rate should be considered with caution, as the value might be higher than the actual carrier prevalence of the disease, as owners often submit samples of specific bloodlines which leads to a more selective than representative testing.¹⁹⁶

A study published in 2011 calculated a 16% frequency of the putative CA mutation among 804 Arabian horses including 29 affected individuals. Again, this value only represents the frequency calculated from the families of the animals involved in this study. Researchers also suggested that the frequency value differs in regards to the different Arabian bloodlines.

A different research paper published in the same year focused on the detection of the CA associated mutation in different horse breeds with Arabian influence. This study identified carrier animals in three breeds, the Bashkir Curly Horse, the Trakehner and the Welsh Pony. Further pedigree analysis and DNA study confirmed the suggestion that Arabian ancestors introduced this genetic abnormality to the different breeds through cross-breedings.¹⁹⁷ The results of this study are relevant to every breed with Arabian ancestry, as it demonstrates that the genetic testing is indicated for breeding animals which belong to a breed with an Arabian influence.¹⁹⁸

Lavender Foal Syndrome

Research on the prevalence of LFS conducted at Cornell University and the University of Kentucky revealed a high frequency of carriers in pedigrees that showed affected cases within the family. As this disease is most frequently seen in the Egyptian bloodline, the calculation of the frequency of LFS carriers focused on unrelated Egyptian Arab horses and Arabians from other bloodlines. The genome of 114 horses was tested for the LFS mutation and the Egyptian bloodline showed a carrier frequency of 10.3% and the carrier frequency of non-Egyptian Arab horses was 1.8%. These values might not show the actual carrier frequency.

An overestimation might be caused by owners submitting a higher number of samples from horses that are related to LFS affected foals. As three of the identified carriers were active breeding stallions, the calculated values could also be lower than estimated, due to the fear of breeders to lose their reputation. Therefore breeders might decide against a DNA-test to avoid the possible negative impact on the prestige of their stud farms.

Nonetheless, the results of this study show the significance of LFS in the Egyptian Arab bloodline. The Egyptian Arab is very popular in North America and only approximately 49,000 Egyptian Arabs are registered worldwide. This emphasizes the value of every single individual and the possible massive threat of LFS to this bloodline if breeders do not direct the preservation of this line towards a DNA-test assisted breeding system that prevents carrier-carrier mating.¹⁹⁹

A recent study was published in 2012 and dealt with the investigation of the LFS carrier frequency in European horses. Tested horses did not only belong to the Arabian breed but also to breeds that were known to have an Arabian influence. A total of 78 Thoroughbred, 30 Standardbred and 215 Arabian horses were genotyped with PCR-RFLP assay to detect the mutant allele. The Arab horses chosen for this study were unrelated to each other within the first generation. No carrier animals could be identified among the Arabian influenced breeds and seven carriers were detected among the Arabian horses. The carrier frequency of Arab horses in Europe was calculated to be 0.0162%. These results demonstrate that the LFS mutant allele could be primary found in the Arab horse. The absence of carriers in the other tested breeds suggested that the mutation might have occurred after the introduction of Arab blood to the bloodline.²⁰⁰

Data from 2014

This year scientists at the University of Pretoria in South Africa revealed their results on their investigation of carrier horses of SCID, LFS and CA within local Arabian horse population, in order to gain an insight in the possible impact of these genetic diseases on the Arabian horse population in South Africa. In case of SCID the results were also used to gain an insight in the effect of the DNA-test on the prevalence of the disease by comparing the numbers of carriers before and after the introduction of the test.

Samples originated from two different populations and were randomly selected from purebred Arab foals born in the 2004/5 and 2009/10 foaling seasons. DNA-tests for all three diseases involved PCR and fragment analysis. The carrier prevalence was 11.7% for LFS and 5.1% for CA in both foaling seasons. Interestingly, the carrier prevalence for SCID dropped significantly from 6.4% in 2004/5 to 3.4% in the 2009/10 foaling season. These figures demonstrate that genetic tests are a great tool to reduce the carrier frequency of a genetic disease and therefore might lead to the eradication of affected horses.²⁰¹

7. Public education of genetic related diseases in the Arabian breed

Educating horse owners and breeders is a crucial aspect in managing genetic diseases in the Arab horse breed. ‘Translating’ complex genetic mechanisms and research results into a language that can be understood by non professionals and increasing public awareness about genetic disorders and managing options within the equestrian community are the main tasks of many organizations. Ensuring public education in genetic related diseases does not only

help to apply methods that ensure sound managing and breeding but also initiates receiving financial support. Owners and breeders should be encouraged to test their horses and contribute to the transparency of genetic statuses by publishing their test results.

7.1 Arabian Foal Association (FOAL)

The Arabian Foal Association is a non-profit organization that provides general knowledge and guidance in testing genetic disorders of the Arabian horse on their website <http://www.foal.org/index.html>. Main focus is placed on displaying the characteristics of CA with reference to the webpage <http://cerebellar-abiotrophy.org/>, which also is an excellent source to gain an insight in this disorder. A great achievement of FOAL is the generation of a public accessible list that displays the genetic status for all testable disorders. The submission of the test result is on a voluntary basis and should be encouraged by all testing institutes to increase transparency in the field of genetic diseases in Arabian horses.

7.2 Arabian Horse Foundation and Arabian Horse Association (AHA)

The Arabian Horse Foundation is an organization that provides financial support for scholarships, public education, equine rescue and rehoming and equine research on diseases affecting the Arab horse and other breeds. The financial contributions of this organization supported the research projects on CA, LFS and many other equine genetic disorders. More detailed information about this organization and its achievements can be found on the website <http://www.thearabianhorsefoundation.org/home.html>.

The AHA not only raises financial support like the Arabian Horse Foundation, but also represents a forum for public education and interaction for the whole Arabian horse community. Their detailed website www.arabianhorses.org and official magazine called “Modern Arabian Horse” provides answers, guidelines and recommendations for breed specific and general horse related topics. The website also includes a section created by the AHA Equine Stress, Research and Education Subcommittee on Genetic Disorders which not only gives detailed and up to date information about all genetic diseases of the Arab horse, but also educates the reader in the basic genetic background of the disorders. This section can be found under <http://www.arabianhorses.org/education/genetic/default.asp>.

8. Outlook on future breeding strategies after the introduction of genetic tests

The results of the research project in South Africa demonstrated that implementing DNA-tests in the Arabian breeding management can indeed reduce the frequency of the mutant allele. The overall goal to eliminate the appearance of affected individuals in a sound breeding system is now within reach. DNA-tests that identify carriers are the only reliable tool to avoid carrier mating without performing a series of test breedings, which are not only very time consuming, regarding the reproductive characteristics of horses, but also result in the creation of further carrier animals and affected horses. The financial and emotional burden that is accompanied with every horse affected by a fatal genetic disorder can now be prevented by analyzing the genetic status of breeding horses.

As all currently testable genetic disorders of the Arab horse follow an autosomal recessive inheritance pattern, the disclosure of the carrier status can not only assist the breeder in

choosing breeding animals that will not produce an affected offspring, but also allows for a systematic breeding system that includes the careful involvement of carrier animals to preserve the variety of traits that makes the Arabian horse unique. These facts argument against the numerous skeptic horsemen which see the full revelation of the horse's genetic information as a threat to the various unique features of each horse breed.

Also, some breeders fear that receiving a test result positive for the carrier status of a high class breeding animal can damage the reputation and prestige of the breeding facility. But these genetic diseases have been known to affect the Arabian horse breed over centuries. The new aspect is that their phenotypic presentation can be avoided through DNA-tests now. A positive carrier test result should not be considered as a disease diagnosis and does not exclude the sound breeding of the individual. On the contrary, keeping valuable carrier horses in a well managed breeding system is encouraged if this supports the preservation of the gene pool and typical traits of the breed. The DNA-test should therefore be used as a tool to support the breeder's profound knowledge of the breed and to create a sound breeding system that involves both, the management of the genetic disease and the preservation of the unique features that make the Arabian horse the elegant and majestic animal it is known for.

9. Appendix

9.1 Picture references

9.2 Literature references

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