Incidence of the endothelin receptor B mutation that causes lethal white foal syndrome in white-patterned horses

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Objective—To determine incidence of the Ile118Lys endothelin receptor B (EDNRB) mutation responsible for overo lethal white syndrome (OLWS) and its association with specific types of white patterning.

Animals—945 horses of white-patterned bloodlines and 55 solid-colored horses of other breeds.

Procedure—Horses were genotyped by use of allele-specific polymerase chain reaction to determine incidence of the Ile118Lys EDNRB mutation.

Results—Genotypes detected were homozygous Ile118, homozygous Lys118, and heterozygous. All foals with OLWS were homozygous for the Ile118Lys EDNRB mutation, and adults that were homozygous were not found. White patterning was strongly associated with EDNRB genotype. Color patterns with highest incidence (> 94%) of heterozygotes were frame overo, highly white calico overo, and frame blend overo. White-patterned bloodlines with lowest incidence of heterozygotes (< 21%) were tobiano, sabino, minimally white calico overo, splashed white overo, nonframe blend overo, and breeding-stock solid. The mutation was not detected in solid-colored horses from breeds without white patterning.

Conclusions and Clinical Relevance—In homozygotes, the Ile118Lys EDNRB mutation causes OLWS. In heterozygotes, the mutation is usually responsible for a frame overo phenotype. The frame pattern can be combined with other white patterns, making accurate estimation of EDNRB genotype by visual inspection difficult. Wide range of incidence of heterozygotes in various subtypes of white-patterned horses indicates different genetic control of these color patterns. Determination of EDNRB genotype by use of a DNA-based test is the only way to determine with certainty whether white-patterned horses can produce a foal affected with OLWS. (Am J Vet Res 2001;62:97–103)
at residue 118 of EDNRB is responsible for OLWS in Paint horses,12 and this mutation has been associated with the parental frame overo phenotype.13 Most solid-colored horses are homozygous for the Ile118 allele of EDNRB (wild type), all parents of OLWS foals are heterozygous, and all OLWS foals are homozygous for the Lys118 allele.12-14

If all overo horses carried 1 copy of the Lys118 allele, as has been suggested,14 the predicted incidence of OLWS from overo breeding would be 25%. However, Paint breeders report that the incidence of OLWS from overo breeding is much lower, and that some overo horses never produce foals that are affected with OLWS. In a small breeding trial conducted in 1978, 6 of 76 (7.9%) overo breedings resulted in the birth of a foal affected with OLWS.1 One explanation for the finding that the incidence of foals with OLWS is < 25% could be that not all overo horses carry the Lys118 allele, suggesting that overo white patterning is the result of more than 1 gene. The purpose of the study reported here was to determine incidence of the Ile118Lys EDNRB mutation responsible for OLWS and its association with specific types of white patterning.

Materials and Methods

Horses—Genomic DNA from white-patterned horses was obtained from horses admitted to the University of Minnesota Veterinary Teaching hospital for treatment, from samples submitted for detection of the Lys118 allele, and from cooperating breeders. The vast majority (> 90%) of samples were obtained from registered Paint horses. A small number of samples were obtained from Pintos (white-patterned horses from lineages other than Paint, Quarter Horse, or Thoroughbred), Thoroughbreds, Mustangs, and Miniature horses. Genomic DNA was obtained from horses residing in 35 states, the District of Columbia, and all Canadian provinces. Cooperating Paint breeding farms included those that had a high incidence of births of foals with OLWS and those that had a zero incidence. Only horses that could be phenotyped were included. The solid-colored horses tested were of Quarter Horse and Thoroughbred breeding.

Genotyping—Genomic DNA was obtained from blood by use of a kit. Deoxyribonucleic acid from hair root bulbs was obtained by heating 3 roots in 20 µl of 200 mM NaOH for 15 minutes at 70°C, followed by neutralization with 20 µl of 200 mM HCl and 20 µl of 100 mM Tris (pH 8.5). The EDNRB alleles were detected by use of allele-specific polymerase chain reaction, as described,14 allowing each horse to be genotyped as homozygous for Lys118 or Ile118 or as heterozygous.

Figure 1—Photograph of a horse with tobiano white patterning.

Figure 2—Photograph of a horse with frame overo white patterning.

Figure 3—Photograph of a horse with loud calico overo white patterning.

Figure 4—Photograph of a horse with splashed white overo white patterning.
Phenotyping—Horses were phenotyped by consensus of 2 individuals who observed the live horse or pictures taken of both sides of the horse, which included facial markings. Horses without white patterning that did not descend from white-patterned lines were designated as solid. Horses of white-patterned bloodlines were first classified phenotypically by use of the APHA color classifications of tobiano, overo, tovero, or breeding stock. Overo horses were further classified into 6 subtypes, and breeding-stock horses were classified as solid breeding stock or white breeding stock.

Tobiano—White markings on the head are similar to those of solid-colored horses (Fig 1). Typically, all 4 limbs are white. Body markings are distinct and regular, often with round or oval patterns. Flanks are dark, and the tail is often of 2 colors. White patches are often vertically oriented, cross the dorsal midline, and can appear draped over shoulders.

Overo—The head is often extensively marked with white (Fig 2). Typically, at least 1 limb is dark. White markings vary from distinct crisp white patches on the body to large irregular or roan areas. White coloration does not cross the back between the withers and tail. The tail is 1 color.

Tovero—These horses have tobiano and overo characteristics. The overo characteristic is most commonly apparent on the head but can also be expressed by irregular body markings.

Breeding stock—These are solid-colored horses of Paint lineage, including Miniature horses. Horses were subtyped as breeding-stock white if they were adult all-white horses of Paint breeding.

Subtypes of overo—Horses that fit the general description of overo have great variation in color pattern that allows further division into 6 distinct phenotypic subtypes: frame, calico, splashed white, sabino, medicine hat, and bald-faced.

Frame—A frame is an overo horse with body spots on the lateral aspect of the body (neck, shoulder, abdomen, flank, and hip; Fig 2). Body spots are distinct with sharp borders and do not connect with limb markings. At least 1 limb (often more) is dark. White limb markings do not usually extend up the carpus or tarsus. The head is usually extensively marked with white, which often extends laterally to the eyes. White spots that are primarily on the ventral midline or only involve the axilla are not typical of frame horses, especially when associated with extensively marked white limbs.

Calico—A calico is an overo horse with white markings that are scattered and have irregular borders (Fig 3). One or more limbs are white, and the white limb markings have narrow extensions up the limb. White markings on the limbs often connect to white markings on the body. Head markings are irregular and may be wide but do not usually

Figure 5—Photograph of a horse with sabino overo white patterning.

Figure 6—Photograph of a horse with medicine hat overo white patterning.

Figure 7—Photograph of a horse with frame blend overo white patterning.

Figure 8—Photograph of a horse with frame overo white patterning and a foal affected by overo lethal white syndrome.
involve the eyes. Calico horses have the widest variation in the amount of white patterning. White-patterned horses without white patterning on the body but with irregular white limb markings extending above the tarsus and carpus were considered minimally marked calico overos. Horses with irregular limb markings above the tarsus and carpus and irregular white body patches on < 75% of their body were considered calico overos, and horses with irregular limb markings above the tarsus and carpus and irregular white markings on > 75% of their body were considered loud calico overos.

Splashed white—A splashed white horse is an overo horse with extensive white markings on the head and large crisp white patches on the ventral aspect of the neck and thorax that often connect with extensive white patches on the forelimbs (Fig 4). The hind limbs also have white markings that extend dorsally above the tarsus, and the ventral aspect of the abdomen is white. A splashed white horse is often described as a horse dipped in white.

Sabino—A sabino is an overo horse with 1 or more white limbs and white markings on the face (Fig 5). Extensive areas of roan coloring (mixing of white hairs within colored patches) is the major characteristic of sabinos. Colored areas are also irregularly shaped and flecked with white that blends with small white patches.

Bald-face—These are overo horses with white face markings that extend ventral to a line that connects the point of the mouth and the poll. The body coloring is solid, and white limb markings distal to the tarsus and carpus may be present.

Medicine hat—A medicine hat is a horse with a rare color pattern in which the coat is almost entirely white (Fig 6). Pigmented areas are found primarily on the ears and poll but may also appear on the thorax, flank, dorsal midline, and tail head. Medicine hat horses can arise from overo or tovero bloodlines; when of overo bloodlines, medicine hat horses may have pigment that is quite faint on the dorsal midline.

Overo blend—Overo blends are overo horses that have evidence of 2 or more overo white patterns (Fig 7). There are 2 recognizable subdivisions of overo blends. The most common is the frame blend, which has frame characteristics such as lateral body spots, solidly colored limbs, and wide blazes, in addition to patterning of another overo subtype, which is usually calico. Less common are the other (nonframe) overo blends, which have 2 other types of overo patterning.

Overo lethal white syndrome—Foals affected with OLWS are almost entirely white (Fig 8). In most foals with OLWS, pigmentation is restricted to the retina. However, small pigmented areas may be present, usually in the mane or tail.

Nonpaint—These are registered Thoroughbreds and Quarter Horses that do not have excessive white on their faces or white limb markings proximal to the carpus or tarsus.

Statistical analyses—Data were summarized by use of 2-way tables. Horses were categorized by pattern versus 3 genotypes (homozygous Ile118, homozygous Lys118, and heterozygous). *χ*2 Analysis was used to test for association between pattern and genotype. Subtables were analyzed by use of *χ*2 analysis or the Fisher exact test. Significance was established as *P* < 0.05.

Results

Incidence of the Lys118 EDNRB allele was determined in horses with various white patterns (Table 1). White patterning and genotype were associated (*P* < 0.001). Genotype of foals affected with OLWS was significantly (*P* < 0.001) different from that of all other horses. No living Lys118 homozygotes were found, and all foals with OLWS (confirmed by use of histologic examination, gross necropsy, or clinical signs) were homozygous for the Lys118 allele.

The strong association between phenotype and genotype persisted after removing foals with OLWS from the analysis (*P* < 0.001). The incidence of heterozygotes within white pattern groups varied from 0 to 100%. Certain color patterns had similar (*P* > 0.05) incidence of heterozygotes (Fig 9).

### Table 1—Distribution of genotypes for endothelin receptor B (homozygous for the Ile 188 allele, heterozygous, and homozygous for the Lys 188 allele) in 945 white-patterned horses and 55 solid-colored horses

<table>
<thead>
<tr>
<th>Color pattern</th>
<th>No. of horses</th>
<th>Homozygous Ile 118</th>
<th>Heterozygotes (%)</th>
<th>Homozygous Lys 118</th>
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<tbody>
<tr>
<td>Tobiano</td>
<td>109</td>
<td>98</td>
<td>11 (10)</td>
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<tr>
<td>Overos</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Frame</td>
<td>188</td>
<td>10</td>
<td>178 (95)</td>
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<td>Calico Loud</td>
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<td>37 (100)</td>
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<td>Calico Minimal</td>
<td>67</td>
<td>61</td>
<td>6 (9)</td>
<td>0</td>
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<tr>
<td>Splashed white</td>
<td>26</td>
<td>23</td>
<td>3 (12)</td>
<td>0</td>
</tr>
<tr>
<td>Sabino</td>
<td>15</td>
<td>12</td>
<td>3 (20)</td>
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</tr>
<tr>
<td>Bald-faced</td>
<td>17</td>
<td>11</td>
<td>6 (35)</td>
<td>0</td>
</tr>
<tr>
<td>Medicine hat</td>
<td>13</td>
<td>4</td>
<td>9 (69)</td>
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<td>Overo blends</td>
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<td>Solid-colored horses</td>
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<td>55</td>
<td>0 (0)</td>
<td>0</td>
</tr>
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</table>

OLWS = Overo lethal white syndrome.
Discussion

Overo lethal white syndrome has been associated with observation with the frame overo subtype,5 and frame overo has recently been linked with horses that are heterozygous for the Ile118Lys EDNRB mutation.13 Ninety-six percent of all frame and frame-blend overos tested in the study reported here possessed the Lys118 allele, strongly supporting the conclusion that in the heterozygous state, the Ile118Lys EDNRB mutation commonly causes the frame phenotype. However, calico overos with a large amount of white coloring also had high (100%) incidence of the Lys118 allele. These largely white calico horses are actually frame blends, but the frame patterning is usually not apparent because of the presence of the calico white pattern. The high incidence of heterozygotes in this subgroup of calicos was unexpected and is useful information for horse breeders.

The association between the heterozygous state and frame overo phenotype is not 100%. Some horses are heterozygous for the Lys118 allele yet do not express frame white patterning, and there are 2 probable explanations for this phenomenon. First, in some white-patterned horses, the frame patterning is not apparent, because it is contiguous with prominent white patterning caused by other color genes. This occurs in tobianos (10% heterozygotes), splashed white overos (12%), calico overos (55%), and sabino overos (20%). In a second group of nonframe heterozygotes, (bald-faced and breeding-stock solid), the frame phenotype is absent. Variable expression of the frame phenotype, possibly because of the influence of other genes, is the most probable explanation for the lack of white patterning.3 In mice, quantitative genetic analysis has identified 6 loci that affect the amount of white coat spotting caused by an EDNRB mutation,4 suggesting that modifier genes could also be present in horses and affect the phenotypic expression of the Lys118 allele. Breeding-stock horses of Paint lineage that carry the Lys118 allele always had some white markings, but 2 heterozygote Miniature horses had no white markings, suggesting that there is a modifier gene present in the Miniature horse population that strongly suppresses white patterning.

In addition to the nonframe heterozygotes, there are a few horses of apparent frame phenotype (5% of frame overos) that do not carry the Lys118 allele. The most common marking on these horses is a single distinct white patch on the ventral aspect of the abdomen that extends laterally up the frame. Variable expression of other white patterning genes is most likely the cause for these patterns. For example, splashed white horses have white body patches with sharp borders, and minimal expression of this pattern could look like frame patterning.

Regarding incidence of heterozygotes, white-patterned horses appear to form 3 groups (Fig 1). In the first group (frame, frame blends, and loud calicos), the high frequency of the Lys118 allele indicates it is essential to production of the pattern. In the second group (all-white breeding stock, medicine hat, tovero, calico, and bald-faced), the moderate frequency of the Lys118 allele indicates it may contribute to the phenotype but is not essential. In the third group (sabino, splashed white, breeding-stock solids, tobiano, calico minimal, and nonframe blends), the uncommon occurrence of the Lys118 allele indicates it rarely contributes to the white patterning.
It has been suggested that there are 4 distinct overo patterns: frame, calico, splashed white, and sabino, and the subtypes of overo are under the control of different, independently assorting genes. Our data support that hypothesis. The Lys118 allele causes the frame pattern, and splashed white, calico, and sabino horses have a significantly different incidence of the Lys118 allele, indicating a distinct genetic basis. Additionally, these 4 overo patterns commonly appear blended, as would be expected from independently segregating overo genes. When combined with other overo white patterns, the Lys118 allele appears to add more white on the head and frame of the body. This is best seen in calico overos in which the percentage of heterozygotes increases from 9% in minimally white calicos to 55% in moderately white individuals to 100% in the whitest calicos.

There are 4 white patterns (breeding-stock white, medicine hat, tovero, and bald-face) that are associated with an intermediate incidence (35 to 80%) of heterozygosity for the Lys118 allele and a less-distinct genetic basis. We believe that these patterns are the result of blending of white patterning genes and that the Lys118 allele may contribute but is not essential. Tovero white patterning has long been known to be the result of breeding tobiano and overo horses. The intermediate incidence of the Lys118 allele in tovero horses results from the fact that the overo pattern may come from nonframe overo genes such as calico. We believe that breeding-stock white and medicine hat patterns similarly arise from a combination of tobiano and overo. In our study, 80% of all-white breeding-stock horses were heterozygous, but in a previous study, 2 all-white breeding-stock horses did not possess the Lys118 allele. The low number of horses tested with this rare pattern indicates that caution should be used in interpretation of breeding-stock white genotypes. Bald-faced horses probably result from minimal expression of the frame gene or because of prominent expression of genes that control white face markings.

There are 3 white patterns (breeding-stock solid, tobiano, and nonframe blends) that have a low incidence (0 to 18%) of heterozygotes. Tobiano is known to have a genetic basis distinct from overo, and there is low incidence (10%) of the Lys118 allele in these horses. However, if breeders choose a tobiano mate for a horse that is heterozygous for Lys118 to avoid OLWS, they will not always achieve their goal. Our experience is that tobiano horses are only heterozygous for the Lys118 allele when their pedigree contains overo horses. The 18% incidence of heterozygotes in breeding-stock solid horses again suggests that the frame phenotype either has variable expression or may be suppressed by other genes. Nonframe blends are horses that have 2 overo patterns other than frame, most commonly sabino and calico. Their existence and lack of heterozygotes among them support the hypothesis that overo horses are genetically heterogeneous.

Additional support for genetic heterogeneity of overo patterning can also be inferred from the incidence (< 23%) of births of foals affected by OLWS from overo X overo breeding. In the study reported here, 73% percent of all overo and overo blend horses were heterozygous for the Lys118 allele. If our sample is representative of overos in general, the predicted incidence of births of foals affected by OLWS in overo X overo breeding would be 13.3% (ie, 0.73 × 0.73 × 25%). This figure agrees with anecdotal reports from breeders of Paint horses but is slightly higher than the figure of 7.9% detected in a small breeding trial. Our finding that 27% of overo horses did not possess the Lys118 allele explains why some overo breedings never produce foals affected by OLWS. Every attempt was made to get a phenotypic and a geographic sampling of Paint horses, but the samples received for testing may have been biased toward excessively white horses, which may have raised the incidence of the Lys118 allele. However, because of the recent popularity of Paints, it is also possible that the incidence of the Lys118 allele is increasing in the breed because of its efficacy in producing and enhancing white patterning.

A few white-patterned horses of breeds other than Paint (Thoroughbreds, Miniature horses, and half Arabians) were heterozygous for the Lys118 allele. This may be a deliberate result, because breeders may arrange outcrosses to Paints to add white patterning. When heterozygotes are used for outcrossing, this gene will be introduced into the breed along with the possibility of producing foals affected by OLWS. However, the appearance of the Lys118 allele in breeds unrelated to Paints is more difficult to explain. Undocumented outcrosses to white-patterned horses are a possibility. De novo mutations have also been suggested to be responsible for the birth of white-patterned horses from solid parents, but this is yet unproved. We believe that these horses may also result from white patterning genes in solid breeds that are minimally expressed because of variable expression or suppression by other genes. If this is true, the Lys118 allele entered these breeds many years ago.

Of greater importance, at least to some breeders, is the occurrence of white patterning in Quarter Horses. Foals with excessive white markings born to registered Quarter Horse parents are known as crop-outs and are ineligible for registration by the American Quarter Horse Association. Quarter Horses are closely related to Paints, and the APHA was formed in part because the AQHA refused to register stock-type horses with excessive white coloring. It is known or suggested by breeding results that at least some crop-out Quarter Horses are heterozygous for the Lys118 allele. This study tested 25 Quarter Horses that were either crop-outs or parents of a white-patterned horse and did not find any heterozygotes, lending further support to the concept that other genes control overo coloration. As genetic testing becomes available for more overo genes, testing of crop-outs and their parents will help determine whether the crop-out white patterning is the result of de novo mutations or whether white patterning genes exist in solid-colored Quarter Horses.

Results of the study reported here indicate that the lle118lys EDNRB mutation is responsible for the frame overo pattern in heterozygotes. Determination of the incidence of the Lys118 allele in white-patterned horses indicates that several genes may interact to result in an overo phenotype that causes overo pat-
tending. Because there are horses that are heterozygous and do not express the frame phenotype or appear to be frame overos and do not carry the Lys118 allele, allele-specific genotyping of DNA must be performed to accurately determine an individual horse's potential to produce a foal affected by OLWS.


*Puregene, Gentra Systems Inc, Research Triangle Park, NC.

References