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A *cis*-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse

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In horses, graying with age is an autosomal dominant trait associated with a high incidence of melanoma and vitiligo-like depigmentation. Here we show that the Gray phenotype is caused by a 4.6-kb duplication in intron 6 of *STX17* (syntaxin-17) that constitutes a *cis*-acting regulatory mutation. Both *STX17* and the neighboring *NR4A3* gene are overexpressed in melanomas from Gray horses. Gray horses carrying a loss-of-function mutation in *ASIP* (agouti signaling protein) had a higher incidence of melanoma, implying that increased melanocortin-1 receptor signaling promotes melanoma development in Gray horses. The Gray horse provides a notable example of how humans have cherry-picked mutations with favorable phenotypic effects in domestic animals.

Horses with the mutation causing the Gray phenotype are born colored but gradually lose hair pigmentation and, by the age of 6–8 years, become white. The manifestation of this mutation as a white horse has had a strong impact on human culture and has left numerous traces in art and literature from Asia and Europe (for example, Pegasus and the unicorn). The oldest written record of the presence of white horses, to our knowledge, is by the Greek historian Herodotus, who describes the Persian emperor Xerxes (who reigned from 485 to 465 BC) as keeping sacred white horses. The prestige of riding a white horse (**Fig. 1a**) has thus led to selection of the Gray-causing mutation by humans; this mutation is by far the most common cause of white color in horses[1].

Gray horses experience a gradual loss of hair pigmentation, while dark skin pigmentation is maintained (**Fig. 1b**). Gray horses also show a very high incidence of dermal melanomas (70–80% of Gray horses older than 15 years have melanomas[2,3]) and reduced longevity[4]. The melanomas occur primarily as jet-black, firm nodules well circumscribed in the dermis of glabrous skin under the tail root and in the anal, perianal and genital regions, perineum, lips and eyelids[5]. The primary multiple melanomas are benign, but some metastasize to several internal organs. Because both loss of hair pigmentation and development of melanomas involve melanocytes and are associated with the Gray phenotype across breeds, we hypothesized that both phenotypes are caused by the same mutation. A large proportion of Gray horses develop vitiligo-like skin depigmentation[2]. Gray horses often show speckling (**Fig. 1c**), and some develop distinct, large patches of red pigmentation known as 'blood marks' (ref. 1 and **Fig. 1d**).

The Gray-causing mutation was previously assigned to horse chromosome 25 (refs. 6–8) and subsequently fine-mapped to a region corresponding to 6.9 Mb on human chromosome 9q (ref. 9), which does not harbor any obvious candidate genes for a pigmentation phenotype. We hypothesized that all Gray horses have inherited the mutated *G* allele from a common ancestor, because of its unique phenotypic manifestations. SNPs in the 6.9-Mb region were screened on a panel of Gray and non-Gray horses (a non-Gray horse is a of any color not carrying the *Gray* allele). SNPs in the interval corresponding to positions 28.7 to 29.1 Mb (~350 kb) on horse chromosome 25 (EquCab1.0) defined the crucial interval, as markers within this interval showed complete linkage disequilibrium with the Gray phenotype across eight breeds (**Supplementary Table 1** online). SNPs flanking the interval did not show complete linkage disequilibrium, implying that historical recombination events have occurred and that regions outside these flanking markers can be excluded

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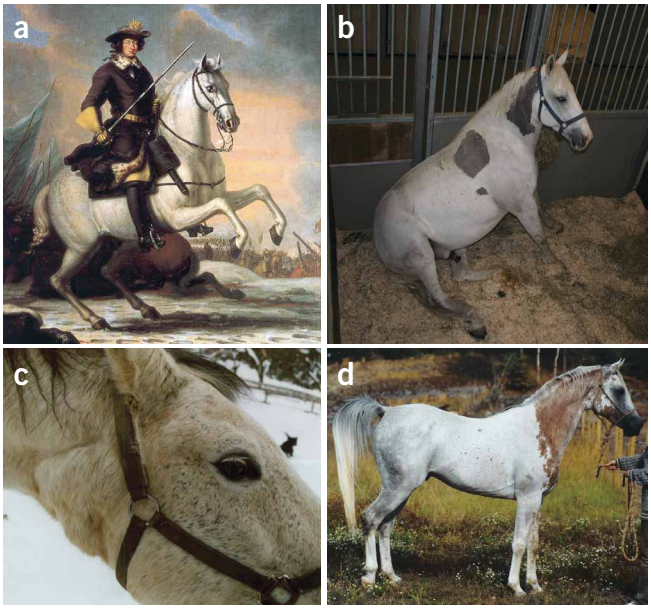

Figure 1 Graying with age in horses. (**a**) Swedish king Karl XI on his Gray horse named Brilliant, painted by David Klöcker Ehrenstrahl after the battle in Lund, December 4, 1676. Photo reproduced with permission of The National Museum of Fine Arts in Sweden. (**b**) Partially paralyzed Gray horse diagnosed with multiple internal melanomas. The shaved areas show the full maintenance of dark skin pigmentation. (**c**) Gray horse with characteristic speckling (numerous small spots of pigmented hair, also called 'flea-bitten' Gray). Photos in **b** and **c** used with permission of Monika H. Seltenhammer. (**d**) Gray horse with red 'blood marks'. There is notable contrast between the remaining black pigmentation in the areas showing graying and the red pigmentation in the blood marks. Photo used with permission of Emilie Kajle.

(**Supplementary Fig. 1** online). The interval is surprisingly large, given that our material included populations as divergent as Icelandic and Arabian horses, which have been separated for at least 1,000 years. This implies a low rate of recombination in the region, also indicated in our previous linkage study[9]. We conclude that the causal mutation is located in this interval and that all Gray horses tested (>700 from eight breeds) have inherited this mutation from a common ancestor. One non-Gray haplotype was identical to the Gray haplotype for all tested SNPs in the interval, suggesting that it represents the 'ancestral' haplotype for Gray (**Supplementary Fig. 1**).

The region of the Gray-causing mutation contains four genes: *NR4A3* (nuclear receptor subfamily 4, group A, member 3), *STX17*, *TXNDC4* (thioredoxin domain–containing-4′) and *INVS* (inversin) (**Fig. 2**). None of these genes has previously been associated with pigmentation defects or melanoma. Northern blot and RT-PCR analysis showed that all four genes are expressed in melanomas, and no variant transcripts thereof were detected in Gray horses (**Fig. 2c**). There was, however, markedly high *NR4A3* expression in Gray melanomas. Sequence analysis of all annotated exons from the four genes revealed no polymorphisms uniquely associated with the Gray phenotype. Southern blot analysis of genomic DNA revealed no polymorphisms for *NR4A3*, *TXNDC4* or *INVS*, but a ~4.6-kb insertion was present in *STX17*. Long-range PCR analysis revealed that the insertion is a duplication located in intron 6. The intron was sequenced to determine the exact position of the duplication. The Gray haplotype showed 38 SNPs in intron 6 of *STX17* compared to non-Gray haplotypes (**Supplementary Fig. 2** online). Notably, the ancestral non-Gray haplotype mentioned above had a sequence identical to that of the Gray haplotype but did not include the duplication. A diagnostic PCR-based test for the duplication was used to screen Gray and non-Gray horses representing 14 breeds.

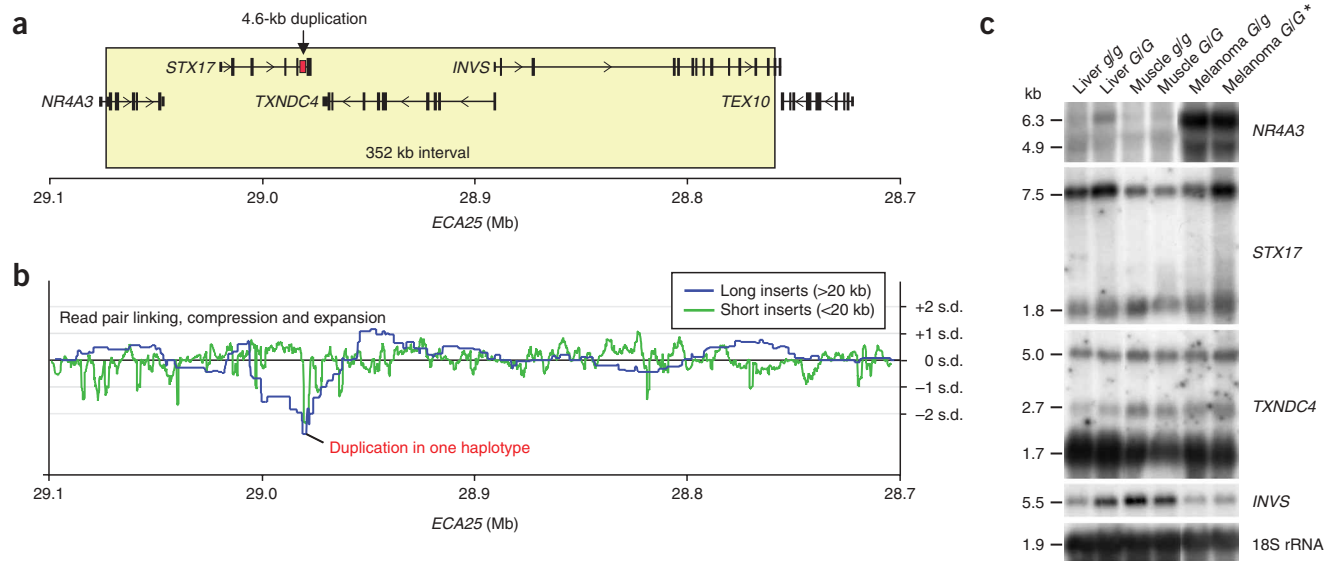

Figure 2 Molecular characterization of the locus of the Gray-causing mutation in horses. (**a**) Gene content of the mutation interval. The 352-kb region showing complete association with the Gray phenotype is indicated by a box; the location of the 4.6-kb duplication in *STX17* intron 6 is marked with an arrow. The annotation is based on the horse genome assembly as presented on the UCSC server (build Jan. 2007, EquCab1 assembly). (**b**) Average paired-end read compression and expansion in the horse genome assembly across the region in standard deviations (s.d.), broken down by insert size. Green, 4.5–10 kb (plasmids); blue, 40 kb (fosmids) and 180 kb (BAC ends). The only spot in which both short and long inserts are significantly compressed (by more than two s.d.) coincides with the 4.6-kb duplication in *STX17* intron 6. (**c**) Multiple-tissue northern blot analysis of genes in the mutation interval; 18S rRNA was used as an internal control. *G*, *Gray* mutant allele; *g*, wild-type allele. *, mRNA from a melanoma cell line derived from a heterozygous (*G/g*) horse (M.H.S., unpublished data) is shown in this lane for hybridization with the NR4A3 probe. The estimated transcript sizes are given to the left.

Table 1 Complete association between Gray phenotype and the 4.6-kb duplication in *STX17* intron 6 across breeds of horses

		Duplication		
Breed	*n*	+/+	+/–	–/–
Gray				
Arabian	22	4	18	0
Connemara	3	0	3	0
Icelandic	1	0	1	0
Lipizzaner	694	467	227	0
New Forest pony	1	0	1	0
Shetland pony	1	0	1	0
Thoroughbred	3	0	3	0
Welsh	2	1	1	0
Total	727	472	255	0
Non-Gray				
Arabian	18	0	0	18
Connemara	4	0	0	4
Fjord	10	0	0	10
Friesian	5	0	0	5
Haflinger	10	0	0	10
Icelandic	11	0	0	11
Lipizzaner	18	0	0	18
Morgan	10	0	0	10
New Forest pony	10	0	0	10
North Swedish	10	0	0	10
Shetland pony	10	0	0	10
Swedish Warmblood	4	0	0	4
Thoroughbred	7	0	0	7
Welsh	4	0	0	4
Total	131	0	0	131

+, presence of duplication; –, absence of duplication.

The duplication was detected in all Gray horses but in none of the non-Gray horses (**Table 1**) and thus qualifies as a candidate causal mutation.

The genome assembly of the horse is derived from the thoroughbred mare Twilight, who is heterozygous for the Gray-causing mutation. A bioinformatics analysis of these data confirmed the presence of a 4.6-kb duplication (**Fig. 2b**), the only notable structural difference between Gray and non-Gray haplotypes (**Supplementary Note** online). This analysis also ruled out the possibility that Gray is caused by a translocation of a gene from another chromosomal region. Further analysis revealed 17 polymorphisms near evolutionary conserved sites between the Gray and non-Gray haplotypes, but none of these was uniquely associated with the Gray phenotype when additional horses were tested (**Supplementary Note** and **Supplementary Table 2** online).

The loss of hair pigmentation in Gray horses is fully dominant. In contrast, the speed of graying, amount of speckling, incidence of melanomas and presence of vitiligo-like depigmentation show considerable variation among Gray horses. A collection of 694 Gray Lipizzaner horses in which these four traits have been observed were genotyped for the *STX17* duplication. Horses homozygous for the mutation showed more rapid graying and were more homogenously white in the final stage of the process compared with Gray heterozygotes (**Fig. 3a**). They also had significantly higher incidence of melanoma (**Fig. 3b**) and vitiligo (**Fig. 3c**) and almost no speckling (**Fig. 3d**). The pigmented spots (speckling) may represent somatic events in which the duplicated copy has been lost or inactivated, considering that in homozygotes both chromosomes must be affected whereas a single event is sufficient in heterozygotes.

Many Gray Lipizzaners carry the recessive black allele, caused by an 11-bp deletion in exon 2 of *ASIP* (agouti signaling protein; ref. 10). This allowed us to test whether an increase in melanocortin-1 receptor (MC1R) signaling has any effect on the incidence of melanomas in Gray horses, as *ASIP* encodes an MC1R antagonist. The frequency of the recessive $ASIP^{a}$ allele was 0.50, and 26% of the tested Gray Lipizzaners were non-agouti ($ASIP^{a/a}$). We conducted a statistical analysis of horses older than 6 years, including polynomial (cubic) regression on age and the *Gray* and *ASIP* genotypes. A highly significant association between *ASIP* genotype and the incidence of melanomas was revealed. The least-squares means (±s.e.) of melanoma grades were 0.88 ± 0.06 for $ASIP^{A/A}$, 1.06 ± 0.04 for $ASIP^{A/a}$ and 1.22 ± 0.07 for $ASIP^{a/a}$ ($P = 0.0006$); these estimates are conditional on the presence of the *Gray* allele. For comparison, the *Gray* locus has a much stronger effect (least-squares means of 1.43 ± 0.04 for *Gray* homozygotes (*G/G*) and 0.67 ± 0.05 for *Gray* heterozygotes (*G/g*)). A similar analysis did not reveal any significant effects of *ASIP* on graying, vitiligo or speckling.

The higher incidence of melanomas in horses carrying an *ASIP* null mutation implies that increased MC1R signaling promotes melanoma development in Gray horses. This result was unexpected because the most well-characterized function of agouti is to modulate MC1R signaling and thereby pigment switching in hair-follicle melanocytes[11]. However, mice that are homozygous for the null mutation causing extreme agouti have much darker pigmentation in the glabrous skin of their ears and tails. This mouse phenotype and our observation that Gray horses carrying the recessive mutant agouti allele have a higher incidence of melanomas in glabrous skin show that ASIP also influences dermal melanocytes. The association between increased MC1R signaling and melanoma development in Gray horses is important because stimulation of the pigmentation machinery downstream of MC1R has been proposed as a strategy to protect MC1R-deficient humans from UV-induced melanoma[12]. Unfortunately, we were not able to further study the relationship between MC1R signaling and melanoma by analyzing Gray horses carrying the recessive chestnut allele[13] because the frequency of this *MC1R* allele was too low in the Lipizzaner breed.

We further investigated *STX17* and *NR4A3* because of the presence of the duplication in the former and the markedly high expression of the latter in Gray melanomas (**Fig. 2c**). Syntaxins contain SNARE domains and are involved in intracellular membrane trafficking[14]. STX17, a divergent member of the syntaxin family, has a broad tissue distribution[15]. STX17 is partially associated with the endoplasmic reticulum and shows nuclear localization in some malignant cells[16]. NR4A3 belongs to the NR4A subgroup of the nuclear hormone receptor superfamily[17]. The NR4A members are classified as early-response genes and have been implicated in several biological processes, including cell cycle regulation, apoptosis and carcinogenesis.

The *STX17* duplication is located in intron 6 just upstream of the initiation of a short alternative transcript (**Supplementary Fig. 3** online). The short form encodes the transmembrane domain and C terminus but lacks the SNARE domain. The short transcript has not been described before but is evolutionarily conserved. Bioinformatics analyses of the duplicated region did not reveal any obvious protein- or microRNA-coding sequences. However, the region contains sequences that are well conserved among mammals (**Supplementary Fig. 3c**) and may thus include regulatory elements. We assessed the relative expression of the long and short transcripts of *STX17* in

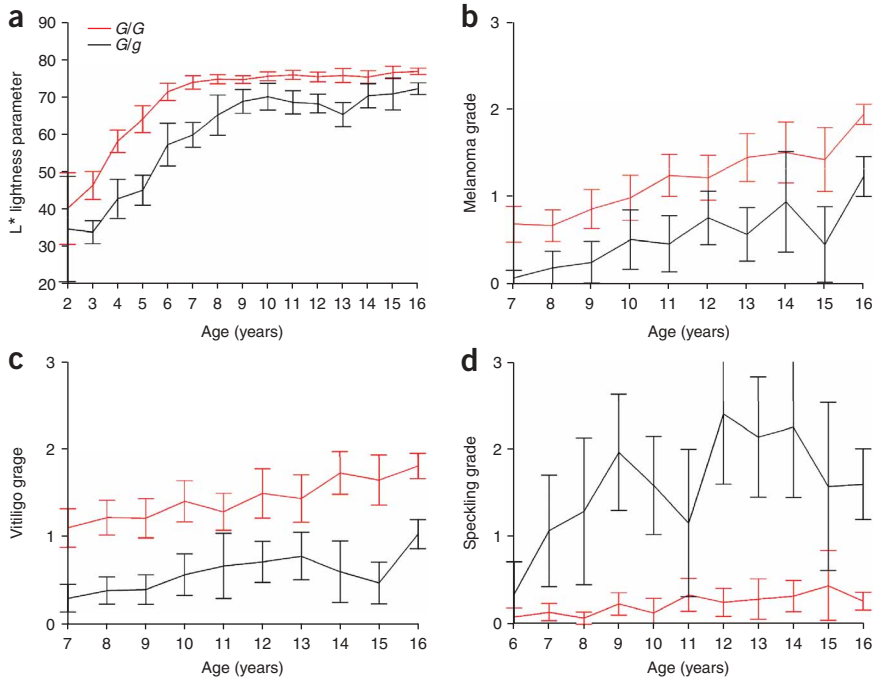

Figure 3 Analysis of phenotypic differences between heterozygous (*G/g*; black lines) and homozygous (*G/G*; red lines) Gray Lipizzaner horses. Shown are degree of lightness (**a**), measured as light reflectance, and grades of melanoma (**b**), vitiligo (**c**) and speckling (**d**). The analysis included 467 *G/g* and 227 *G/G* Lipizzaner horses, which were genotyped for the *STX17* duplication by a PCR-based method. The data show means ±2 s.e.; that is, nonoverlapping bars indicate a statistically significant difference. Overall $P < 0.0001$ for all comparisons.

various tissues from Gray and non-Gray horses by real-time PCR (**Fig. 4a**). The long transcript was the predominant form, but the two forms showed very similar expression patterns. Both the long and short *STX17* transcripts showed high expression in Gray melanomas compared with liver and skin from both Gray and non-Gray horses (**Fig. 4a**). To study this differential expression more directly, we quantified the relative expression of alleles in melanomas from three *G/g* horses using SNPs located in the 5′ UTR of the short transcript (this analysis was not possible for the long form because of the lack of suitable polymorphisms). cDNA sequences from all three melanoma samples revealed expression of only one allele, indicating differential expression of the short isoform (**Fig. 4b**). The strong association between the expression levels of the two transcripts implies that the long form is also likely to be differentially expressed.

Northern blot analysis revealed high *NR4A3* expression in Gray melanomas (**Fig. 2c**), a result confirmed by real-time PCR analysis (**Fig. 4a**). Notably, sequence analysis of cDNA from two *G/g* horses revealed *NR4A3* expression from only the Gray haplotype, indicating that a *cis*-acting regulatory mutation is underlying the upregulation of expression (**Fig. 4c**). Genomic DNA from the same melanoma tissue was used as a control to ensure that differential expression of *STX17* and *NR4A3* are not caused by chromosome loss in the tumor. Cyclin D1 (*CCND1*) and cyclin D2 (*CCND2*) have both been identified as targets of *NR4A3* (ref. 18), which prompted us to investigate whether these genes are also upregulated in Gray melanomas. Comparative northern blot analysis of horse melanoma tissue and cell lines showed high expression of *CCND2* but not *CCND1* in Gray melanomas (**Fig. 4d**). In contrast, analysis of three human melanoma cell lines revealed low expression of *STX17*, *NR4A3* and *CCND2* but high expression of *CCND1*. Thus, the expression phenotype of Gray melanomas is clearly different from that associated with

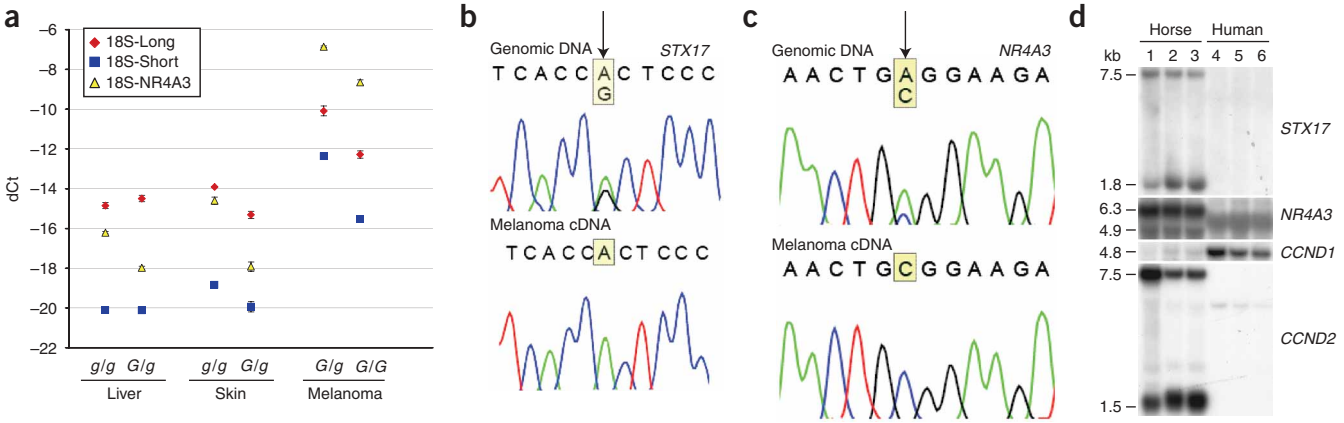

Figure 4 Expression analysis of *STX17* and *NR4A3*. (**a**) Real-time PCR analysis showing expression of *STX17* (short and long isoforms) and *NR4A3* in relation to the expression of 18S rRNA in various tissues from Gray and non-Gray horses. dC_t, difference in C_t value (threshold cycle) for the control gene (18S in this case) and the target transcript. (**b**,**c**) Differential expression analysis for *STX17* (**b**) and *NR4A3* (**c**) using melanoma tissue from *G/g* heterozygotes; the nucleotide sites for the SNPs in *STX17* and *NR4A3* correspond to positions 28,972,811 bp (intron 6, 5′ UTR of the alternative transcript) and 29,063,351 bp (exon 8), respectively. Genomic DNA was used as a reference. (**d**) Northern blot analysis showing that enhanced expression of *STX17* and *NR4A3* are associated with high expression of *CCND2* but not *CCND1* in Gray melanomas. 1, melanoma tissue from *G/g* horse; 2, melanoma cell line from *G/g* Lipizzaner horse; 3, melanoma cell line from *G/g* Arabian horse; 4, human melanoma cell line A375; 5, human melanoma cell line M5; 6, human melanoma cell line BL. The horse melanoma cell lines were established by M.H.S. (unpublished data).

the three human melanomas included in this comparison. A similar analysis of *TXNDC4* and *INVS* using Gray melanomas did not reveal any differential expression (data not shown).

Our data show that graying with age in horses is caused by a *cis*-acting regulatory mutation, as *STX17* and *NR4A3* both show differential expression in melanoma tissue. We propose that the 4.6-kb duplication in intron 6 of *STX17* constitutes this regulatory mutation because (i) it is completely associated with the Gray phenotype in >800 horses, (ii) it is the only observed difference between the Gray haplotype and the non-Gray ancestral haplotype and (iii) it seems unlikely that a complete association between the duplication and the Gray phenotype could have been maintained over thousands of years unless it is the causative mutation, as tandem duplications are notoriously unstable[19]. It is possible that the observed ancestral haplotype may not actually be ancestral, but rather a Gray haplotype that has lost the duplication and thereby the association with Gray. To the best of our knowledge, there are no documented cases of revertants, but such events are difficult to verify in an outbred species such as the horse. Somatic revertants are expected to cause pigmented spots and, notably, speckling is common in *G/g* but not in *G/G* horses (**Figs. 1c** and **3d**). The rare occurrence of blood marks in Gray horses (**Fig. 1d**) is also consistent with a somatically unstable mutation.

It is not yet clear whether the range of phenotypic effects observed in Gray horses is caused by the combined effect of overexpression of *STX17* and *NR4A3*, or if only one of these is the sole causal agent. *STX17* encodes a poorly characterized member of the syntaxin family. Syntaxins are involved in vesicle transport, suggesting that a mutation in *STX17* could influence pigmentation by altering melanosome production or transport. This seems unlikely, as hair and skin pigmentation in Gray horses is perfectly normal at birth and dark skin pigmentation is maintained throughout life. We propose that upregulated expression of *NR4A3* and/or *STX17* cause the Gray phenotype by promoting melanocyte proliferation. *NR4A3* has a firm association with cell cycle regulation and an established link with carcinogenesis, as chimeric fusions of *NR4A3* and *EWSR1*, *TCF12* or *TAF15* cause extraskeletal myxoid chondrosarcoma[17]. Furthermore, *CCND2*, which is a target gene for *NR4A3* (ref. 18), showed pronounced expression in Gray melanomas. Cyclins are crucial regulators of the cell cycle, and upregulation of cyclin expression is associated with tumor development[20]. It has recently been reported that MC1R signaling induces expression of NR4A genes, including *NR4A3* (ref. 21); this may explain why Gray horses carrying an *ASIP* null mutation have a higher incidence of melanomas. A possible link between *STX17* and tumor development is less obvious, but a previous study reported nuclear localization of STX17 in some malignant cells and suggested that STX17 interacts with RAS[16]. The RAS signaling pathway is of utmost importance in melanoma development, and more than 50% of human melanomas have mutations in *RAS* or *BRAF*[22,23].

Since the first description of melanomas in Gray horses in 1903 (ref. 24), researchers have questioned how a mutation causing loss of hair pigmentation can also cause melanomas with a massive production of melanin. Our results suggest a possible explanation. Hair-follicle and dermal melanocytes have different life cycles[25,26]. When a new hair grows, melanocytes are recruited from a pool of stem cells. Hair-follicle melanocytes are terminally differentiated and undergo apoptosis when pigment synthesis of the new hair is complete. Incomplete maintenance of melanocyte stem cells in $Bcl^{-/-}$ mice leads to premature hair greying[27]. Notably, melanocytes in the epidermis and dermis of glabrous skin (areas in which horse melanomas primarily occur) from these mice survived throughout the hair cycle. We propose that the *STX17* duplication leads to proliferation of dermal melanocytes in glabrous skin, thus predisposing to melanoma development. In contrast, hyperproliferation of hair-follicle melanocytes may cause premature depletion of stem cells. This interpretation is supported by the fact that young Gray horses undergo a darkening of coat color before the graying process is initiated[1].

METHODS

Genotyping. Long-range PCR with Expand Long Template PCR System Mix 1 (Roche) was used to genotype the 4.6-kb duplication. One forward primer (DupForward; **Supplementary Table 3** online) and two different reverse primers (DupReverseN for the normal copy and DupReverseD for the duplicated copy) were used in the same reaction. The PCR was run using 125 ng of genomic DNA, and the primer content was 3.75 pmol of DupForward, 2.5 pmol of DupReverseN and 5 pmol of DupReverseD. The 11-bp deletion in *ASIP* was genotyped essentially as previously described[10].

Northern blot hybridizations. Total RNA from horse tissues or cell lines was extracted using the TRIzol (Life Technologies) protocol. mRNA was prepared using an Oligotex mRNA kit (Qiagen). Poly A+ RNA was electrophoretically separated on a denaturing formaldehyde agarose gel, transferred to a nylon membrane (Nybond N+, Amersham) and immobilized by UV irradiation. Random-primed ^{32}P-labeled probes were generated using the full-length coding region for each of *NR4A3*, *STX17*, *TXNDC4*, *INVS*, *CCND1*, *CCND2* and 18S rRNA. Hybridizations and washings were done using the ExpressHyb (Clontech) hybridization protocol.

Real-time PCR. Expression of *STX17*, *NR4A3* and 18S rRNA was analyzed by the comparative C_t method using the primers and probes given in **Supplementary Table 3**. PCR was performed in 25-µl reaction volumes using TaqMan Buffer A (Applied Biosystems), 0.7 µM each of forward and reverse primer, 0.25 µM of TaqMan probe, 3.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.625 units of AmpliTaq Gold DNA polymerase (Applied Biosystems). The PCR reactions were done using an ABI7700 instrument (Applied Biosystems). All samples were analyzed in triplicate.

Recording and statistical analysis of Lipizzaner data. Horses from five national studs in Austria, Croatia, Hungary, Slovakia and Slovenia were examined up to three times during annual visits. Coat color was measured with a CR-300 Chroma-Meter (Minolta), with the parameter L* of the CIE L*a*b* color system indicating lightness. Melanomas were graded according to a previously described classification system[28], as described in detail in **Supplementary Methods** online. The grading of vitiligo and speckling are also described in **Supplementary Methods**. Statistical analysis involved the comparison of heterozygous and homozygous Gray horses within age categories and a linear model including age, *STX17* and *ASIP* genotypes and their interaction; the interaction was not significant and was removed from the model.

Analysis of allelic imbalance of *NR4A3* and *STX17* expression in *Gray* heterozygotes (*G/g*). Total RNA was extracted from melanoma tissue or cell lines using TRIzol (Life Technologies). cDNA was made using the Advantage RT-for-PCR kit (Clontech) and purified with a Chroma Spin TE-10 column (Clontech). PCR reactions were carried out in a total volume of 25 µl containing 75 ng genomic DNA or 100 ng cDNA. PCR fragments were gel-purified with an EZNA Gel Extraction kit (Omega Bio-tek) and sequenced.

Database accession numbers. The sequence data presented in this paper have been submitted to GenBank with the following accession numbers: EU595709–EU595728 and EU595728–EU606027.

URLs. The genome assembly of the horse is available at http://www.broad.mit.edu/mammals/horse/. Further information on the EquCab1 genome is available at http://genome.ucsc.edu/cgi-bin/hgGateway.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

G.R.P. was responsible for marker development, positional cloning, characterization of the *STX17* transcripts and real-time PCR analysis; A.G. was responsible for generation of antibodies to STX17, immunohistochemistry and northern blot analysis; E.S. was responsible for genotyping the Lipizzaner population material and analyzing allelic imbalance in melanoma tissue; I.C., M.H.S., T.D., R.B. and J.S. collected phenotypic data and blood samples from Lipizzaners; J.S. did the statistical analysis of genotype-phenotype relationships; J.L. and C.-H.H. took part in the functional characterization of *STX17* and *NR4A3*; M.H.S. and M.V. established Gray melanoma cell lines and provided skin samples from Gray and non-Gray horses; M.B. provided samples from Gray tumors and helped isolate BAC clones; C.F. assisted with northern blot analysis; G.L. assisted with characterization of BAC clones; K.S. provided samples from Gray and non-Gray horses; S.S. and F.P. assisted with immunohistochemistry analysis; M.G., C.W. and K.L.-T. did the bioinformatics analysis of the horse genome assembly; L.A. planned the study and prepared the manuscript with input from the other authors.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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