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Report

The Genetic Basis of White Tigers

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Summary

The white tiger, an elusive Bengal tiger (*Panthera tigris tigris*) variant with white fur and dark stripes, has fascinated humans for centuries ever since its discovery in the jungles of India [1]. Many white tigers in captivity are inbred in order to maintain this autosomal recessive trait [2–5] and consequently suffer some health problems, leading to the controversial speculation that the white tiger mutation is perhaps a genetic defect [6]. However, the genetic basis of this phenotype remains unknown. Here, we conducted genome-wide association mapping with restriction-site-associated DNA sequencing (RAD-seq) in a pedigree of 16 captive tigers segregating at the putative *white* locus, followed by whole-genome sequencing (WGS) of the three parents. Validation in 130 unrelated tigers identified the causative mutation to be an amino acid change (A477V) in the transporter protein SLC45A2. Three-dimensional homology modeling suggests that the substitution may partially block the transporter channel cavity and thus affect melanogenesis. We demonstrate the feasibility of combining RAD-seq and WGS to rapidly map exotic variants in nonmodel organisms. Our results identify the basis of the longstanding white tiger mystery as the same gene underlying color variation in human, horse, and chicken and highlight its significance as part of the species' natural polymorphism that is viable in the wild.

Results and Discussion

The White Tiger Is Not a True Albino

Coat color and pattern are prominent morphological features in mammals and play an essential role in survival. Individuals of the same species are often defined by shared morphological characteristics, and most species present an overall uniform coat color. Tigers are characterized by their iconic pattern of black stripes against an orange background; however, they are also among the several known mammalian taxa that display natural intraspecific coat color polymorphism (Figure 1A). The white tiger is a rare variant of the Bengal tiger (*Panthera tigris tigris*) that has dark or sepia brown stripes on white fur, blue eyes, a pink nose, and pink paw pads [2, 4, 5]. They were once observed sporadically in the wild on the Indian subcontinent, with the oldest record dating back to the 1500s [1]. In 1951, a male white tiger named Mohan was captured in Rewa, now part of Madhya Pradesh in India, from which numerous white tigers were bred for captivity [2–6]. The white tiger provides a precious opportunity to better understand mammalian coat color formation and adaptive pigmentation; however, except for the monogenetic autosomal recessive mode of inheritance, its genetic basis remains unknown.

Melanin is the pigment determining skin, hair, and eye color and has two major types: pheomelanin produces red to yellow colors, and eumelanin produces black to brown. Repression of either of these pigments influences specific color formation [7]. Melanin can be found in a variety of pigmented tissues and plays diverse roles in multiple biological pathways. Severe melanin deficiency or alterations have been connected to a number of human diseases such as oculocutaneous albinism, Hermansky-Pudlak syndrome, Griscelli syndrome, and melanoma [7].

The white tiger is not a true albino, in that although pheomelanin is largely absent, eumelanin is present in the eyes and in the hairs of stripes [2, 5]. Some white tigers also show strabismus, probably due to the reduction of pigment in the retinal epithelium and iris during eye development [8]. Robinson [2, 3] postulated that the white tiger mutation resembles a *chinchilla* allele at the *albino* locus because of its phenotypic similarity to the chinchilla variant in rodents and the Burmese breed of domestic cat. We therefore began by examining previously reported mammalian coat color-determining genes, including *MC1R*, *ASIP*, *TYR* (the *albino* gene), *TYRP1*, and *SLC7A11*, in both white and orange tigers. No variation associated with the white tiger was observed (see Table S2 available online), disproving Robinson's proposed genetic mechanism.

A Single Amino Acid Change in SLC45A2 Causes the White Tiger Phenotype

We refer to the white tiger-determining gene as a distinct "*white* locus" with two alleles: *W*, the wild-type, is dominant over *w*, the recessive mutant [5]. We recruited a *Ww* × *ww* captive tiger pedigree (n = 16) including seven white and nine wild-type tigers (Figures 1B and S1; Table S1). We performed whole-genome sequencing (WGS) in the three parents (Tables S3 and S4) at 30× genome coverage each and restriction-site-associated DNA sequencing (RAD-seq) [9–12] in the 13 offspring (Table S4). A total of 509,220 SNP markers were identified and aligned to the tiger reference genome (http://tigergenome.org), among which 172,554 contained data from at least 15 of the 16 individuals and were used for a genome-wide association study (GWAS) at an approximate marker density of 1 SNP per 14 kb. SNPs from scaffolds 75, 188, and 1458 showed significant association ($p < 0.001$) with the white phenotype (Figure 2A; Table S5). Scaffold 188 was discarded, as it failed to fit the recessive inheritance pattern. Scaffolds 75 and 1458 demonstrate conserved synteny with human chromosome 5 (19.71–36.04 Mb, hg19) and cat

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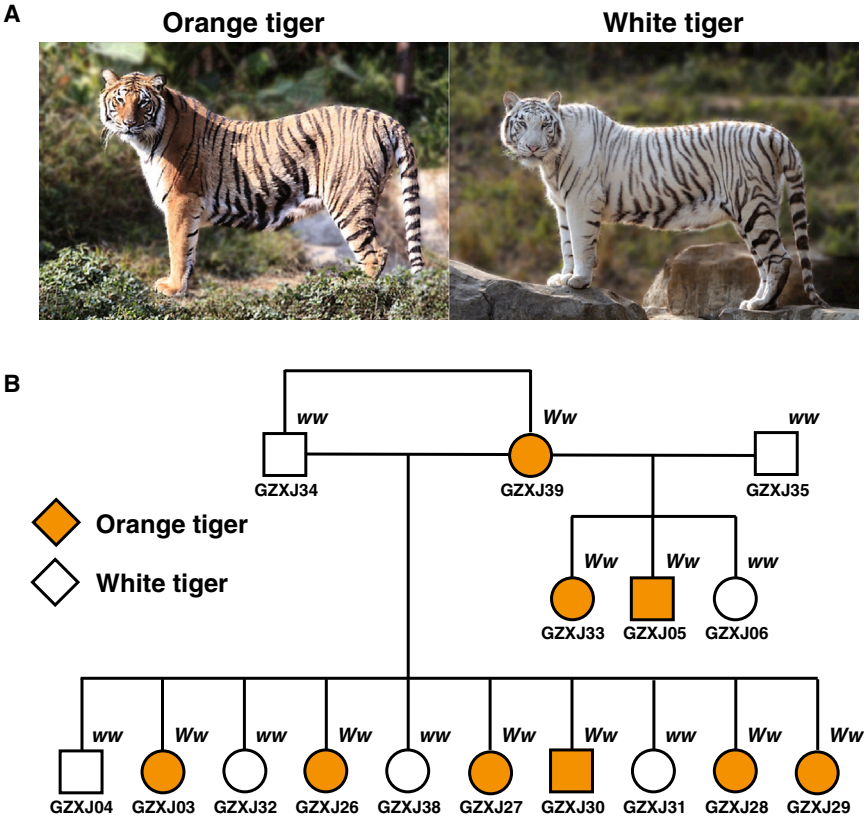

Figure 1. Tiger Coat Color and Pedigree

(A) The white tiger mutant (*ww*, right) is recessive to the orange (*WW* or *Ww*).

(B) The SLC45A2 A477V substitution cosegregates with the white phenotype in a pedigree that includes seven white and nine orange tigers (*W* = wild-type A477 allele; *w* = mutant A477V allele).

See also Table S1.

chromosome A1 (210.57–223.49 Mb, felCat5) and are adjacent to each other on the same chromosome (Figure 2B).

Linkage analysis in these two candidate scaffolds revealed one haplotype block of 3.3 Mb at complete linkage disequilibrium (LD, r^2 = 1; Figure 2C). All white tigers examined in the pedigree were fixed for a single haplotype spanning 52 SNPs from position 871,133 to 4,192,353 in scaffold 75 (Figure 2C; Table S6). All wild-type tigers (*Ww* at the *white* locus) from the pedigree were heterozygous for this haplotype.

Annotation of the tiger reference genome suggested 23 genes in this candidate LD interval (Figure 2C). Through scanning all SNPs among the three WGS parent genomes within the candidate region, we identified seven genes displaying polymorphisms in the coding regions between white and wild-type tigers (Table S7). Nonsynonymous substitutions in two genes were considered putative mutations, including the C-to-T transition in exon 7 in *SLC45A2* (solute carrier family 45 member 2, also known as *MATP* or *AIM-1*), which corresponds to alanine-to-valine substitution at amino acid residue 477 (A477V), and the D1125A substitution in *ADAMTS12*.

The two genes are tightly linked, are approximately 300 kb apart on the chromosome, and are perfectly segregated in the five white and eight wild-type first-generation offspring. In an extended study that included 130 unrelated tigers from various sources, the phenotype correlated exactly with the A477V substitution in *SLC45A2*, but not the D1125A substitution in *ADAMTS12* (Table 1). *SLC45A2* is a pigmentation-related gene in humans, whose polymorphisms are associated with light skin color in modern Europeans and pathogenic mutations known to cause oculocutaneous albinism type 4 (OCA4; Figure 3A) [13–18]. Mutations in *SLC45A2* also cause lightened skin and/or hair pigmentation in several other vertebrate species, including mouse, horse, chicken, and medaka fish (Figure 3A) [18–21]. These results support that *SLC45A2* is the tiger *white* gene, and that the single amino acid change A477V is causative for the recessive white phenotype.

SLC45A2 Homology Modeling Suggests the Functional Effect of the A477V Substitution

Residue A477 of the 560 amino acids of SLC45A2 is highly conserved among vertebrates (Figures 3A and 3B), and a mutation at the same position (A477T) has been reported only once in humans, in an OCA4 German with pale skin and dark blonde hair [15]. To decipher the potential functional impact of the A477V substitution, we generated a three-dimensional protein structure homology model of SLC45A2. The modeled SLC45A2 structure consists of 12 transmembrane helices connected by loop regions, forming a transporter protein-like structure (Figure 3C). The substrate transportation cavity is surrounded by four transmembrane helices: TM4, TM5, TM10, and TM11 (Figure 3D). Residue A477 is located on TM11 facing the inner surface of the transporter cavity toward the cytoplasm, and the two additional methyl groups introduced in the valine substitution of A477 lead to a reduction in cavity size (Figure 3D). We speculate that the A477V substitution might partially block the cavity, hindering substrate transport of SLC45A2.

Mutation in *SLC45A2* Primarily Affects Pheomelanin Pigmentation in the Tiger

The *SLC45A2* missense mutation in the white tiger primarily inhibits the synthesis of red/yellow pheomelanin, with no or only minor effect on black eumelanin, a specific phenotypic feature also observed in *SLC45A2* mutant cream horses and silver chickens [20, 21]. It is intriguing that some recessive null allele mutations in *SLC45A2*, such as the *underwhite* allele in mouse and the *sex-linked imperfect albinism* allele in chicken [18, 20], inhibit both eumelanin and pheomelanin pigmentation, whereas some missense mutations, such as the chicken *Silver* allele, are dominant and cause a specific inhibition of pheomelanin in homozygotes or heterozygotes. The *Cream* allele of *SLC45A2* in horses primarily affects pheomelanin in heterozygotes, yet it inhibits both eumelanin and pheomelanin synthesis in homozygotes [21]. Although the white tiger mutation mimics the effects of *SLC45A2* mutations in chickens and horses, it is apparently a missense yet recessive allele. Further studies on these missense mutations in the tiger,

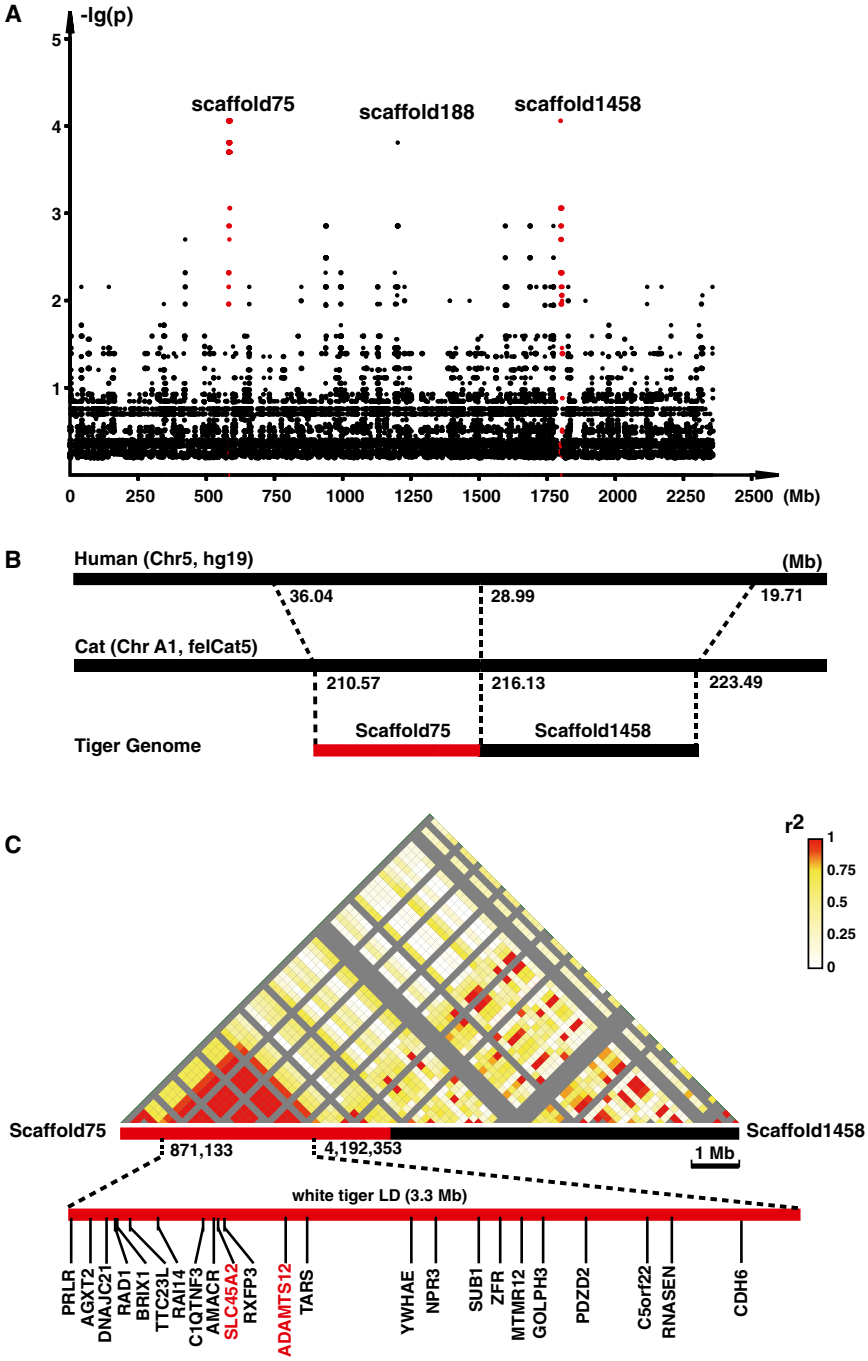

Figure 2. Genetic Mapping of the White Tiger Mutation Based on RAD-Seq and Whole-Genome Sequencing

(A) Genome-wide p values (y axis) of 172,554 SNPs are plotted every 500 kb based on the reference tiger genome scaffold numeric order (x axis). SNPs within one scaffold are arranged by level of significance, and those from highly associated scaffolds are marked in red.

(B) Conserved synteny of tiger genome scaffolds 75 and 1458 to the cat and human genomes.

(C) Haplotype block at linkage disequilibrium (LD) with the white phenotype based on 186 restriction-site-associated DNA SNPs. Regions without SNP coverage are gray. The diagram below indicates all genes within the candidate region in the tiger genome. See also Figure S1 and Tables S3, S4, S5, S6, and S7.

chicken, and horse may shed light on the specific function of SLC45A2 associated with the eumelanin and pheomelanin pathways.

The various phenotypic outcomes of *SLC45A2* mutations cannot be explained based on the current limited understanding of its function. Because cysteine is essential for pheomelanin production, it has been speculated that one function of SLC45A2 might be to transport cysteine into the melanosome, and that this function may be disrupted by missense mutations at the locus [20]. Alternatively, mouse SLC45A2 was reported to be involved in pigmentation by regulating the processing and trafficking of tyrosinase, the enzyme critical for melanin biosynthesis [22]. Previous studies have suggested that SLC45A2 may be a sucrose or proton transporter because it shares structural similarity and the signature RXGRR motif with sucrose/proton symporters in plants [18, 19]. This is supported by the recent discovery of the *Drosophila melanogaster* sucrose/proton cotransporter SCRT, which phylogenetically clusters with the SLC45 protein family and exhibits the highest degree of amino acid similarity with SLC45A2 [23]. Tyrosinase processing and trafficking is regulated by organellar pH [24], and in addition, the crenate melanosome found in the *uw* mouse mutant [22, 25] implies disruption of osmotic balancing that is probably due to the disruption of sucrose transportation [22]. Taking the evidence together, it is plausible that SLC45A2 is a sucrose/proton symporter and mediates melanin synthesis by regulating organellar pH and/or osmotic balancing.

A recent study on feline coat patterns proposed that the patterned coat is sustained by the comparatively high expression of *EDN3* in dark marking regions (e.g., cats' tabby stripes and cheetahs' spots), which stimulates region-specific eumelanogenesis (*TYR*, *TYRP1*, *DCT*, *SILV* upregulated) through the EDNRB pathway [26]. The mechanism may be conserved in the Felidae and explains why the white tiger retains dark stripes despite its melanogenesis being affected by SLC45A2.

Implications for Tiger Conservation

All tested white tigers were homozygous for the SLC45A2 A477V allele, and only one orange tiger carried the mutant allele in the heterozygous form (Table 1; see also Table S1), consistent with the idea that the *white* mutation has evolved only once and that its frequency is probably never high. The last known free-ranging white tiger was shot in 1958, before which sporadic sightings were made in India [27].

Table 1. Correlation between Tiger Coat Color Phenotypes and *SLC45A2* Genotypes

Gene	Genotype	Phenotype	
		White	Wild-Type
SLC45A2	A477V/A477V	20 (7)	0
	A477V/+	0	1 (9)
	+/+	0	109
ADAMTS12	D1125A/D1125A	20 (7)	19
	D1125A/+	0	30 (9)
	+/+	0	60
	missing data	0	1
Total		20 (7)	110 (9)

Numbers represent unrelated individuals used in the extended validation study. Numbers in parentheses represent related individuals from the pedigree shown in Figure 1B. See also Tables S1, S2, and S7.

Reasons for the extinction of wild white tigers were likely the same as those accounting for the dramatic decline in wild tigers in general: uncontrolled trophy hunting, habitat loss, and habitat fragmentation [28].

Public admiration for exotic animals has driven the captive breeding of white tigers from only a few individuals, which are highly inbred in order to preserve this recessive trait. Inbreeding depression has thus become the primary cause of many health problems for white tigers in captivity, such as premature death, stillbirth, and deformities [6, 8]. This has led to speculation that the white tiger trait is a genetic deformity. However, the fact that many white tigers captured or shot in the wild were mature adults suggests that a white tiger in the wild is able to survive without its fitness being substantially compromised [6]. The undesirable traits often associated with captive white tigers are thus most likely due to human-induced inbreeding. Indeed, *SLC45A2* mutations in human and chicken (e.g., the White Leghorn breed) worldwide rarely cause phenotypic features other than hypopigmentation [13–18, 20]. Therefore, we argue that the SLC45A2 A477V substitution in the tiger primarily affects only pigmentation, and that the white tiger morph is a viable natural genetic polymorphism. Despite its low frequency, this polymorphism has persisted for at least several hundred years and should be considered a part of the genetic diversity of tigers that is worth conserving.

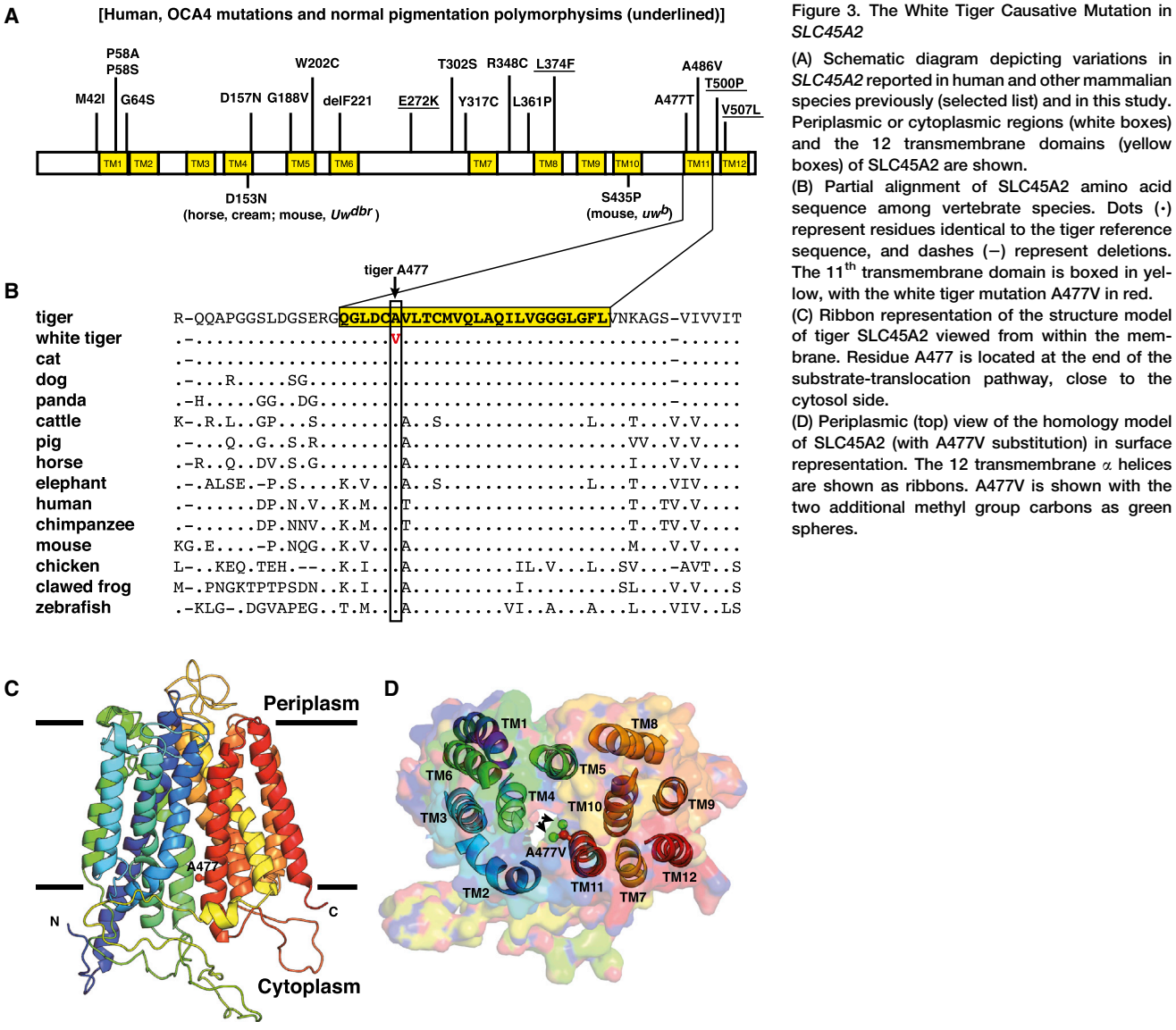

Figure 3. The White Tiger Causative Mutation in *SLC45A2*

(A) Schematic diagram depicting variations in *SLC45A2* reported in human and other mammalian species previously (selected list) and in this study. Periplasmic or cytoplasmic regions (white boxes) and the 12 transmembrane domains (yellow boxes) of SLC45A2 are shown.

(B) Partial alignment of SLC45A2 amino acid sequence among vertebrate species. Dots (·) represent residues identical to the tiger reference sequence, and dashes (–) represent deletions. The 11th transmembrane domain is boxed in yellow, with the white tiger mutation A477V in red.

(C) Ribbon representation of the structure model of tiger SLC45A2 viewed from within the membrane. Residue A477 is located at the end of the substrate-translocation pathway, close to the cytosol side.

(D) Periplasmic (top) view of the homology model of SLC45A2 (with A477V substitution) in surface representation. The 12 transmembrane α helices are shown as ribbons. A477V is shown with the two additional methyl group carbons as green spheres.

Conclusion

Mapping the *white* mutation in the tiger to the A477V substitution in the transporter protein SLC45A2 proves the efficiency of using pedigree-based GWAS followed by WGS to identify causative genes and mutations based upon next-generation RAD-seq in an exotic species. For the variety of charismatic nonmodel organisms carrying unique or significant phenotypes that are controlled by single genes, this approach promises to be a simple, efficient, and cost-effective means of gene and mutation mapping. Because the SLC45A2 A477V substitution affects the white tiger's pigmentation only, without causing severe physiological defects, we argue that the white tiger morph is a naturally occurring and viable feature of genetic diversity in tigers.

Accession Numbers

Genome resequencing and RAD-seq reads have been deposited in the NCBI BioProject database (SRP017677) under the accession numbers SRS381413–SRS381428 and SRS381821.

Supplemental Information

Supplemental Information includes one figure, seven tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.04.054.

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