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**Donskoy cats as a new model of oculocutaneous albinism with the identification of a splice-site variant in *Hermansky-Pudlak Syndrome 5* gene**

**Running Title:** Feline *HPS5* splice-site variant

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**Abstract**

In the feline Donskoy breed, a phenotype that breeders call "pink-eye", with associated light-brown skin, yellow irises and red-eye effect, has been described. Genealogical data indicated an autosomal recessive inheritance pattern. A single candidate region was identified by genome-wide association study and SNP-based homozygosity mapping. Within that region, we further identified *HPS5* (*HPS5 Biogenesis Of Lysosomal Organelles Complex 2 Subunit 2*) as a strong candidate gene, since *HPS5* variants have been identified in humans and animals with Hermansky-Pudlak syndrome 5 or oculocutaneous albinism. A homozygous c.2571-1G>A acceptor splice-site variant located in intron 16 of *HPS5* was identified in pink-eye cats. Segregation of the variant was 100% consistent with the inheritance pattern. Genotyping of 170 cats from 19 breeds failed to identify a single carrier in non-Donskoy cats. The c.2571-1G>A variant leads to *HPS5* exon-16 splicing, that is predicted to produce a 52 amino-acids in-frame deletion in the protein.

These results support an association of the pink-eye phenotype with the c.2571-1G>A variant. The pink-eye Donskoy cat extends the panel of reported *HPS5* variants and offers an opportunity for in-depth exploration of the phenotypic consequences of a new *HPS5* variant.

## Significance

Models in large animals, rather than rodents, have proved relevant to model human conditions and to fill the gap between preclinical and clinical research. Companion animals exhibiting spontaneous disease and traits provide potential animal models to better characterize phenotypes and identify new genetic variants. Here we describe the genetic characterization of the first spontaneous model of Hermansky Pudlak Syndrome 5 in the domestic cat (*Felis catus*). The identification of a new variant in *HPS5* will allow further study of the genotype-phenotype correlation in Hermansky Pudlak Syndrome 5.

## Keywords

*Felis catus*, Hermansky-Pudlak Syndrome 5, albinism, BLOC2S2, ruby-eye 2

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Feline DNA samples are part of the Feli-DNA biobank, which is part of the CRB-Anim infrastructure [ANR-11-INBS-0003, in the framework of the "Investing for the Future" program (PIA)].

## Funding information

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## **Abstract**

In the feline Donskoy breed, a phenotype that breeders call "pink-eye", with associated light-brown skin, yellow irises and red-eye effect, has been described. Genealogical data indicated an autosomal recessive inheritance pattern. A single candidate region was identified by genome-wide association study and SNP-based homozygosity mapping. Within that region, we further identified *HPS5* (*HPS5 Biogenesis Of Lysosomal Organelles Complex 2 Subunit 2*) as a strong candidate gene, since *HPS5* variants have been identified in humans and animals with Hermansky-Pudlak syndrome 5 or oculocutaneous albinism. A homozygous c.2571-1G>A acceptor splice-site variant located in intron 16 of *HPS5* was identified in pink-eye cats. Segregation of the variant was 100% consistent with the inheritance pattern. Genotyping of 170 cats from 19 breeds failed to identify a single carrier in non-Donskoy cats. The c.2571-1G>A variant leads to *HPS5* exon-16 splicing, that is predicted to produce a 52 amino-acids in-frame deletion in the protein.

These results support an association of the pink-eye phenotype with the c.2571-1G>A variant. The pink-eye Donskoy cat extends the panel of reported *HPS5* variants and offers an opportunity for in-depth exploration of the phenotypic consequences of a new *HPS5* variant.

## **Keywords**

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## **1 Introduction**

Pedigree cats have been selected for aesthetic traits including coat colour, and progress has been made over the past twenty years to identify the genes involved in these traits. Initial strategies used candidate gene approaches and feline linkage maps developed at the end of the nineties. Several variants were identified by genome-wide linkage analysis, but this approach have some limitation. Individuals from large extended pedigrees need to be genotyped for the markers. Thus, classical linkage studies are often impossible to conduct in feline breeds with small size population or when a limited number of individuals exhibit the trait of interest. Tremendous progress was made in 2007 when the first cat genome sequence obtained from an Abyssinian female was published

(Pontius et al., 2007). Additional sequencing of numerous cats led to the identification of millions of SNPs (single nucleotide polymorphisms) that were then used to develop a feline SNP array, which became available in 2012 (Illumina 63K SNP array). Since this decisive breakthrough, genome-wide association studies (GWAS) became the standard method to search for genes and variants underlying feline traits. Indeed, GWAS requires fewer cases and controls, compared to linkage analyses. Finally, whole-genome sequencing became affordable. This new genomic tool allows to characterize variants by sequencing a single case and comparing it to a reference database of genomes (Gandolfi et al., 2015).

By using a candidate gene strategy, five variants have been shown to cause non-syndromic complete or partial albinism in cats. All these variants lie within the *TYR* (tyrosinase) gene. Three variants cause temperature-sensitive tyrosinase activity with peripheral pigmentation (Lyons et al., 2005a; Schmidt-Küntzel et al., 2005; Yu et al., 2019), similar to the oculocutaneous albinism (OCA) reported in a patient in 1991 (King et al., 1991; OCA1B, OMIM: 606952). The other two alleles are loss-of-function variants that cause complete oculocutaneous albinism (Imes et al., 2006; Abitbol et al., 2017), a phenotype homologous to human OCA1A (OMIM: 203100).

Seven loci are involved in non-syndromic oculocutaneous albinism in humans (OCA1 to OCA7, Frederico et al., 2019). Syndromic OCA includes Chediak-Higashi Syndrome (OMIM: 214500), Hermansky-Pudlak Syndrome (HPS, OMIM: 203300), Griscelli Syndrome (OMIM: 214450), Elejalde Syndrome (OMIM: 256710) and Cross Syndrome (OMIM: 257800).

HPS associates OCA and various features including a bleeding diathesis, and, in some patients, pulmonary fibrosis, granulomatous colitis, or immunodeficiency. Variants in *AP3B1* (*Adaptor Related Protein Complex 3 Subunit Beta 1*, HPS2), *AP3D1* (*AP3 Subunit Delta 1*, HPS10), *BLOC1S3* (*Biogenesis Of Lysosomal Organelles Complex 1 Subunit 3*, HPS8), *BLOC1S6* (HPS9), *BLOC2S1* (*Biogenesis Of Lysosomal Organelles Complex 2 Subunit 1*, HPS3), *BLOC2S2* (HPS5), *BLOC2S3* (HPS6), *BLOC3S1* (*Biogenesis Of Lysosomal Organelles Complex 3 Subunit 1*, HPS1), *BLOC3S2* (HPS4) and *DTNBP1* (*Dystrobrevin Binding Protein 1*, HPS7) human genes have been identified (OMIM: 203300).

HPS5 is described as a mild form of HPS since patients lack the severe complications present in other HPS, in particular pulmonary fibrosis. The typical clinical presentation includes hypopigmentation, reduced visual acuity and mild prolonged bleeding time.

In animals, HPS5 has been described in the mouse (*ruby-eye2*, Zhang et al., 2003, MGI:1856144), stickleback fish (Hart et al., 2017), zebrafish (Daly et al., 2013), silkworm (Fujii et al., 2012) and drosophila (Falcón-Pérez et al., 2007, Syrzycka et al., 2007).

A new coat colour was reported in 2007 in Donskoy cats in Ukraine and in 2015 in the United States of America (USA). Cats showed reduced pigmentation, yellow irises with red-eye effect under direct light, suggesting a new phenotype of OCA. The Donskoy (or Don Sphynx) is a Russian breed whose main characteristic is hypotrichosis. The Donskoy history started in the 1980s in Russian city of Rostov-on-Don, where a hairless female kitten born to outbred cats was found. This female is the founder of the breed, that was first recognized in 1997 by the World Cat Federation ([wcf-online.de](http://wcf-online.de)).

Here we report the identification of a splice-site recessive variant in *HPS5*, suggesting that the Donskoy OCA is a Hermansky-Pudlak syndrome 5, the first one described in cats.

## 2 Material and Methods

### 2.1 Animals

A total of 198 cats from 19 breeds were included in the genetic study. They were sampled in Europe and the USA, from September 2010 to June 2019 and included individuals from the following breeds: Donskoy (n = 35 including eight pink-eye cats and 27 non pink-eye cats), Birman (n = 25), Domestic shorthair and longhair (n = 24), Maine Coon (n = 15), Devon Rex (n = 11), Persian (n = 11), Exotic shorthair (n = 9), Chartreux (n = 9), British shorthair and longhair (n = 7), Oriental shorthair and longhair (n = 7), Ragdoll (n = 7), Sphynx (n = 6), Norwegian Forest (n = 6), Russian Blue shorthair and longhair (n = 6), Bengal (n = 6), Siamese (n = 5), Abyssinian (n = 4), Cornish Rex (n = 4) and Siberian (n = 1). All cats were included following their owners' consent. Donskoy cats were collected specifically for this study. Non-Donskoy control cats were collected for genetic studies through a feline DNA banking initiative. Non-invasive buccal swabs were sent back directly by owners or collected by a veterinarian. Four Donskoy cats were spontaneously presented to their regular veterinarian. They were clinically evaluated prior to blood collection.

Pedigrees were collected from the owners. Genealogical data were drawn using GenoPro ([www.genopro.com](http://www.genopro.com)).

### 2.2 Ethics Statement

All animals were client-owned cats on which no harmful invasive procedures were performed, so there was no animal experimentation according to the legal definition in Europe (Subject 5f of Article1, Chapter I of the Directive 2010/63/UE of the European Parliament and of the Council).

In four Donskoy cats, blood was obtained as part of routine clinical procedures for diagnostic purposes, at the request and with the consent of the owners.

DNA from other cats were obtained non-invasively (cheek swabs) specifically for this study or for zootechnical purposes, at the request of their owners who consented that their cats' DNA could be used in research projects aiming at improving animal health and welfare.

### **2.3 Eye examination**

Kitten eye examination was performed without any sedation by a certified ophthalmology specialist, at the request and with the consent of the owner.



## 2.4 Nucleic acid extractions

DNA was extracted from whole blood and buccal swabs according to the manufacturers' protocols, using either a Maxwell® 16 Instrument (Promega Corporation, Madison, USA), or the NucleoSpin 96 Tissue DNA Kit (Macherey-Nagel EURL, Hoerdt, France).

Total RNA from a pink-eye cat and from a control Devon Rex cat was extracted from buccal cells collected by owners using cheek swabs and preserved in RNAlater (Ambion Europe Ltd, Cambridge, UK). NucleoZOL and NucleoSpin RNA Set for NucleoZOL (Macherey-Nagel, Düren, Germany) were used. RNA was treated with RQ1 RNase-Free DNase (Promega Corporation, Madison, WI, USA) and stored at -80°C.

## 2.5 SNP genotyping and genome-wide association study

Twenty-three Donkoy cats were genotyped using the Illumina Infinium iSelect 63k Cat DNA SNP genotyping array (Illumina, Inc., San Diego, CA). For each cat, 700 ng of genomic DNA was sent to the Neogen laboratory ([www.neogen.com](http://www.neogen.com)). Arrays were processed according to the manufacturer's protocol. SNP genomic positions were inferred according to the felCat5 SNP manifest for the Illumina Feline 63k SNP array (Willet & Haase, 2014).

SNP genotyping rate and minor allele frequency were assessed using the PLINK software (Purcell et al., 2007; <http://pngu.mgh.harvard.edu/purcell/plink/>). SNPs with a MAF < 5%, genotyping rate < 95%, and individuals genotyped for < 95% of SNPs were excluded from downstream analyses. Case-control association analyses were performed using PLINK. The *p* values were corrected according to Bonferroni procedure. A multi-dimensional scaling (MDS) with two dimensions was performed on 59,146 SNP in PLINK to evaluate population substructure within cases and controls. Clustering of individuals and permutation testing based on 2x2xK Cochran-Mantel-Haenszel statistics (CMH) were performed using PLINK. Inflation of *p* values was evaluated using the inflation factor calculated by PLINK (Purcell et al., 2007; <http://pngu.mgh.harvard.edu/purcell/plink/>). Manhattan plots of the results were generated using R ([www.r-project.org/](http://www.r-project.org/)).

## 2.6 Whole genome sequencing

An Illumina TruSeq PCR-free DNA library with 350 bp insert size of a pink-eye Donskoy cat was prepared by GATC Biotech (GATC Biotech AG, Konstanz, Germany). GATC generated 150 bp paired-end reads on an Illumina HiSeq 3000 instrument (30x coverage). Mapping and alignment

were performed using the *Felis\_catus* 8.0 genome reference. The sequence data were deposited under the BioProject accession ID PRJNA632484 and the BioSample accession ID SAMN14910606 at the sequence read archive (SRA, <https://www.ncbi.nlm.nih.gov/sra>). Variant calling and filtering were performed by GATC using GATK (McKenna et al., 2010). To predict the functional effects of the called variants, SnpEFF (Cingolani et al., 2012) software, together with annotation release 102 for *Felis\_catus* 8.0 assembly, was used.

## 2.7 *HPS5* genotyping by sequencing

Reference genomic sequence was collected from Ensembl: [www.ensembl.org](http://www.ensembl.org); feline *HPS5* gene (ENSFCAG00000011158, annotation release 104 for *Felis\_catus* 9.0 genome assembly).

PCR and sequencing primers were designed using Primer3 (Untergasser et al., 2012). Genomic sequence for exon 17 and intron 16 splice acceptor site was amplified and sequenced using the following forward and reverse of primers and an annealing temperature of 60°C: 5'-GCTTAACACCTGAGCCAC-3' and 5'-ACCGTTGATCTTCAGGCCT-3'. The sequence was amplified individually for each cat from 100 ng of their genomic DNA according to the manufacturers' protocol, with GoTaq G2 Hot Start Polymerase and 35 cycles (Promega Corporation, Madison, WI, USA). Four hundred ng of each PCR amplicon were sent to GATC Biotech (GATC Biotech AG, Konstanz, Germany); purified and Sanger-sequenced in both forward and reverse directions. Electropherograms were manually inspected with Chromas Lite (Technelysium Pty Ltd, South Brisbane, Australia).

Genomic coding sequences of *HPS5* intron 16 with splice acceptor site from wild-type and pink-eye (variant) Donskoy cats (*Felis catus*) were submitted to GenBank. Accession numbers are (GenBank: MT339222) for the wild-type allele and (GenBank: MT339221) for the c.[2571-1G>A] variant allele.

## 2.8 Protein sequence comparisons and structural prediction

Cat, human and mouse, *HPS5* sequences were collected from Ensembl [[www.ensembl.org](http://www.ensembl.org); cat: (ENSFCAT00000011162.6, annotation release 104 for *Felis\_catus* 9.0 genome assembly), human: (ENST00000349215.7), mouse: (ENSMUST00000014562.13)]. Multiple alignments were performed using Multalin (Corpet 1988; <http://multalin.toulouse.inra.fr/>; BLOSUM-62 and identity matrix). The predicted impact of missense variants was assessed using PROVEAN (Choi et al., 2012; [http://provean.jcvi.org/seq\\_submit.php](http://provean.jcvi.org/seq_submit.php)). Prediction of exons and exon-intron

structures in feline genomic sequences were assessed using NNSPLICE 0.9 (Reese et al., 1997; [http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)). Human HPS5 protein domains were extracted from Genome3D (Sillitoe et al., 2019; <http://www.genome3d.eu>).

## 2.9 RT-PCR and cDNA sequencing

Reverse transcription was performed using 250 ng of total RNA, an exon 19 *HPS5* specific primer (5'-ACCCAGGAAAGCAAGTG-3') and a GoScript Reverse Transcriptase kit, according to manufacturer's protocol (Promega Corporation, Madison, WI, USA).

Amplification by PCR of a sequence spanning from exon 16 to exon 18 of *HPS5* was achieved using a couple of primers located in *HPS5* exon 16 (5'- AAATGGCAAGTTCCAGTCC-3') and *HPS5* exon 18 (5'- AGCATCGTGGCTCACTGC-3'), 200 ng of cDNA from the pink-eye and the control cats, GoTaq G2 Hot Start Polymerase and 45 cycles (Promega Corporation, Madison, WI, USA).

Four hundred ng of each PCR amplicon were sent to GATC Biotech (GATC Biotech AG, Konstanz, Germany); there, they were purified and Sanger-sequenced in both forward and reverse directions. Electropherograms were manually inspected with Chromas Lite (Technelysium Pty Ltd, South Brisbane, Australia).

## 2.10 Accession numbers

The whole-genome sequence data from the pink-eye cat were deposited under the BioProject accession ID PRJNA632484 and the BioSample accession ID SAMN14910606 at the sequence read archive (SRA, <https://www.ncbi.nlm.nih.gov/sra>). Genomic sequences of *HPS5* intron 16 splice acceptor site from wild-type and pink-eye (variant) Donskoy cats (*Felis catus*) were submitted to GenBank. Accession numbers are (GenBank: MT339222) for the wild-type allele and (GenBank: MT339221) for the c.[2571-1G>A] variant allele.

## 3 Results

### 3.1 An OCA phenotype in a cat family

In July 2015, a Donskoy cat breeder contacted us regarding a male kitten born to two black parents and showing a pale skin colour different from the usual skin colours observed in the breed. The kitten had three black littermates (Figure 1a). The skin colour has persisted throughout growth

and at adulthood and the cat had a light-brown / beige skin, a light-brown nose leather, yellow / light-green irises with a strong red-eye effect under direct light. Coat colour DNA tests performed by the breeder revealed that the kitten was homozygous for the non-agouti *a* allele at the *Agouti* locus (*a/a*, solid colour, Eizirik et al., 2003), homozygous for the dominant black allele at the *Brown* locus (*B/B*, black eumelanin, Lyons et al., 2005b; Schmidt-Küntzel et al., 2005), homozygous for the non-diluted dominant allele at the *Dilute* locus (*D/D*, intense pigmentation, Ishida et al., 2006) and homozygous for the dominant full-colour allele at the *Colour* (*tyrosinase*) locus (*C/C*, no albinism and no colour-restriction, Lyons et al., 2005a; Schmidt-Küntzel et al., 2005). Thus, the kitten was wild type at the *B*, *D* and *C* loci which together with the *a/a* genotype was expected to cause a solid black colour.

Physical examination of the kitten performed by the breeder's regular veterinarian did not reveal any abnormality. The eye examination was normal and indicated yellow irises. The fundus lacked any pigment and tapetum and therefore appeared as an albinotic type with only visible retinal and choroidal blood vessels with normal white scleral background.

Altogether, the data were consistent with an OCA with residual skin and eye pigmentation, distinct from the colourpoint, sepia, mink and mocha temperature-sensitive colour-restrictions (Lyons et al., 2005a; Schmidt-Küntzel et al., 2005; Yu et al., 2019) and distinct from the complete OCA previously reported in cats (Imes et al., 2006; Abitbol et al., 2017).

Towards the end of 2015, a second breeder, who owned Donskoy cats related to the parents of the male kitten, informed us of additional cats showing the same phenotype (Figure 1b). Breeders called these cats with pale skin colour and red-eye effect "pink-eye" cats. Pedigree examination revealed close common ancestors and was consistent with an autosomal recessive inheritance pattern (Figure 1c). Eight pink-eye cats were reported to us, none of which had presented any apparent deleterious phenotype during the physical examination performed by their regular veterinarian. In particular they lacked colitis and lung fibrosis. Neutropenia was not evaluated.

To initiate the identification process of the molecular aetiology of this new phenotype, DNA was collected from relevant available cats.

Prior to whole genome analyses, candidate gene sequencing was performed using DNA from a pink-eye cat (Supporting Information Supplementary Material and Methods, Table S1). Exons and exon-intron boundaries from six OCA candidate genes (*TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, *C10orf11*) were sequenced and compared to the reference feline sequence. No candidate variant was found (Supporting Information Table S2).

### 3.2 GWAS and homozygosity mapping revealed a single locus on chromosome D1

GWAS using SNP arrays have proved successful for identifying recessive variants in felines when cohorts of several affected cases and controls are available. In the present study, eight pink-eye cats were available for the genotyping assay. To circumvent this potential limitation, we used a combined GWAS and homozygosity mapping strategy. Twenty-three Donskoy cats (including the eight pink-eye cats) were genotyped using the Illumina Feline 63k SNP array, among which 59,146 SNPs yielded usable results (minor allele frequency MAF > 5%, genotyping rate > 95%). All 23 cats had genotyping rates > 95 % and all were conserved for the analysis. Samples were evaluated for population stratification by multi-dimensional scaling. The analysis revealed three main clusters of samples that did not reflected clustering based on case and control status (Supporting Information Figure S1). Following basic case-control analysis, the highest significant association was identified for SNP chrD1.112279828 ( $p_{raw} = 2.6 \times 10^{-6}$ , Felis\_catus 6.2 genome sequence/felCat5 SNP manifest) located on feline chromosome D1 at position 76,996,428 (Felis\_catus 9.0 genome sequence) (Figure 2a). After Bonferroni correction of the  $p_{raw}$  values for multiple tests, 27 SNPs had  $p_{Bonferroni} < 0.1$  (Figure 2b). These were 25 SNP located on chromosome D1, one SNP located on chromosome B2 (chrB2.15987060,  $p_{raw} = 2.6 \times 10^{-6}$ ,  $p_{Bonferroni} = 0.11$ ) and one SNP located on chromosome C2 (chrC2.23037192,  $p_{raw} = 1.6 \times 10^{-5}$ ,  $p_{Bonferroni} = 0.66$ ). Three unassigned SNP (chromosome "unknown") with  $p_{Bonferroni} < 0.1$  (chrUn18.8141785, chrUn18.7193177 and chrUn18.7217697) were relocated on chromosome D1 according to the Felis\_catus 9.0 genome sequence (Supporting Information Table S3). No SNP had a  $p_{Bonferroni} < 0.05$  (Figure 2b). The  $p$  values and chromosomal locations of the 15 most associated SNP, according to the Felis\_catus 6.2 and Felis\_catus 9.0 genome sequences, are shown in Table 1. We then performed a case-control analysis to control for stratification. We first performed clustering of individuals on the basis of autosomal genome-wide SNP data. Given the case-control phenotype and the set of three clusters defined, we performed permutation testing based on 2x2xK Cochran-Mantel-Haenszel statistics. The best SNP were the same as in the initial association analysis and pointed the same locus on chromosome D1 (Supporting Information Table S3). The inflation factor was reduced from 2.76 (basic case-control analysis) to 1.03. We also performed an association analysis with genomic-controlled  $p$  values. Results led to the same best SNP markers (Supporting Information Table S3).

In addition to the GWAS, genotypes for each chromosome were manually inspected to identify homozygous regions shared by the eight pink-eye cats. Two regions located on chromosomes B1 and D1 were identified (Supporting Information Table S4). Only one region from chromosome D1 was consistent with the non-homozygously mutated status of the control cats and the highly probable heterozygous status of the four carrier cats who gave birth to pink-eye kittens (Figure 1c). This region encompassing 2.8 Mb lay between SNP chrD1.111090678 and SNP chrD1.114601810 and included the most significantly associated SNP identified in the GWAS (Figure 2c). These two SNPs were located at positions 76,063,418 and 78,842,539 respectively in the *Felis\_catus* 9.0 genome assembly. Comparison of genes within or in the vicinity of the identified region with the list of genes possibly causing OCA (OMIM: 203100), Chediak-Higashi Syndrome (OMIM: 214500), HPS (OMIM: 203300), Griscelli Syndrome (OMIM: 214450) or Elejalde Syndrome (OMIM: 256710) revealed *HPS5* as the best candidate gene (chromosome D1: 76,175,177-76,241,946 bp, GenBank ID: 101099749, *Felis\_catus* 9.0 genome sequence, GenBank annotation release 104). *HPS5* encodes BLOC2S2, the deficient protein in human HPS type 5.

### 3.3 A splice-site variant in *HPS5* segregates in the Donskoy family

Along with the GWAS and homozygosity mapping we analyzed the whole genome sequence of a pink-eye cat to screen for variants across the candidate region, as well as to survey albinism candidate genes across the entire genome. Paired-end reads ( $2 \times 150$  bp) were collected from a shotgun DNA fragment library from a pink-eye cat (BioProject ID: PRJNA632484; BioSample ID: SAMN14910606), achieving genome-wide coverage of 30x. SNPs and indels were called against the *Felis\_catus* 8.0 reference genome. We identified 212 homozygous variants with a high predicted impact and 7827 homozygous variants with a moderate predicted impact (Supporting Information Table S5). Three variants from chromosome D1 drew our attention, namely two missense variants and a splice-site variant located in *HPS5*, the best candidate gene. The two missense variants were predicted by PROVEAN to be neutral variants: c.817A>G (T273A change predicted in the protein, score: -1.096) and c.1753T>G (rs785849714, Y585D change predicted in the protein, score: 1.405). The c.2571-1G>A splice-site variant was located in intron16 and was predicted to modify a consensus acceptor splice-site (NNSPLICE 0.9 score = 0). Available members of the Donskoy family segregating the pink-eye phenotype were genotyped for the c.2571-1G>A *HPS5* variant. In addition to the sequenced cat, this validation cohort included 27

Donskoy cats from the family. Genotypes showed a perfect concordance with the phenotypes and all pink-eye carrier cats were heterozygous for the variant (Figure 1c).

Finally, we genotyped 170 control cats from 19 breeds, including seven Donskoy cats not linked to the family - none of them carried the *A* allele for the c.2571-1G>A variant (Table 2). Due to the rarity of Donskoy cats (34 kittens registered in France in 2019, French Feline Stud Book LOOF: www.loof.asso.fr), we were not able to assess the frequency of the c.2571-1G>A variant in the breed (Table 2).

Screening for putative deleterious SNPs or Indels in the six OCA genes (*OCA1* to *OCA4*, *OCA6*, *OCA7*), eight HPS genes (*HPS1* to *HPS4*, *HPS6*, *HPS7*, *HPS9*, *HPS10*) and *LYST* failed to identify candidate variants in the whole sequence of the pink-eye cat (Supporting Information Table S6).

Hence the 100% concordance observed between the autosomal recessive mode of inheritance of the pink-eye phenotype and the genotypes of cats from and outside our pedigree, corroborated our hypothesis of full association between the c.2571-1G>A variant in *HPS5* and the OCA reported in Donskoy cats.

### **3.4 The associated allele causes *HPS5* exon 17 splicing and is predicted to code a shortened protein**

As the c.2571-1G>A variant was predicted to modify a consensus acceptor splice-site, we performed an *in silico* prediction of exons and exon-intron structures in *HPS5* genomic sequences from a wild-type cat and from a pink-eye cat (Figure 3a). The c.2571-1G>A variant was confirmed to disrupt the acceptor splice-site in *HPS5* intron 16. Three acceptor splice-sites were predicted in *HPS5* intron 17. Two of them were alternative sites located within intron 17. Their use was predicted to lead to frameshifts. The third one was the consensus acceptor splice-site of intron 17. Splicing of *HPS5* exon 17 was predicted to lead to an in-frame deletion in the protein (Figure 3a).

To evaluate the relevance of the three alternative splicing hypotheses, *HPS5* partial coding sequences were obtained from one pink-eye cat and one control cat using mRNA extracted from buccal cells, RT-PCR and sequencing. The sequence of the RNA transcript revealed that the consensus intron 17 acceptor splice-site was employed, leading to exon 17 splicing (Figures 3b and 3c). *In silico* translation predicted the loss of 52 amino acids in the *HPS5* protein. The functional importance of these 52 amino acids was evaluated using an alignment of the feline

HPS5 protein sequence with HPS5 sequences from human and mouse (Figure 3d) and indicated a 89% identity between feline and human HPS5 proteins. The predicted 52 amino acid deletion lay in the leucin-rich repeat variant domain of HPS5. This domain spans from amino acid 758 to 1129 in the human protein and from amino acid 761 to 1132 in the feline protein (genome3d.eu, Figure 3d). The predicted deletion removed amino acids 857 to 908 in the cat protein (Figure 3d).

Altogether, these results strongly suggest that the OCA observed in Donskoy cats is an autosomal recessive trait associated with a splice-site variant in *HPS5*.

## 4 Discussion

### 4.1 Mild OCA observed in Donskoy cats is consistent with a Hermansky-Pudlak syndrome type 5

According to breeders, the first cats with a light-brown skin were observed in progeny from Ukrainian catteries who imported founders from the first Russian cattery of the breed. The initial variant Donskoy cats were judged as "chocolate" in cat shows but unlike chocolate cats from other breeds their eyes were bright red under certain lighting conditions. The feline chocolate coat colour is governed by the *B* locus (*b* recessive chocolate allele). It rapidly became apparent that mating variant cats with cats known through laboratory testing to be homozygous *b/b* for the *b* allele, did not produce chocolate kittens.

We identified a c.2571-1G>A variant in the intronic sequence of *HPS5* in pink-eye cats, leading to exon 17 splicing and predicted *in silico* to produce an in-frame 52 amino acid deletion in HPS5. Our results suggest that the identified c.[2571-1G>A] allele yields a HPS5 protein with deficient protein-protein interactions.

Among the eight pink-eye cats included in this study, we were warned that two male cats had undergone surgical neutering. None of them had exhibited prolonged bleeding time or any complication. But no specific exploration of blood clotting has been performed.

Breeders reported no visual impairment, but as Donskoy cats are mainly indoor cats, visual defects are not easy to assess.

Altogether, reduced skin and iris pigmentation, red-eye effect and reduced retinal pigment on fundoscopic examination, and the apparent absence of bleeding tendency are consistent with a mild OCA in pink-eye cats. This phenotype is also consistent with a HPS type 5, as variants in *HPS5* in human patients are associated with a spectrum of phenotypes combining OCA or OA



with or without visual impairment, bleeding diathesis and colitis (Michaud et al., 2017; Botero et al., 2018; Wei et al., 2019).

#### **4.2 The *HPS5* variant is restricted to Donskoy cats**

By using the combined approach, we were able to identify a c.2571-1G>A splice-site variant in *HPS5*. Genotyping of an additional cohort of 163 cats from 18 non-Donskoy breeds for the *HPS5* c.[2571-1G>A] allele failed to identify carriers (Table 2). The Donskoy hairless variant appeared spontaneously and was first reported in Russia in the late 1980s. Before official recognition of the breed, Donskoy cats were mated with Domestic Shorthair cats. Other breeds were included in the genetic pool, including Siamese and Oriental Shorthair during creation of the Peterbald, a breed resulting from crossing Oriental and Donskoy cats. Outcrossing was stopped 15 years ago (tica.org). In our feline cohort, the c.[2571-1G>A] *HPS5* allele was restricted to Donskoy. To our knowledge, pink-eye cats have not been described in Oriental or Siamese cats, or in any other breed of cats. A single pink-eyed cat was described in 1961. This outbred domestic shorthair female, found in Springfield (USA), had a light tan coat, yellow irises with a red-eye effect and likely suffered from visual impairment. She was described as pink eyed. She died from intestinal obstruction before that in-depth exploration of her case could be performed (Todd, 1961).

We can therefore hypothesize that the *HPS5* variant was present at the beginning or during the first years of development of the Donskoy. The allele could i - have been introduced by a Domestic Shorthair cat used to increase the genetic pool of the breed, ii - could have been present in the foundation female or iii - may have arisen spontaneously in a cat from the breeding stock during the expansion of the breed. A haplotype analysis performed in a large cohort of Donskoy and Domestic Shorthair cats from Russia and Ukraine would be necessary to explore the origin of the c.[2571-1G>A] *HPS5* allele.

#### **4.3 The *HPS5* deficient cat: a potential novel large animal model for HPSs**

HPS type 5 is a rare condition described as a mild form of HPS. It is likely underdiagnosed because of the mild phenotype and this condition is often hard to distinguish from mild OCA or ocular albinism. Since the publication of the first *HPS5* mutation in 2003 (Zhang et al., 2003), 29 patients and 31 variants in human *HPS5* have been reported (Michaud et al., 2017; Stephen et al., 2017; Botero et al., 2018; Wei et al., 2019). Most patients exhibit mild hypopigmentation, mild bleeding and no pulmonary fibrosis or colitis (Michaud et al., 2017). Thanks to animal models, the

HPS proteins have been identified to be subunits of four-protein complexes: adaptor protein-3 (AP-3) and BLOC-1, -2 and -3 (biogenesis of lysosome-related organelles complex). These complexes are involved in the biology of lysosome-related organelles, particularly in pathways required for content delivery and function of melanosomes, dense granules, lung lamellar bodies and organelles from immune cells (Wei 2006; Bowman et al., 2019). HPS5 encodes BLOC2S2, a subunit protein that localizes to tubular endosomes and which interacts with HPS3 (BLOC2S1) and HPS6 (BLOC2S3) proteins to form BLOC-2, (Zhang et al., 2003; Gautam et al., 2004). In melanocytes, BLOC-2 is involved in melanosome cargo delivery, that is required for endomembrane organelle maturation (Dennis et al., 2015). BLOC-2, in association with other actors, could control the generation and stabilization of endosomal tubular transport intermediates, i.e. recycling endosomes (Shakya et al., 2018). The roles of BLOC-2 in other cell types are not yet known precisely (Bowman et al., 2019). The predicted 52 amino acid deletion of pink-eye cats lies in the leucin-rich repeat variant (or Armadillo repeat) domain of HPS5, probably involved in protein-protein interactions (genome3d.eu). BLOC-2 architecture might thereby be destabilized by the deletion. Indeed, destabilization of BLOC-2 has been shown in HPS3, HPS5 or HPS6 defective cells from human patients (Nazarian et al., 2008; Wei et al., 2019).

Animal models of HPS5 include only one mammalian model, the *ruby-eye2* mouse. The interest of the feline HPS5 model may reside not in the molecular dissection of the lysosome-related organelles biology but rather in study of the genotype-phenotype correlation. Pink-eye cats need to be phenotypically characterized in-depth. Their mild OCA does not seem to be associated with deleterious characteristics. But additional clinical examinations are required to better characterize the model. The required analyses have been postponed by the rarity of Donskoy cats. An experimental cross could help in generating pink-eye individuals. These cats might provide a complementary large animal model that mimics the orthologous human condition. Such cats could be used for comparative analyses and to fill the gap between rodent models and humans in the evaluation of future therapeutic strategies for HPS, especially severe forms of HPS as HPS proteins share common biological pathways. Additionally, the pink-eye cat may serve as a non-rodent model to evaluate splicing modulation therapy. Of course, ethical issues related to the use of a companion animal in experimental procedures and the cost of husbandry will have to be carefully balanced with scientific data. Consent of the owners and ethical committees will be a prerequisite to any preclinical trial performed in cats.

In conclusion, we described the first *HPS5* variant reported in cats. The recessive c.[2571-1G>A] allele, which can easily be detected by a genetic test, is associated with the pink-eye phenotype, a feline model of OCA and HPS.

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## **Author Contribution**

MM performed experiments, analysed and interpreted data. CH and MR analysed genotypic data. CDDC recruited control cats. VG supervised the study. MA conceived and supervised the study, recruited cats, performed experiments, analysed and interpreted data, prepared figures and wrote the manuscript. All authors revised the manuscript.

## **Conflict of Interest Statement**

Antagene is a for-profit company selling DNA tests for animals.

The authors declare no conflict of interest.

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## Figure Legends

### Figure 1. Mild oculocutaneous albinism in a Donskoy cat family

(a) Picture of a Donskoy male kitten showing a light-brown skin and red-eye effect. Breeders call this particular phenotype "pink-eye". The kitten stands with three black littermates, their black mother and a non-Donskoy cat. (b) Picture of an adult pink-eye Donskoy cat. Notice the light-brown colour of the nose and the yellow irises. (c) Pedigree-tree of a Donskoy cat family segregating the pink-eye phenotype. Circles represent females, squares represent males. Pink-eye cats are depicted with fully filled symbols. Carriers are depicted with two-toned symbols. When available, result of the genotyping assay for the c.2571-1G>A variant is mentioned. Stars indicate two cats with a chocolate coat colour registration on their pedigrees but which were known by breeders to be pink-eye cats. PE and C refer to cats from the family who were genotyped for the GWAS (Figure 2). PE: pink-eye cat. C: control cat.

### Figure 2. Genome-wide association study and homozygosity mapping

Manhattan plot of the GWAS. The plots represent the  $p_{raw}$  (a) and  $p_{Bonferroni}$  (b) values of each SNP included in the case-control association study. The association study compared the eight pink-eye Donskoy cats with 15 control Donskoy cats. A suggestive association with chromosome D1 was detected. The four SNPs with the highest association were D1.112279828, D1.112359805, D1.111233416 and B2.15987060, with a  $p_{raw}$  value of  $2.6 \times 10^{-6}$  and  $p_{Bonferroni}$  value of 0.11 (Table 1). UN: unknown. (c) Genotypes for chromosome D1 candidate region. SNP genotypes for each cat were manually inspected in Excel to identify homozygous regions shared by the pink-eye cats. Only one region from chromosome D1 spanning from position 111,090,678 bp to position 114,601,810 bp (according to the updated felCat5 SNP manifest for the Illumina Feline 63k SNP genotyping array) was consistent with the non-homozygously mutated status of the control cats and the likely heterozygous status of the four carrier cats. This region encompassed 2.8 Mb. Homozygosity for the allele shared by the pink-eye cats is shown in pink. Heterozygosity or homozygosity for the opposite allele is shown in light green. Missing genotypes are noted as 0. bp: base pairs. PE: pink-eye cat. C: control cat.

### Figure 3. Consequences of the c.2571-1G>A variant

(a) *In silico* prediction of exons and exon-intron structures in *HPS5* exon 16 to exon 18 genomic sequence from a wild-type cat and from a pink-eye cat. The wild-type sequence (WT cat) was excerpted from Ensembl (ENSFCAG00000011158, annotation release 104 for *Felis\_catus* 9.0 genome assembly). Structures were predicted using NNSPLICE 0.9. Wild type splice sites are shown according to Ensembl (ENSFCAG00000011158). The c.2571-1G>A variant (shown in red) was predicted to abolish the intron 16 acceptor site (score = 0). The two alternative acceptor sites located in intron 17 and exhibiting the strongest scores (0.98 and 1) are shown for the pink-eye cat sequence. Predicted consequences of the three splicing hypotheses for the pink-eye cat are shown below. The third hypothesis, further confirmed by RT-PCR and sequencing, is shown in red. (b) Electropherograms from partial sequences of feline *HPS5* cDNA. A cDNA sequence spanning from exon 16 to exon 19 was amplified in a control cat and in a pink-eye cat. Sequences showing junctions between exons are shown. (c) Alignment of cDNA sequences of *HPS5* from exon 16 to exon 19. A cDNA sequence spanning from exon 16 to exon 19 was amplified in a control cat and in a pink-eye cat using primers located in exon 16 and exon 19 (grey boxes). Sequencing revealed the complete deletion of exon 17 in the pink-eye cat. The feline reference sequence (Ref-ex16-19) was excerpted from Ensembl (ENSFCAG00000011158). Exon 16 is shown in green, exon 17 is shown in pink, exon 18 is shown in blue and exon 19 is shown in brown. Dots represent absent nucleotides (d) Alignment of protein sequences of HPS5, translated from the wild type alleles reported in human; (ENST00000349215.7), mouse (ENSMUST00000014562.13) and cat (WTcat, ENSFCAT00000011162.6, annotation release 104 for *Felis\_catus* 9.0 genome assembly). The YVTN/WD40 domain of the protein, shown by the blue bar, starts with amino acid number 29 in human and cat proteins and ends with amino acid number 358 (<http://www.genome3d.eu>). The leucin-rich repeat variant domain of the protein, shown by the pink bar, starts with amino acid number 758 in human protein and with amino acid 761 in cat protein and ends with amino acid numbers 1129 and 1132 in human and cat proteins respectively (<http://www.genome3d.eu>). The position of the 52 amino acids predicted to be deleted in the pink-eye HPS5 protein (amino acids 857 to 908) is shown in red. The feline HPS5 sequence was used as the reference sequence. Conserved residues are represented by dots in human and mouse sequences. Dashes represent deletions.

## Tables

**Table 1. Fifteen highest associated SNPs in the GWAS**

CHR	SNP	6.2 position	9.0 position	$P_{raw}$	$P_{Bonferroni}$
D1	chrD1.112279828	112279828	76996428	$2.615 \times 10^{-6}$	0.111
D1	chrD1.112359805	112359805	77065787	$2.615 \times 10^{-6}$	0.111
D1	chrD1.111233416	111233416	76170702	$2.615 \times 10^{-6}$	0.111
B2	chrB2.15987060	15987060	14165743	$2.615 \times 10^{-6}$	0.111
D1	chrD1.124596371	124596371	79818023	$5.636 \times 10^{-6}$	0.2391
UN	chrUn18.8141785	Un18.8141785	D1: 86854996	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.124331264	124331264	80057162	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.123452060	123452060	80786285	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.122438243	122438243	81642395	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.118504450	118504450	84997231	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.124211967	124211967	80148926	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.113172621	113172621	77687070	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.123294595	123294595	80915406	$6.593 \times 10^{-6}$	0.2798
D1	chrD1.110124037	110124037	75549888	$7.254 \times 10^{-6}$	0.3078
D1	chrD1.118687561	118687561	84835665	$8.378 \times 10^{-6}$	0.3555

CHR: chromosome. The 6.2 location is based on cat assembly version 6.2 (felCat5 SNP manifest for the Illumina Feline 63k SNP array). The 9.0 location is based on cat assembly version 9.0.

**Table 2. Genotypes for the c.2571-1G>A variant in 19 breeds of cats**

Breed	G/G	G/A	A/A	Total
Control Donskoy cats	7	0	0	7
Birman	25	0	0	25
Outbred Domestic SH and LH	24	0	0	24
Maine Coon	15	0	0	15
Devon Rex	11	0	0	11

Persian	11	0	0	11
Exotic SH	9	0	0	9
Chartreux	9	0	0	9
British SH and LH	7	0	0	7
Oriental SH and LH	7	0	0	7
Ragdoll	7	0	0	7
Sphynx	6	0	0	6
Norwegian Forest	6	0	0	6
Russian Blue SH and LH	6	0	0	6
Bengal	6	0	0	6
Siamese	5	0	0	5
Abyssinian	4	0	0	4
Cornish Rex	4	0	0	4
Siberian	1	0	0	1
Total	170	0	0	170

SH: shorthair, LH: longhair

## Supporting Information

### Supplementary Material and Methods

**Figure S1.** Multi-dimensional scaling (MDS) graph

**Table S1.** PCR and sequencing primers for OCA candidate genes

Sequences from the intronic primers that were used to amplify and sequence the coding exons

**Table S2.** Genomic variations in OCA candidate genes identified between pink-eye cat exonic sequences and the Abyssinian coding reference sequence

**Table S3.** Twenty-seven SNP markers with  $p_{Bonferroni} < 0.1$  in the GWAS

Positions are shown according to the *Felis\_catus* 6.2 and *Felis\_catus* 9.0 reference sequences.

CHR: chromosome. UN: unknown.

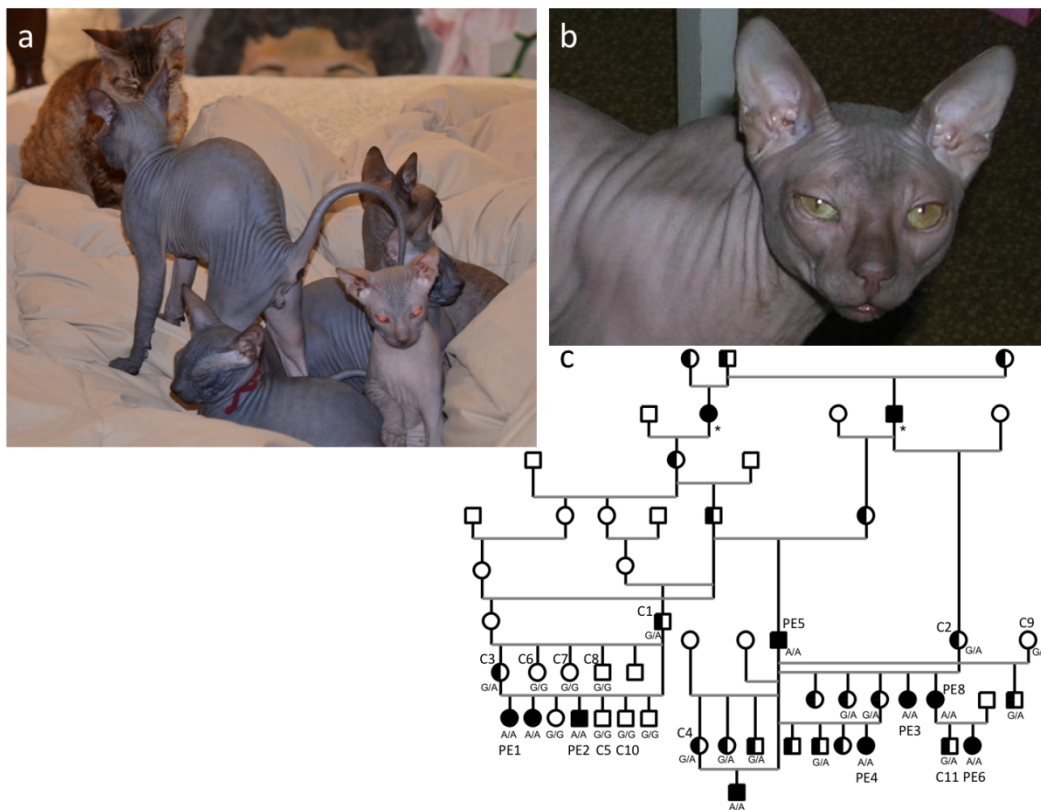
**Table S4.** Homozygous regions shared by pink-eye cats on chromosomes B1 and D1

SNP genotypes for each cat were manually inspected with Excel to identify homozygous regions shared by the pink-eye cats. A single region from chromosome D1 was consistent with the non-homozygously mutated status of the control cats. Homozygosity is shown in pink in pink-eye cats and in yellow in control cats. Heterozygosity is shown in white. Missing genotypes are noted as 0.

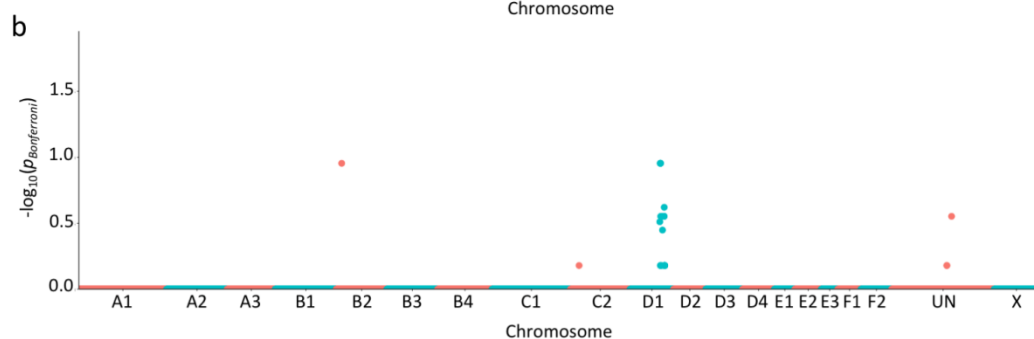
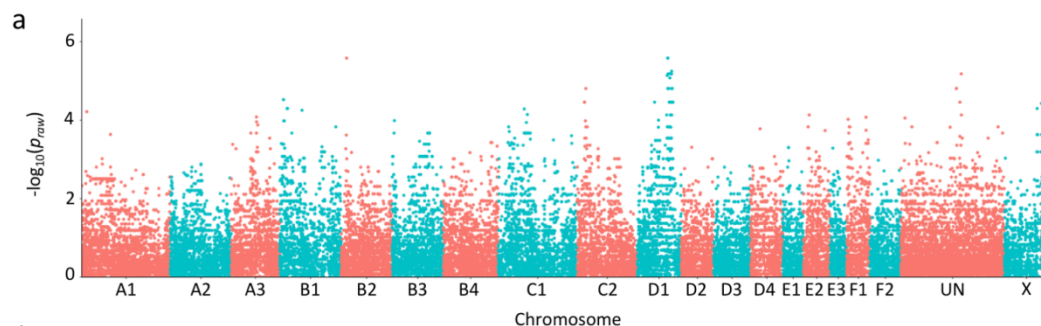
Chr: chromosome. bp: base pairs. PE: pink-eye cat. C: control cat.

**Table S5.** Homozygous variants with a predicted impact, identified in the whole genome sequence of a pink-eye cat

**Table S6.** Variants in OCA and HPS genes identified in the whole genome sequence of a pink-eye cat



pcmr\_12906\_f1.tiff



**c**

SNP	Position (bp)	Pink-eye							Carrier				Control												
		PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	
chrD1.110762019	110762019	CT	CT	TT	TT	TT	CT	TT	TT	CT	TT	CT	TT	CT	TT	TT	CT	CC	CT	CT	CT	TT	TT		
chrD1.110838505	110838505	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	CT	CT	CT	TT	
chrD1.110863636	110863636	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.110944935	110944935	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.110971012	110971012	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	
chrD1.111003337	111003337	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	
chrD1.111031931	111031931	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	GA	GA	GA	AA
chrD1.111090678	111090678	00	AG	GG	00	GG	GG	GG	GG	AG	AG	GG	GG	AG	AG	GG	GG	GG	GG	GG	GG	00	GG	AG	
chrD1.111233416	111233416	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CT	CT	CT	TT	CT	CT	TT	TT	TT	TT	
chrD1.111327742	111327742	AA	AA	AA	AA	AA	AA	AA	AA	AG	AG	AA	AG	AG	AA	AG	AG	AG	AG	AG	AG	AG	AG	GG	
chrD1.111355343	111355343	AA	AA	AA	AA	AA	AA	AA	AA	AG	AG	AA	AG	AG	AA	AG	AG	AG	AG	AG	AG	AG	AG	GG	
chrD1.111576337	111576337	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	CT	TT	CT	TT	CT	CT	TT	CT	TT	CC	CC	TT	TT	
chrD1.111678625	111678625	GG	GG	AG	AG	GG	AG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.111688919	111688919	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	TC	CC	CC	CC	CC	00	CC	TC	TT	TT	CC	CC	CC	
chrD1.111781023	111781023	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.111811623	111811623	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	TC	CC	TC	CC	TC	CC	TC	CC	CC	CC	CC	TT	
chrD1.111898825	111898825	AA	AA	AA	AA	AA	AA	AA	AA	GA	GA	GA	GA	GG	GA	GA	GA	GG	GA	GG	GA	GG	GA	GG	
chrD1.111905172	111905172	TT	TT	TT	TT	TT	TT	TT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT	CT	CT	CT	CT	CT	
chrD1.112028287	112028287	GG	GG	GG	GG	GG	GG	GG	GG	AG	AG	GG	GG	AA	AG	AG	AG	AA	GG	AG	AG	AG	AG	AG	
chrD1.112140179	112140179	TT	TT	TT	TT	TT	TT	TT	TT	CT	TT	TT	TT	CT	CT	TT	CT	TT	CT	TT	TT	TT	TT	TT	
chrD1.112214128	112214128	AA	AA	AA	AA	AA	AA	AA	AA	AG	AG	AG	AG	GG	AG	AG	GG	GG	AG	GG	AG	AG	AG	GG	
chrD1.112279828	112279828	GG	GG	GG	GG	GG	GG	GG	GG	GA	GA	GA	GA	AA	GA	GA	GA	AA	GA	AA	AA	AA	AA	AA	
chrD1.112359805	112359805	CC	CC	CC	CC	CC	CC	CC	CC	TC	TC	TC	TC	TT	TC	TC	TC	TT	TC	TC	TT	TT	TT	TT	
chrD1.112384041	112384041	AA	AA	AA	AA	AA	AA	AA	AA	GA	AA	AA	AA	GA	AA	AA	AA	GA	AA	AA	AA	AA	AA	AA	
chrD1.112411099	112411099	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.112499098	112499098	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	
chrD1.112581388	112581388	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.112858454	112858454	CC	CC	CC	CC	CC	CC	CC	CC	TC	TC	CC	CC	TC	TC	CC	TC	TC	CC	CC	CC	CC	TC	TC	
chrD1.11290339	11290339	AA	AA	AA	AA	AA	AA	AA	AA	AG	AG	AA	AA	AG	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	
chrD1.113172621	113172621	CC	CC	CC	CC	CC	CC	CC	CC	CT	CT	CT	TT	CC	CT	CT	TT	TT	CT	TT	TT	TT	TT	TT	
chrD1.11331266	11331266	GG	GG	GG	GG	GG	GG	GG	GG	GG	GT	GT	GT	GG	GT	GG	GT	GT	GT	TT	TT	TT	TT	TT	
chrD1.113355776	113355776	AA	AA	AA	AA	AA	AA	AA	AA	AA	AC	AC	AC	AA	AC	AA	CC	AC	AC	CC	AC	CC	CC	CC	
chrD1.113387079	113387079	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CT	CT	CT	CC	CT	CC	CT	CT	CT	TT	TT	TT	TT	
chrD1.113576547	113576547	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	
chrD1.113813419	113813419	GG	GG	GG	GG	GG	GG	GG	GG	AG	GG	GG	GG	AG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.113862177	113862177	CC	CC	CC	CC	CC	CC	CC	CC	TC	CC	CC	CC	TC	CC	CC	TC	CC	CC	CC	CC	CC	CC	CC	
chrD1.113890513	113890513	CC	CC	CC	CC	CC	CC	CC	CC	TC	CC	CC	CC	TC	CC	CC	TC	CC	CC	CC	CC	CC	CC	CC	
chrD1.113989943	113989943	CC	CC	CC	CC	CC	CC	CC	CC	CC	TC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	
chrD1.114148990	114148990	TT	TT	TT	TT	TT	TT	TT	TT	TT	CT	CT	CT	CT	TT	CT	TT	CC	CT	CC	CT	TT	CC	CC	
chrD1.114211536	114211536	AA	AA	AA	AA	AA	AA	AA	AA	AA	GA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	
chrD1.114249696	114249696	AA	AA	AA	AA	AA	AA	AA	AA	AA	CA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	CA	
chrD1.114294365	114294365	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GA	GA	GG	AA	GA	GA	AA	GA	AA	AA	AA	AA	GA	
chrD1.114333077	114333077	GG	GG	GG	GG	GG	GG	GG	GG	GG	AG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	
chrD1.114385748	114385748	CC	CC	CC	CC	CC	CC	CC	CC	CC	AC	AC	AC	CC	CC	AA	AC	CC	AA	AA	AA	AA	AA	AA	
chrD1.114415282	114415282	TT	TT	TT	TT	TT	TT	TT	TT	TT	TC	TC	TC	TT	TC	TC	TC	TC	TC	CC	CC	TC	CC	CC	
chrD1.114441239	114441239	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	CT	CT	CT	TT	CT	CT	CT	CT	CC	CT	TT	CT	CT	
chrD1.114510406	114510406	GG	GG	GG	GG	GG	GG	GG	GG	GG	GT	GT	GT	GG	GT	GG	GT	GT	GT	GT	GT	GT	GT	GT	
chrD1.114535351	114535351	TT	TT	TT	TT	TT	TT	TT	TT	TT	CT	CT	CT	CT	TT	CT	CT	CT	CT	CC	CT	CC	CT	CC	
chrD1.114601810	114601810	00	GA	AA	00	AA	AA	AA	AA	00	00	GA	GA	GA	00	GA	AA	GG	GA	GA	GG	GG	GA	GA	

