Yellow parrots
M1 Genomes and Phenotypes Exam – 4 December 2023
1h30
Questions 7, 8 and 9 are independent.

The rose-ringed parakeet (***Psittacula krameri, perruche à collier***) is a popular pet species and escaped birds have colonized a number of cities around the world including Paris. The yellow form is characterized by an absence of grey and blue pigments (Fig. 1).

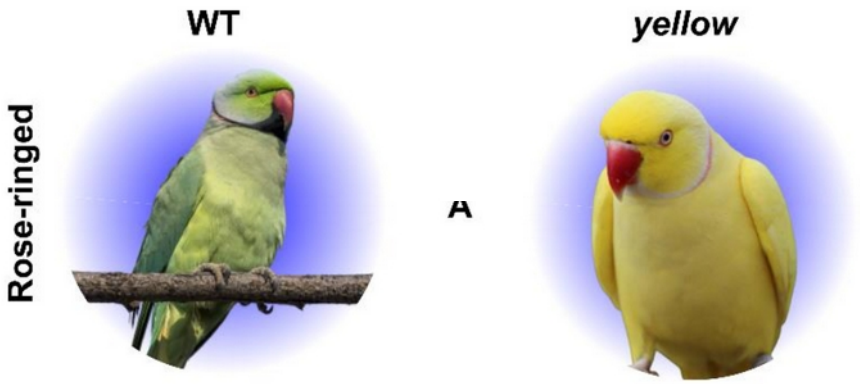

Fig. 1. Picture of two rose-ringed parakeets, a wild-type (left) and a yellow mutant (right).

Crosses were performed between birds and their progeny was examined.

Cross	Phenotype of the female parent	Phenotype of the male parent	Progeny
1	yellow	yellow	100 % yellow
2	WT	WT	100 % WT
3	yellow	WT	100 % WT
4	yellow	WT	Females: 50% yellow and 50% WT Males: 50% yellow and 50% WT
5	WT	yellow	All females yellow and all males WT

1. Under the hypothesis that the yellow phenotype is due to a single locus, what can you conclude about the *yellow (y)* allele based on the results of the crosses? Justify and write down the results of the five crosses. (3 pts)

Whole genome sequencing of two yellow birds and one WT bird was performed. Three candidate pigmentation genes were analyzed and protein-coding changes were found only in one of them, named *PkSLC45A2*. The coding region of *PkSLC45A2* was amplified by PCR from genomic DNA of a wild-type male and of a yellow male. Results of the Sanger sequencing of the PCR fragments are shown in Fig. 2.

2. Complete the sequence of the yellow male (yellow rectangles) using the genetic code table (Fig. 3). Interpret the results. (2 pts)

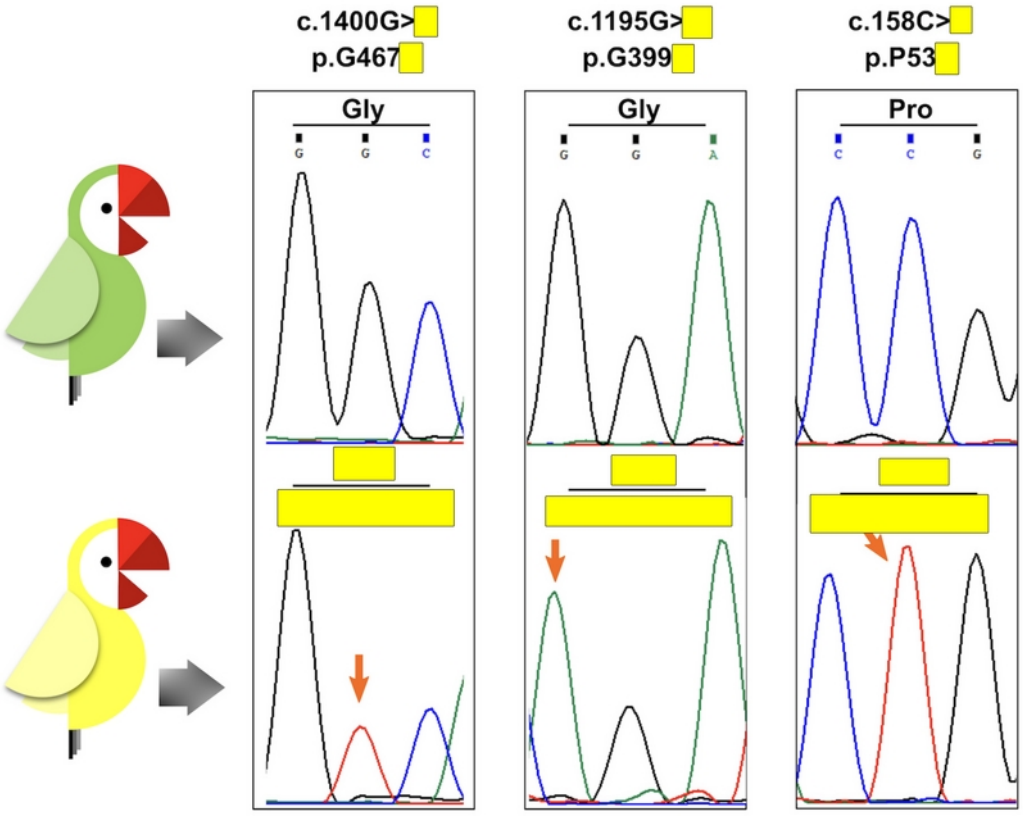

Fig. 2. Protein-coding mutations found in *PkSLC45A2* in a yellow bird (bottom) compared to a wild-type bird, for species *Psittacula krameri*.

	U	C	A	G
U	UUU Phe F	UCU Ser S	UAU Tyr Y	UGU Cys C
	UUC Phe F	UCC Ser S	UAC Tyr Y	UGC Cys C
	UUA Leu L	UCA Ser S	UAA Ter *	UGA Ter *
	UUG Leu L	UCG Ser S	UAG Ter *	UGG Trp W
C	CUU Leu L	CCU Pro P	CAU His H	CGU Arg R
	CUC Leu L	CCC Pro P	CAC His H	CGC Arg R
	CUA Leu L	CCA Pro P	CAA Gln Q	CGA Arg R
	CUG Leu L	CCG Pro P	CAG Gln Q	CGG Arg R
A	AUU Ile I	ACU Thr U	AAU Asn N	AGU Ser S
	AUC Ile I	ACC Thr U	AAC Asn N	AGC Ser S
	AUA Ile I	ACA Thr U	AAA Lys K	AGA Arg R
	AUG Met M	ACG Thr U	AAG Lys K	AGG Arg R
G	GUU Val V	GCU Ala A	GAU Asp D	GGU Gly G
	GUC Val V	GCC Ala A	GAC Asp D	GGC Gly G
	GUA Val V	GCA Ala A	GAA Glu E	GGA Gly G
	GUG Val V	GCG Ala A	GAG Glu E	GGG Gly G

Fig. 3. Table of the canonical genetic code.

The same genomic region was amplified and sequenced from a yellow mutant of another species of the same genus, *P. cyanocephala.*

3. Complete the sequence of the yellow male (yellow rectangles) in Fig. 4 using the genetic code table (Fig. 3). Interpret the results. (1 pt)

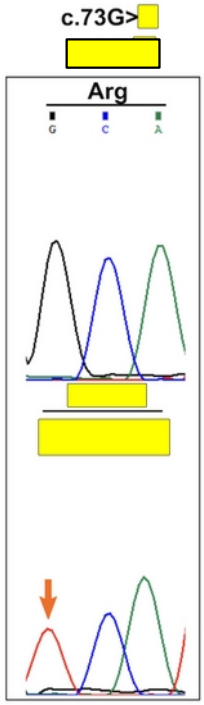

Fig. 4. Protein-coding mutation found in *PkSLC45A2* in a yellow bird (bottom) compared to a wild-type bird, for species *Psittacula cyanocephala*.

The amino acid sequence of the *P. krameri* SLC45A protein was compared with the homolog protein from various species (Fig. 5).

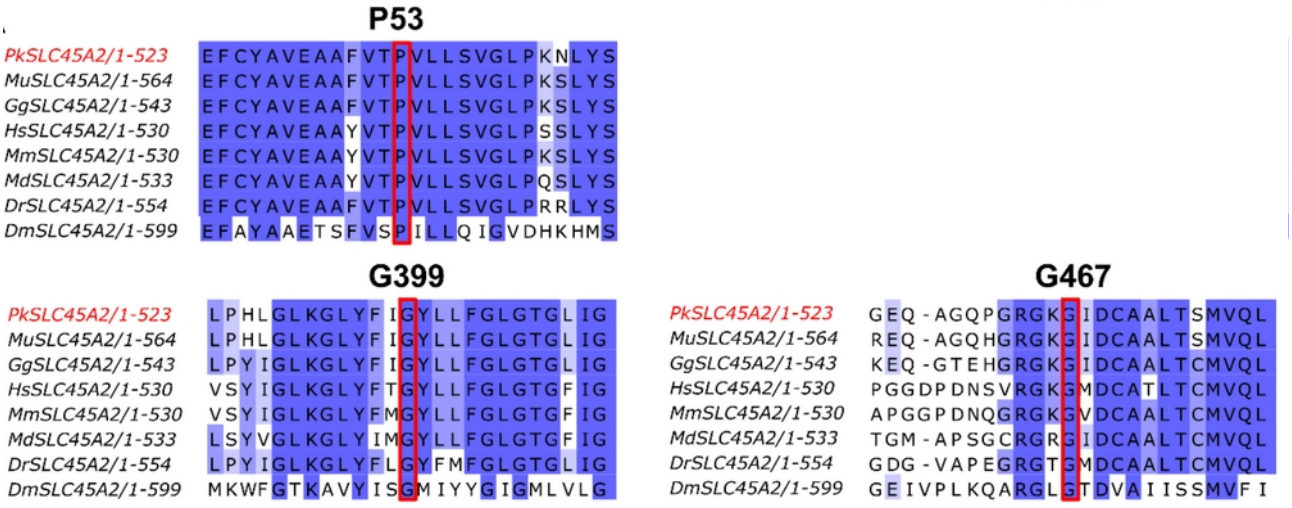

Fig. 5. Sequence alignment of parts of the SLC45A protein from several species: Pk: rose-ringed parakeet (*Psittacula krameri*); Mu: budgerigar (*Melopsittacus undulatus*); Gg: chicken (*Gallus gallus*); Hs: human (*Homo sapiens*); Mm: house mouse (*Mus musculus*); Md: gray short-tailed opossum (*Monodelphis domestica*); Dr: zebra fish (*Danio rerio*); Dm: fruit fly (*Drosophila melanogaster*).

4. Interpret the results of Fig. 5. (1 pt)

SLC45A2 is a transmembrane protein present in distinct punctate microdomains associated with the nucleus. The P53L mutation was introduced into a *PkSLC45A2* plasmid by site-directed mutagenesis and the protein was expressed in Hela cells under a constitutive promoter.

5. What is a plasmid?
Explain the different steps of the protocol, from plasmids to the images shown in Fig. 6. (3 pts)

6. Interpret the results of Fig. 6 (both images). (2 pts)

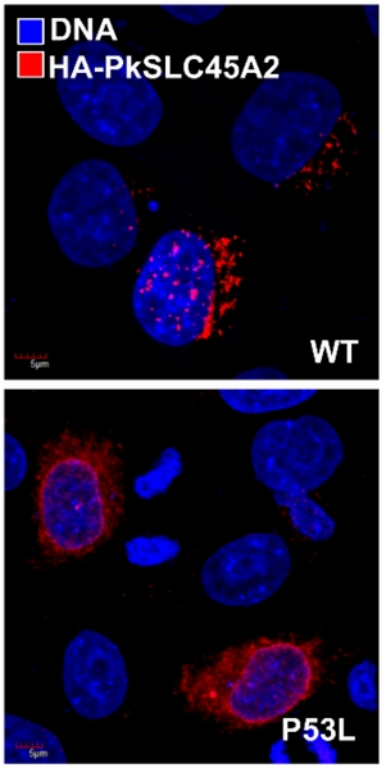

Fig. 6. Localization of wild type (top) and mutant (bottom) SLC45A2 proteins upon ectopic expression in HeLa cells. DNA is stained in blue and SLC45A2 in red.

In humans the *SLC45A* gene is associated with reduced pigmentation and oculocutaneous albinism type 4 (OCA4).

7. Interpret the results of Fig. 7.

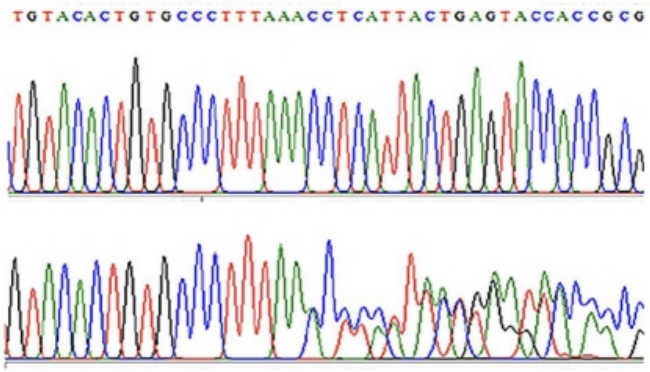

Fig. 7. Nucleotide sequence of part of the *SLC45A2* coding region from two individuals: an unaffected healthy man (top) and a man suffering from oculocutaneous albinism type 4 (bottom).

SLC genes code for membrane transporters. Phylogenetic trees can be used to identify the different *SLC* gene families and to reconstruct their evolutionary history.

8. What can you infer regarding the *SLC* genes of the common carp (diamonds) from the phylogenetic tree presented in Fig. 8? (2 pts)

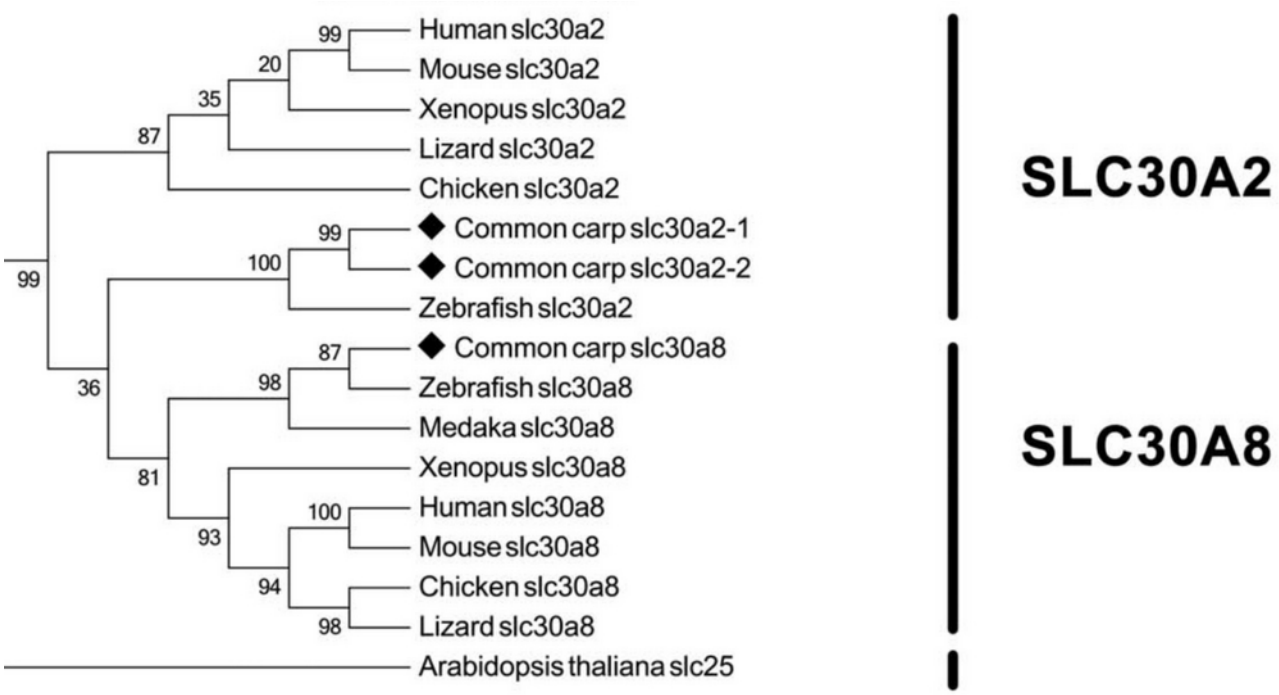

Fig. 8. Phylogenetic tree of the SLC30A8 and SLC30A2 proteins from various Vertebrates. Numbers indicate bootstrap values. SLC25 protein from the plant *Arabidopsis taliana* was used to root the tree.

Question 9 on the next page
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9. What are the arguments for and against the use of CRISPR-Cas9 to generate red Nile Tilapia fishes for consumption? You can use the text below to find some of them. (4 pts)

Genome Editing Using the CRISPR-Cas9 System to Generate a Solid-Red Germline of Nile Tilapia (*Oreochromis niloticus*)

Segev-Hadar, A., Slosman, T., Rozen, A., Sherman, A., Cnaani, A., & Biran, J. - 16 Aug 2021
The CRISPR Journal

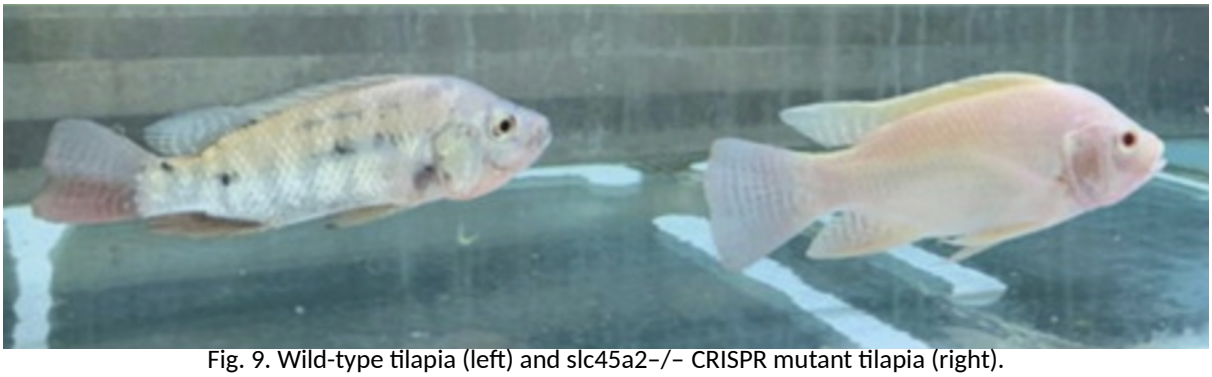

Fig. 9. Wild-type tilapia (left) and slc45a2−/− CRISPR mutant tilapia (right).

Tilapias are the second most aquacultured fish group in the world, with a global production of roughly 5.5 million tons per year, mostly of Nile tilapia (*Oreochromis niloticus*). In recent years, red tilapias have been gaining worldwide popularity, and their prices have increased accordingly. It has been suggested that the red coloration is due to faulty melanophore development. However, the resulting phenotype is unstable, as black or dark-red spots reduce the fish market value. An alternative approach of phenotypic selection for red coloration has resulted in a significant loss of genetic variability due to a founder effect. Moreover, current red tilapia strains are more sensitive to cold stress induced by overwintering and display reduced growth compared to other commercially used Nile tilapia strains.

In the present work, we describe the identification of *slc45a2* in Nile tilapia and utilization of CRISPR for induction of somatic and germline indels in the tilapia *slc45a2* exon 1. We show that when gRNAs are multiplexed and delivered into Nile tilapia zygotes, they induce OCA in this species, resulting in a solid-red phenotype. Lastly, we demonstrate the generation of several germline-transmitted null *slc45a2* alleles, and that the cross of allele-specific F1 fishes can establish stable and genetically trackable solid-red tilapia germlines. Our *slc45a2* mutant tilapia does not express melanin in its eyes or have black blotched skin, as opposed to the currently known red tilapia strains.

Taken together, our findings highlight the applicative potential of the CRISPR-Cas9 system in Nile tilapia culture. Moreover, Nile tilapia is one of the most widely cultured species in global aquaculture and is often hybridized with other Oreochromines species. Thus, the generation of heritable and trackable *slc45a2−/−* alleles with a red phenotype in Nile tilapia may affect the global tilapia aquaculture. Nonetheless, this would be tested by the attitude of farmers, consumers, and regulators toward consumption of genome-edited fish.

Corrections
Exam 4 Dec 2024 – Yellow parrots

Adapted from:
Ghosh Roy, S., Abdu, M., Bakhrat, A., Afonso, S., Pereira, P., Carneiro, M., & Abdu, U. (2023). Mutation in SLC45A2 leads to loss of melanin in parrot feathers. *BioRxiv*, 2023-08.
https://www.biorxiv.org/content/10.1101/2023.08.29.555428v1.abstract

Bibi, N., Ullah, A., Darwesh, L., Khan, W., Khan, T., Ullah, K., ... & Umm-e-Kalsoom. (2020). Identification and computational analysis of novel TYR and SLC45A2 gene mutations in pakistani families with identical non-syndromic oculocutaneous albinism. *Frontiers in Genetics*, *11*, 749.
https://www.frontiersin.org/files/Articles/549346/fgene-11-00749-HTML-r1/image_m/fgene-11-00749-g001.jpg
Jiang, Y., Zhang, S., Feng, S., Sun, J., & Xu, P. (2014). Genome wide identification, phylogeny and expression of zinc transporter genes in common carp. *PLoS One*, *9*(12), e116043.
https://www.researchgate.net/publication/270342598_Genome_Wide_Identification_Phylogeny_and_Expression_of_Zinc_Transporter_Genes_in_Common_Carp/figures?lo=1
Segev-Hadar, A., Slosman, T., Rozen, A., Sherman, A., Cnaani, A., & Biran, J. (2021). Genome editing using the CRISPR-Cas9 system to generate a solid-red germline of Nile tilapia (Oreochromis niloticus). *The CRISPR Journal*, *4*(4), 583-594.
https://www.liebertpub.com/doi/10.1089/crispr.2020.0115

1. The *yellow (y)* allele is recessive for plumage color relative to the WT allele (according to cross 3). It is sex-linked because the phenotype of the progeny of cross 5 differs between males and females. It cannot be X-linked (with females being XX and males XY) because this cannot explain the results of cross 3 or cross 4.
So we have to assume that the y allele is on the Z chromosome. Females are ZW and males are ZZ.
Cross 1: Z^yW x Z^yZ^y → all are [y] : Z^yW and Z^yZ^y
Cross 2: Z^+W x Z^+Z^+ → all are [WT] : Z^+W and Z^+Z^+
Cross 3: Z^yW x Z^+Z^+ → all females are [WT] Z^+W and all males are [WT] Z^+Z^y
Cross 4: Z^yW x Z^+Z^y → females are either [WT] Z^+W or [y] Z^yW; males are either [WT] Z^+Z^y or [y] Z^yZ^y
Cross 5: Z^+W x Z^yZ^y → all females are [y] Z^yW and all males are [WT] Z^+Z^y.

2.
- c. 1400G>T
p. G467V
Val
GTC

- c. 1195G>A
p. G399R
Arg
AGA

- c. 158C>T
p. P53L
Leu
CTG
The all are substitutions (one-letter change) and they change one amino acid into another = non-synonymous, missense mutations. **The three males are homozygous.**

3. C. 73G>T
p. A25S
Ser
TCA
missense mutation. Mutation in the same gene, which reinforces the hypothesis that this mutation causes the yellow phenotype.

4. The three missense mutations present in *SLC45A2* in the rose-ringed parakeet yellow mutant affect amino acids that are highly conserved, not only in birds but also in mammals and insects. These amino acids are probably important for the function of the protein. The 3 mutations associated with the yellow phenotype are thus likely to disrupt the chemical properties of SLC45A2 proteins.

5. A plasmid is a small, extrachromosomal circular DNA molecule which can replicate independently from chromosomal DNA in a cell. Plasmids are widely used as vectors in molecular cloning, to amplify a DNA of interest in large amounts.
Protocol:
- amplify the plasmids in cells (bacteria)
- extract plasmid DNA
- transform Hela cells with the plasmid DNA
- culture the cells in specific media (and select the ones that received the plasmid)
- fix the cells and stain them with antibodies that can recognize SLC45A2 proteins: either anti-SLC45A2 antibodies (if there are available), or antibodies directed against the tag HA= hemaglutinin = tag added as a protein fusion to SLC45A2.
- use fluorescently labelled secondary antibodies that can recognize the primary antibodies
- add a DNA stain
- examine under a fluorescent microscope

6. The control experiment (top panel) shows that the SLC45A2 proteins are accumulating in HeLa cells and that they are detected in distinct punctate microdomains associated with the nucleus, as in wild-type cells. The bottom panel shows that the P53L mutation disrupts the localization of the protein, it looses its punctuate aspect and appears to locate all over the cytoplasm and the nuclear membrane.

7. The healthy man appears to be homozygous. The man suffering from oculocutaneous albinism type 4 harbors a wild-type allele and another allele that looks like an indel. Since the man has two *SLC45A2* alleles, the *SLC45A2* gene is not sex-linked in humans. One defective allele appears to be sufficient to get the disease. The mutated allele is thus dominant for the OCA4 disease.
If the indel is not a multiple of 3, it will lead to a frameshift and thus is expected to fully disrupt the function of the SLC45A2 protein.
Bonus of 1 pt: this is a 1-bp deletion.

8. The common carp possesses three copies: *SLC20A2-1, SLC20A2-2* and *SLC20A8*. The ancestor of common carp and zebrafish probably had two copies of *SLC30*: *A2* and *A8*, which arose by duplication in a common ancestor of humans and fishes. Then, after divergence between common carp and zebrafish, in the branch leading to common carp, the *SLC30A2* duplicated and gave rise to two copies of this gene.

9. Pros:

- economical: second most aquacultured fish group
- auqacultured fish has the potential to feed a lot of people
- better mutation than the available ones: no black spots, stable phenotype, potentially less sensitive to cold
- no need to have a selection process and its potentially damaging effects on other traits
- can be followed by PCR
- can allow the maintenance of genetic variability (this argument is proposed in the text but CRISPR-mutations have the same issue, unless the mutation is introduced in multiple genomic backgrounds)

Cons:
- the mutation may have unexpected effects: loss of survival/growth, more susceptible to disease, effect on human health, etc. (if it is not present in nature, it is probably because it has detrimental effects in the wild)
- there might be off-target mutations
- people (consumers and politics) do not want to eat GMO food
- the mutation may spread to natural populations of this species (and body color can affect behavior), plus maybe hybridization with other Oreochromis species
- potentially more dependance towards biotechnology firms