MULTIPLE OCULAR COLOBOMA (MOC) IN SNOW LEOPARDS

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Modern conservation biology pays a special attention to endangered animal species in the world. International studbooks have been started in the zoos for registration of individual animals belonging to important endangered species. The snow leopard studbook was started in 1976 and is kept by the Helsinki Zoo (Blomqvist 1978a, b, 1980. 1982). This registration system has provided data to construct pedigrees and establish lineages of animals from which Inheritance of genetic defects can be traced.

The snow leopard, native of the high mountains of Central Asia [USSR, Mongolia. China, Nepal, Bhutan. India. Pakistan and Afghanistan), is a rare animal spread over a wide geographical area with a patchy population structure. Very little is known about the wild snow leopard population. There are, however, clear indications that the snow leopard population is constantly diminishing in our days (Blomqvist, 1978a, b: Braden, 1982). The snow leopard has been designated a species of particularly high priority in the Species Survival Plan developed by the American Association of Zoological Parks and Aquariums (Foose, 1982).

A unique congenital eye malformation, multiple ocular coloboma (MOC), is described in sixteen animals in the Helsinki pedigree of snow leopards. This anomaly is also known In snow leopards from the zoos in Amsterdam, Zurich, Omaha and Dublin. The snow leopards in the zoos of Amsterdam and Omaha are unrelated to the Helsinki Zoo snow leopards.

Typical colobomas of the iris, chorioidea and the optic nerve, well known in human beings and domestic animals [McCorniac et al. 1975) are caused by an incomplete closure of the embryonic eye fissure. A frequent cause for the coloboma complex is a dominant gene with a wide range of expression (Sorsby. 1973). Chromosomal abnormalities can furthermore cause the coloboma (Hittner et al., 1979). The clinical variation of this anomaly is considerable, ranging from little or no effect on the vision to fully developed microphthalmia or an ophthalmia leading to total blindness. Other than genetic factors may also determine the failure in closure of the embryonic eye cleft. These include environmental factors, deficiencies and various terato-genic agents.

Multiple ocular coloboma in the snow leopard, however, is a specific clinical entity, different from the coloboma complex described above, and so far unknown outside the felid group (Wahlberg, 1978: Wahlberg and Tarkkanen, 1980: Wahlberg et al., 1982). This entity consists of a coloboma of the upper lid and, in its complete form, of a bilateral microphthalmia with uveal. retinal and optic nerve coloboma, persistent primary hyperplastic vitreous and retinal dyspla-

sia. The upper lid coloboma is the only constant feature in affected animals associated with or without any of the ocular signs.

The same combination of lid and ocular coloboma is previously described in the domestic cat (Bellhorn et al.. 1971).

Lid colobomas are generally regarded to be of nonhereditary origin. They may result from a localized failure of adhesion of the lid folds externally caused during the late phases of embryonic development (Mann. 1957).

In this paper we utilize the known pedigree for examining whether MOC in snow leopards is genetically transmitted or not. We also report cytogentic and biochemical genetic studies of zoo animals.

MATERIAL

The Helsinki pedigree of snow leopards (Figure 1) and that part of the Zurich pedigree (Figure 2) comprising offspring from the male Walo (Hki 8, transferred from the Helsinki Zoo to the Zurich Zoo) [Wahlberg et al., 1982) include eighteen MOC affected animals in total.

The first ancestors of the Helsinki pedigree, Vilma (Hki 1) and Ville (Hki 2). were wildbom animals and trapped In 1964 and 1966, respectively. Their geographical descent is unknown. During the years 1967-1974 Vilma (Hki 1) and Ville (Hki 2) produced seven litters totalling altogether 15 cubs. Ten of the cubs survived past the age of six months. In back-crosses between Ville (Hki 2) and two of his daughters, Vilkku (Hki 3) and Valkky (Hki 4), nine litters with altogether 19 cubs were born between 1971 and 1976. Of these cubs. 13 survived past the age of six months. The first cub affected with MOC was Vainamo (Hki 32). He was born in 1976 as a son of Ville (Hki 2) and his daughter Valkky (Hki 4). Valkky (Hki 4) herself has a slight eye anomaly, described as a weakness in the structure of iris of the left eye leading to an assymmetrical pupil. This assymmetry is not considered as a symptom of MOC, although it was originally described as an iris coloboma (Wahlberg, 1978).

In 1978 Valkky (Hki 4) again gave birth to a male cub Vasili (Hki 48) with MOC. The sire, a related male Vellamo (Hki 30), was bom in 1976 as a son of Valkky's sister Venla (Hkl 61 and Charlie (LPZ 9) from the Lincoln Park Zoo. Chicago. A second cub (Hki 49) in the same litter was found dead and partly eaten. The eye status of this cub is unknown.

During the years 1974-1981 Vilkku's (Hki 3) and Valkky's (Hki 4) two sisters Venla (Hki 6) and Valpuri (Hki 13) gave birth to eight litters comprising 21 cubs, 11 of which were affected with MOC. The sire was Charlie (LPZ 9) from Chicago. Valpuri (Hki 13) was later transferred to the Nettuno Zoo, Italy, where she produced a litter of two unaffected cubs.

In 1983 Venla's (Hki 6) daughter Vieno (Hki 24) gave birth to a litter with three cubs, all affected with MOC. The sire was the unrelated Kara (Cin 6). born in the Cincinnati Zoo.

All MOC affected cubs were born after 1976. Before 1976. 37 unaffected cubs were born in Helsinki Zoo. All cubs in the same litters have been either affected or unaffected; no litters consisted of both affected and unaffected cubs.

The mortality of snow leopard cubs in the zoos has been approximately 30% during the last years (Blornqvist, 1981). Some of the cubs have been stillborn; others, living at birth, have been killed and eaten by their mothers. Offspring of unknown sex represent cases where only parts of the legs or the tail have remained left in the den. The eye status of the early dead cubs is unknown.

The Helsinki pedigree of snow leopards has a total of 75 cubs born between 1967 and 1983. Of these, 50 survived the age of six

months (mortality 33%). Before 1980 only those young snow leopards, which reached the age of six months, were numbered and named. From 1980 on, all captive born cubs have been given their own numbers at birth.

METHODS

Clinical Investigations of the Eyes

Since 1976 all the snow leopards in the Helsinki Zoo have been examined ophthalmologieally.

Animals over three months of age are sedated with a mixture of Xylatsln (Rompun®. Bayer) and Ketamlne-chloride (Ketalar®. Park Davis) given intramuscularly; 500 mg Xylatsin is dissolved in 5 ml Ketalar® (100 mg/ml Ketamine-chloride). The dose is approximately 0.07 ml of the mixture per kilogram body weight. Examinations of younger cubs is performed without sedation. The eyes were anaesthetised with Oxibuprocain HC1 (Oftan Obucain®, Star, 4 mg/ml). The external ocular examination is carried out with magnifying spectacles and spot light. Corneal diameters were measured, pupillary light reactions tested and the lids and anterior ocular structures inspected. Pupils are dilated with Tropicamid (Oftan Tropicamid®, Star

5 mg/ml). Opthalmoscopy is performed with direct as well as indirect methods.

The cubs born in the zoo are examined for the first time at approximately four weeks of age. The examination is repeated at least once at an age over three months.

A full pathological and anatomical examination of the eyes of all those animals that died or were euthanized has been performed at the Helsinki University Eye Hospital.

Analysis of the Helsinki Pedigree

Possible genetic transmission of MOC was analysed assuming that a single-gene character was originally transmitted by either of the ancestors Vilma (Hki 1) or Ville (Hki 2). It was also assumed that only the direct descendants of the ancestral couple in the present pedigree are allowed to carry the allele. It soon became evident that this hypothesis requires incomplete penetrance of the allele (Wahlberg and Tarkkanen, 1980), but only rough approximations were used to estimate the penetrance for two reasons: 1) the number of cases was not large enough to allow reliable estimates, and 2) the segregation patterns suggested that the genetic asssumptions underlying the penetrance estimates were not likely to be valid in this case.

The segregation patterns were tested using chi-square heterogeneity tests.

Chromosome Examinations

Chromosome examinations were performed on twenty animals, twelve of which were healthy subjects. The unaffected animals are the males Hki 2, 38, 43, 44, 53. 54, 56 and Cin 6, and the females Hki 4 and 14. Zu 13 and Okla 18. The MOC affected males studied are Hki 35, 40 and 48. The affected females are Hki 36. 60 and 61, and two euthanized cubs, bom in 1978.

The chromosomes were studied in selected metaphases obtained from blood cultures stimulated by pokeweed mitogen (Gibco), Bacto Phytohaemagglutinin P (Difco) or Concanavalin A (Sigma).

Several band-staining methods were applied: G-. Q-, R-, C-

banding and Ag-NOR stainings. On some slides subsequent G- (GAG) and C- (CBG) bandings were performed.

Serum Protein Investigations

For serum protein studies samples were collected from seventeen animals: Hki 4, 18, 34 (MOC). 41 (MOC), 43, 51, 52. 53. 54, 55. 56, 58. 59, 60 (MOC), 61 (MOC). Cin 6 and Zu 13. Of these animals Hki 43 is the only wild-born animal. The samples were subjected to routine serum electrophoresis in an agarose gel and stained with Coomassie Blue. All samples were run on the same gel.

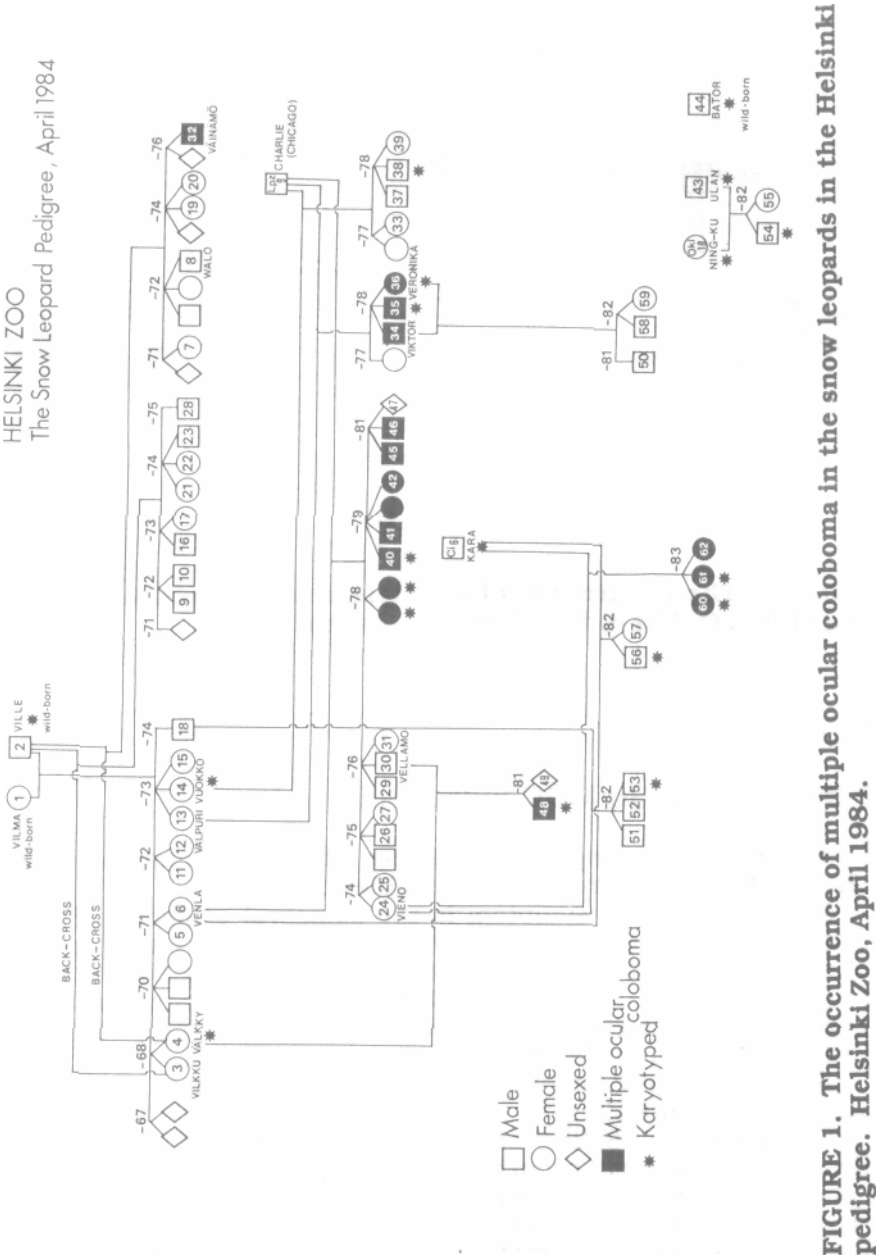

FIGURE 1. The occurrence of multiple ocular coloboma in the snow leopards in the Helsinki pedigree. Helsinki Zoo, April 1984.

RESULTS

The Eye Findings In MOC Individuals

The clinical findings in the sixteen affected animals belonging to the Helsinki pedigree are shown in Table 1.

The predominant symptom is the palpebral coloboma (Figure 3). The appearance of the palpebral coloboma varies from a very small notch in the eyelid to a total absence of the margin of the outer lateral half of the upper lid. The palpebral lesion is found only on the upper lid and is always affecting its central and lateral portion. The lower lid and the medial half of the upper lid are never affected.

In four of the cases no changes were present In the eye globe (Hki 34. 36, 41 and 42).

One case [Ve 78/2) presents the complete MOC syndrome with all described symptoms occurring in one individual. A retinal dyspla-sla, one of the uncommon traits in MOC. is observed In three cases [Ve 78/1. Ve 78/2. see above, and Hki 46). Whether the retinal dysplasia is a sequela to the colobomatous changes, particularly the microph-thalmia. or is considered to be a separate abnormality is not clear.

Results of the Pedigree Analysis

Assuming that MOC is affected by a single gene locus, recessiveness of the allele can be ruled out. This assumption would require that three individuals not descending from this pedigree (Kara (Cin 6), Charlie (LPZ 9) and Andra (Zu 2)) were carriers. Since MOC Is also expressed in a male line (a son of Walo (Hki 8)). the possible inheritance pattern should be autosomal. Because MOC is commonly expressed in cubs of unaffected individuals, the possibility of a dominant allele with complete penetrance can further be eliminated. Thus the only hypothesis left is that of dominance with incomplete penetrance. It has to be assumed that either Vilma (Hki 1) or Ville (Hki 2) has been heterozygous. Those individuals in the pedigree which must be heterozygous (if the hypothesis is correct), can be picked up on the basis of affected offspring. The expected frequency of heterozygous cubs in the outbred crossings of those parents is 0.5. Comparing this expectation with the observed frequency of affected offspring, after removing the index cases, the penetrance estimates are 0.41 (13 out of 32) assuming Vilma (Hki 1), or 0.36 (13 out of 36) assuming Ville (Hki 2) to be the original carrier.

The above estimations and the assumptions based on them are undermined by the segregation patterns among and within the families. First, if the single-gene hypothesis with incomplete penetrance is correct, the penetrance has to be highly variable among families. The proportion of the affected offspring in the progenies of Vilma (Hki 1). Venla (Hki 6) and Walo (Hki 8) differ significantly from each other ($X2=11.8$, $df=2$. $P<0.01$). Second, the affected offspring show a clustered appearance within the families. This is best seen in the offspring of Venla (Hki 6), the probability of complete yearly segregation of affected and unaffected cubs is very low ($P=0.00093$ using a randomization test with the observed progeny sizes and the probability $8/16=0.5$ for colobomal.

Chromosome Findings

The fclids are well known for a rather unique karyotype characterized by conservative traits. The chromosome number is 38. Only a few species in the marginal borders of the original distribution area of the cats have only 36 chromosomes. The chromosomes are dis-

tributed in six classes A-F. A small metacentric satellited marker chromosome El is found in all felids as also in some other carnivores (Wurster-Hill and Gray, 1975). Silver stained NOR regions are found only in the marker El (Figure 4).

The G-banded karyotype of the snow leopard {Soderlund et al. 1980) (Fig. 4) shows numerous homologtes with other felids. The karyotypes of the different cat species have as a rule been compared with the chromosomes of the domestic cat Felts *domestica,* the karyotype of which has thus been used as the standard cat karyotype

Wurster-Hill and Gray. 1973). The banded chromosomes in groups A, C and E are Identical with the corresponding chromosomes of the domestic cat. Homology is also demonstrated In groups B (Bl, B2 and B3), D (Dl, D3 and D4) and F (one pair).

Differences from the standard cat karyotype have been found in *Panthera (Panthera leo. tigris, pardus* and onca) (Wurster-Hill and Gray, 1973). The following changes have been observed: a pericentrlc inversion In B4, a small light region close to the centromere In D2p, the two F group chromosomes represent the pairs 2 and 3 of the three original F group pairs occurring in the karyotype of ancestors to presently living cat species. In the standard cat karyotype the F group is represented by the original felid chromosomes Fl and F2.

The karyotype of the snow leopard Is principally identical with the karyotype of the other *Pantherae,* which fact confirms the close relationship between the snow leopard and the *Panthera* species.

Furthermore the snow **leopard shows the** same slightly pronounced negatively stained **area** in the paracentric region of Alp as Is observed **In** the **lion** (Wurster-Hill **and Gray. 1973;** Gripenberg et al.. 1982).

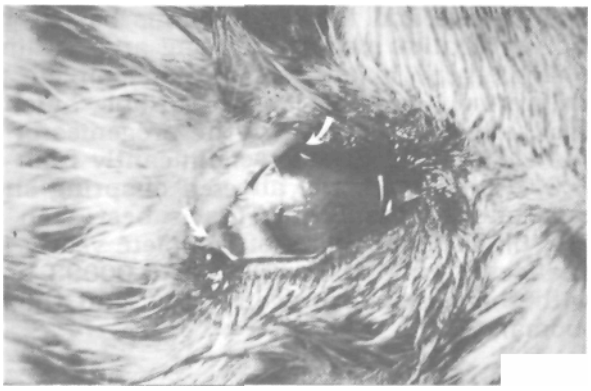

FIGURE 3. A typical coloboma of the upper lid on a snow leopard with multiple ocular coloboma (Vainamo, **Hki 32, right eye). The arrows enclose the coloboma. The lower lid Is normal. The cornea! haze Is also seen.**

The C-band pattern (Gripenberg et al.. 1982) (Figure 4) of the snow leopard shows large dark blocks on the sex chromosomes. The proximal parts of Xp and Xq have large C-bands. most of Yq Is heavily stained. In many autosomes the C-bands, however, are small and dot-like (Al, B2, B3, B4, D group chromosomes, El and E3). On several autosomes C-bands are hardly discernible. The C-band pattern of the snow leopard is in agreement with the general felid C-band pattern (Pathak and Wurster-Hill. 1977). Satellite DNA seems to be lacking In many cat species (Arrhigi et al., 1970).

The R-band pattern (Grlpenberg et al., 1982) shows the expected reverse pattern of the G-band staining.

Comparing the chromosomes of unaffected snow leopards and animals with MOC, neither numerical nor structural differences could be observed.

Serum Protein Results

Transferrin, albumin, complement C3, and several other protein fractions likely to include Gc, Hp and iAT are visible as monomorphic bands. No variant phenotypes have been observed. Also serum catalase shows no variants (Atroshi, personal communication). In other words, there is no evidence of electrophoretically detectable variation in the snow leopards studied so far.

TABLE I. The eye findings In the sixteen MOC affected snow leopards belonging to the Helsinki Zoo pedigree, d, right eye; s, left eye; Colob., coloboma; Ret., retinal.

DISCUSSION

Before we are going to discuss the findings associated with MOC we would like to make some remarks in connection with the karyotype findings of the snow leopards.

The snow leopard is a species detected late in the history. The first descriptions are from the end of the eighteenth century (Rieger, 1980). Taxonomically the position of the snow leopard seems obscure. In the English literature the snow leopard mainly is called *Panthera uncia* while German authors often use the name *Uncia uncia.* Formerly the snow leopard was referred to the genus *Felis* and accordingly named Felis uncia.

The karyotype of the snow leopard has the same characteristics as the chromosomes of the four species generally accepted to belong to the genus Panthera (lion, tiger, leopard and Jaguar). The chromosomal similarities seem thus to justify the genus name Panthera for the snow leopard.

Also other cat species have taxonomically dubious positions in the system. It seems that previous (Wurster-Hill and Meritt. 1974) and more recent (Gripenberg et al.. 1982) chromosome findings in different cat species could be useful in a possible future revision of the systematics tn *Felidae.*

The eye anomaly MOC in the snow leopard deserves special interest for several reasons. The combination of eye coloboma together with coloboma of the upper lid seems obscure since the affected tissues have different origin: the eye tissues are of neuroectodermal origin whereas the lids have developed from the mesoderm. Furthermore the two structures develop at different stages during the embryonic development. Thus the etiology of this entity seems confusing.

As already mentioned, nothing is known about the occurrence of MOC, cub mortality or the causes of the deaths in the wild population.

The understanding of the causes of MOC in the captive animals is most urgent. If hereditary, the breeding of affected animals of an endangered species with a small gene pool would rapidly disseminate the deleterious gene through the zoo populations.

The analysis of the Helsinki pedigree showed that MOC is probably not transmitted by simple genetic rules. The first case in Helsinki resulted from a father-daughter crossing (see above) but later cases do not indicate that MOC would follow from inbreeding (Wahlberg et al.. 1982). The tight clustering of the affected offspring

from the same parents speaks against purely genetic effects. The same holds for the hypothesis that the malformation would depend either on the maternal genotype or on inbreeding depression of non-specific allelic effects. The available evidence strongly points to non-genetic maternal influence, or some other external factor which affects all the embryos similarly.

Serological tests have so far not been able to confirm the involvement of any viruses as the cause of MOC, nor have Investigations

of toxic agents given evidence as to the etiology of MOC (Wahlberg and Tarkkanen, 1980).

The serum proteins showed no differences between any of the individuals examined, indicating high homozygosity in the zoo animals. This should not be taken as an evidence for genetic impoverishment in the zoo population, because low levels of genie variation are commonly found in large mammals (Nevo et al.. 1984). It has to be stressed that protein polymorphism in wild snow leopard populations has not been studied.

The chromosome investigations revealed identical karyotypes in affected and unaffected animals. No numerical or structural differences could be observed. Our opinion is that a chromosomal abnormality most probably is not the cause of this congenital eye anomaly. Analyses of prolonged chromosomes have, however, not yet been performed. A change in some minute parts of a chromosome can thus not be fully excluded.

The investigations here presented have not been able to explain the causes of MOC.

Reports on the occurrence of other familial anomalies, possibly observed but not published because of an unsolved etiology, would be highly desirable.

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