INHERITED DISEASES

Why should veterinary practitioners be interested in inherited diseases, especially diseases that appear as a group to be obscure and untreatable? Most veterinarians learn early in their training that inherited diseases are rare, and therefore by inference not worthy of the same attention given to infectious diseases. In fact, this "dogma" is ingrained so deeply that the words inherited disease are almost never spoken without the modifier rare, and the 3 words thus appear to be 1: rare–inherited–disease. If this concept is incorrect, where did it originate and why does it persist? The concept is borrowed from human medicine, where most inherited diseases indeed are rare. That is to say, in the general human population, the case incidence rate (number of affected patients in a potentially susceptible population) is very low, and with few exceptions, the carrier frequency rate (clinically normal individuals heterozygous for a trait) of recessive traits also is low. For example, the most common human gangliosidosis, Tay–Sachs disease, has a carrier frequency rate in the general population of only 1 in 300 or 0.3 per cent. However, American Jews of Eastern European origin have an ethnic predilection for this disease, and in this group the rate is 1 in 30 or 3 per cent.¹

The basis for the overall low frequency of inherited diseases in human beings relates to religious, moral, and legal restrictions on mating between closely related individuals. Within some human families, however, the appearance of affected individuals and the carrier frequency can be very high nonetheless. The epidemiology of inherited diseases in pure breeds of cats is strikingly different from that in the human general population, and more closely resembles the pattern seen in some families. Some significant differences between affected human families and feline pure breeds are that pure breeds, depending on their popularity, are composed of thousands to tens of thousands individuals. A popular male may mate to hundreds of females. There
is no restriction on matings to closely related individuals, and in fact, this method is preferred by some breeders. Consequently, the same forces that prevent the propagation of inherited diseases in human beings are not being applied to purebred animal populations, and accepted breeding practices actually encourage proliferation of inherited diseases. As a result, the carrier frequency of the gangliosidoses in some groups of pure breeds of cats and dogs has been documented to approach 20 to 40 per cent of breeding stock. If an individual practitioner may never see an animal affected by one of the gangliosidoses, and if patients diagnosed with this disease all die within a year, how can practitioners best serve their clients? The veterinary practitioner should be the first in line of defense for every animal disease, including inherited diseases. Unfortunately, pure-breed enthusiasts frequently are better informed about the inherited diseases of particular importance to their breed than most practitioners. Therefore, the goal of this chapter is to equip veterinary practitioners with the knowledge needed to fulfill their role as guardians of animal health, and to guide them to serve the pure-breed pet breeders and owners by leading the way toward detection and elimination of inherited diseases, using the feline gangliosidoses as a well-characterized example.

**THE GANGLIOSIDOSES**
The gangliosidoses represent a class of inherited diseases known as storage diseases, so-called because they are characterized by the accumulation of undegraded metabolites in hypertrophied lysosomes (see Chapters 51, Neuronal Storage Disorders, and 57, Mucopolysaccharidosis). A comprehensive discussion of the feline lysosomal diseases can be found in a chapter by Wood contained in Volume 1 of this series. 2 The gangliosidoses are progressive, fatal neurological diseases of cats, human beings, and other animals. Gangliosides accumulate principally in neuronal lysosomes, 2,3 and are designated according to their chemical structure as $G_{M1}$ or $G_{M2}$ gangliosidosis, depending on which ganglioside degradative pathway is blocked. These diseases are caused by inherited defects in the genes encoding lysosomal enzymes that degrade gangliosides. $G_{M1}$ gangliosidosis results from a mutation of the $\beta$-galactosidase gene (GLB1) with malfunction of the lysosomal enzyme $\beta$-galactosidase ($\beta$-gal). $G_{M2}$ gangliosidosis results from a mutation of the hexosaminidase gene (HEXB) and malfunction of the $\beta$-hexosaminidase enzyme ($\beta$-hex). It is important to understand that whereas these two diseases involve a common biochemical pathway and induce
similar clinical diseases, they actually are two distinct inherited diseases resulting from mutations of completely different genes (Table 77-1). Diagnosis of affected kittens is key to recognising that these diseases exist in a family or breed, because they are inherited as recessive traits, and "carriers" that are heterozygous for the mutation are completely normal in physical appearance. Therefore, some attention to initial diagnosis of affected cats is appropriate, even though the major emphasis of this chapter is on detection of the carrier state. Diagnosis of a kitten showing clinical signs can be accomplished by neurological examination, histopathology, ganglioside biochemistry, and enzyme activity assays. As always, the clinician must have a high degree of suspicion and take the proper steps to achieve a correct diagnosis, usually with the assistance of centers having expertise in the pathology and biochemistry methods that are needed for definitive diagnosis. The gangliosidoses often are misdiagnosed as cerebellar hypoplasia caused by fetal infection with panleukopenia virus. The key distinguishing features are: (1) the age of onset of clinical signs in the gangliosidoses is 4 months of age or older, whereas ataxia due to cerebellar hypoplasia is present at birth, and (2) neurological signs of the gangliosidoses are progressive, whereas those of cerebellar hypoplasia remain static or actually improve with age. The earliest signs of the gangliosidoses are fine tremors of the head and hind limbs. Owners rarely note these early signs or become concerned enough to seek professional assistance at that point. Signs progress to unsteady gait, wide stance, and inappropriate falling. Even at this stage, some owners will attribute these well-developed signs to a clumsy kitten. Therefore, when presented for diagnosis, many affected cats have advanced cerebellar signs of dysmetria of hind-limb paresis. The onset and rate of progression of clinical signs vary somewhat with the specific type of mutation. \( \text{G}_{M1} \) gangliosidosis typically becomes obvious by 4 to 6 months, is less severe, and progresses slowly over 12 to 14 months. \( \text{G}_{M2} \) gangliosidosis is apparent by 3 to 4 months, is more severe, and progresses more rapidly. Late signs include paraplegia of the hind limbs, raspy vocalisation, blindness, exaggerated startle response, and grand mal seizures.

The second step in defining a potential case of feline gangliosidosis is histopathology of brain, which demonstrates accumulation of high-molecular-weight glycolipids in neurons with lesions characteristic of storage diseases. However, histopathology cannot confirm that the storage material is a ganglioside, or identify the chemical type. Definitive diagnosis can be made only by chemical identification of accumulated ganglioside in brain, and biochemical detection of reduced activity of the appropriate enzyme: \( \beta \)-galactosidase for \( \text{G}_{M1} \) gangliosidosis, and \( \beta \)-hexosaminidase for \( \text{G}_{M2} \)
gangliosidosis. These assays are too specialised for most laboratories, and should be referred to a qualified diagnostic or research laboratory. Enzyme assays on tissue homogenates of affected cats are reliable for diagnosis because enzyme activity normally is high in liver and brain, and affected cats have essentially no enzyme activity. This differs distinctly from using enzyme activity to detect carrier state, because blood has low enzyme activity normally, and results are too variable for clear prediction of genotype in many individuals.

Inheritance of the Gangliosidoses
The gangliosidoses are inherited as simple autosomal recessive traits. That is, 3 genotypes exist: (1) normal, meaning that both alleles are normal and the cat is normal in clinical appearance; (2) carrier or heterozygote, where 1 member of the gene pair is normal, the second is mutant (single dose of the mutation), and the individual is normal in clinical appearance; and (3) affected or recessive, where both members of the gene pair are mutant (double dose of the mutation), and the individual is affected clinically. The affected genotype is important only inasmuch as it shows that the gangliosidoses exist in the family or breed. For this reason, accurate and timely diagnosis is very important. However, affected cats represent only the tip of a potentially very large iceberg. Carriers are the most important genotype because they give no physical clues to the existence of the diseases, but transmit the mutation to half of all their progeny. In addition, the frequency of carriers in a population far exceeds the frequency of affected individuals. For example, a disease that affects just 1 per cent of the population has an estimated carrier frequency of 18 per cent! Therefore, the carrier state makes the recessive diseases the most dangerous of all patterns of inheritance; this is true for families, but is of overwhelming importance in pure breeds.

Typically, a recessive trait is not suspected until an individual shows clinical signs and is diagnosed accurately. If a champion tom is heterozygous for a disease trait, he will pass on this trait to half of his progeny, and they in turn will pass the trait to half of their progeny. The same pattern occurs if the "founder" of the trait is a queen, but the process of dissemination in the breed is slower. Unless 2 carriers mate and have an affected kitten, the dissemination process proceeds silently, involving an ever-expanding number of cats in more and more family lines. Even if an affected kitten is born, delay of definitive diagnosis can be very long if the inherited disease is not well known to practitioners, or if laboratory assistance needed to confirm the disease is not available.
When an accurate diagnosis is made, unless there is a method that can be applied readily for detecting the carrier state, no progress can be made in understanding the breadth of the problem or working toward eliminating carriers. For example, the existence of \( G_{M2} \) gangliosidosis in Korats was demonstrated in 1985. At that time, the only diagnostic procedure available was enzyme assay of peripheral blood leukocytes. This procedure was not adaptable for successful carrier screening because enzyme activity is very labile. Even when samples where processed properly, values for normals and carriers overlapped, and an unambiguous assignment of genotype could not be made consistently. An attempt was made to eliminate carriers using this method in spite of these limitations, but the effort was narrow in scope and of questionable benefit.

**Molecular Characterization of Mutations in the Gangliosidoses**

Before molecular characterization of a mutation, the gene responsible for an inherited disease must be determined, and the DNA of the normal gene must be sequenced. Fortunately, the lysosomal enzymes that catabolize the gangliosides have been characterized for the human diseases. More recently, the genes encoding these enzymes were sequenced for human beings and mice, providing some basis to sequence the cat genes. Hexosaminidase consists of 2 subunits, \( \alpha \) and \( \beta \) which dimerize to form different isoforms of the enzyme: Hex A (\( \alpha \beta \)) and Hex B (\( \beta \beta \)). Each subunit of this enzyme is encoded by a different gene. Deleterious mutations in the gene encoding \( \beta \) subunit of hexosaminidase (HEXB) affect both Hex A and Hex B enzymes, producing \( G_{M2} \) gangliosidosis variant 0 to indicate the loss of both isozymes. In 1978, we described feline \( G_{M2} \) gangliosidosis, variant 0 of shorthair domestic, non-purebred cats (fG\(_{M2}\)Baker). In 1985, a similar clinical disease was described in Korat cats (fG\(_{M2}\)Korat). A partial sequence for the normal feline hexosaminidase gene (HEXB) was reported in 1994. This information was used to discover the mutation site in fG\(_{M2}\)Korats, which results from a single base deletion and frame shift that introduces a premature stop codon early in the coding sequence. Based on this report, we investigated the mutation responsible for fG\(_{M2}\)Baker, sequencing the HEXB cDNA from fG\(_{M2}\)Baker mutants to determine if it differed from the Korat mutation. The data showed that the Baker mutation is different from the Korat mutation, and consists of a 25-base-pair inversion at the extreme 3' end of the coding sequence, which should introduce 3 amino acid substitutions at the carboxyl terminus of the \( \beta \)
subunit and a translational stop that is 8 amino acids premature (see Table 77-1). In contrast to these 2 mutations discovered to date in cats, human G\textsubscript{M2} gangliosidosis, variant 0 (Sandhoff's disease) results from at least 18 different HEXB mutations.\textsuperscript{1}

In 1971, we described G\textsubscript{M1} gangliosidosis in Siamese cats, and similar diseases subsequently were described in non-purebred cats.\textsuperscript{8-11} In 1998, G\textsubscript{M1} gangliosidosis was described in Korats, providing the first evidence of the unexpected occurrence of both gangliosidoses in a single breed.\textsuperscript{12} In all cases, the activity of \(\beta\)-galactosidase (\(\beta\)-gal) was absent or reduced to less than 10 per cent of normal, and G\textsubscript{M1} ganglioside was the predominant storage material in brain. Although the sequence and sites of mutations have been reported for the human structural \(\beta\)-galactosidase gene, this information was lacking for cats. Therefore, we sequenced the full-length feline GLB1 cDNA from normal cat brain, liver, and skin fibroblasts. Based on this normal feline GLB1 sequence, we amplified GLB1 from tissues of Siamese GM1 gangliosidosis mutants and obligate carriers. We identified a single G to C substitution (CGT\textsuperscript{1486}→CCT) at base 1486, resulting in a change of an amino acid from Arg to Pro and loss of hydrolytic activity. This mutation does not correspond to any of the 23 mutations of the GLB1 known to cause human G\textsubscript{M1} gangliosidosis. In collaboration with DeMaria and colleagues,\textsuperscript{12} we sequenced the GLB1 gene tissues from Korats with G\textsubscript{M1} gangliosidosis, and found unexpectedly that this mutation was the same as that responsible for the disease in Siamese cats (see Table 77-1).\textsuperscript{7} Because a given inherited disease in a pure breed usually results from a genetic "error" in a single individual, commonly called the founder effect, it can be assumed that the mutation would be unique to that breed. Even if the same syndrome is recognised in a second breed, the assumption would be that the mutations would be different, such as that observed in the feline G\textsubscript{M2} gangliosidosis. Finding the identical mutation in both Korats and Siamese might contradict this principle, except that both breeds originated from Siam (Thailand), and use of Siamese breeding stock was permitted in the development of the Korat breed in the West. Therefore, it is likely that the mutation of the GLB1 gene originated in Siamese cats and was transmitted to the Korat breed decades ago.

**Molecular Test for Carriers of the Gangliosidoses in Korats**

Characterization of the feline HEXB and GLB1 genes and the mutations responsible for the gangliosidoses of Korats allowed organisation of a Korat gangliosidosis screening program that offered molecular detection of carriers of both G\textsubscript{M1} and G\textsubscript{M2} gangliosidosis. The advantages of a molecular test include: (1) unambiguous assignment of genotype, (2) use of a small volume (0.5 ml) of
uncoagulated blood sample, (3) no requirement for processing outside the molecular testing laboratory, (4) stability of DNA, which allows for shipping without refrigeration, and for long transit times (up to 7 to 10 days at ambient temperature), and (5) ability to store samples in the laboratory for months to years at -70°C.

The first samples were received for testing in March 1998, and as of May 1999 a total of 227 samples have been evaluated, which translates to 454 separate tests, because each sample is tested for G_{M1} and G_{M2} gangliosidosis. Samples have been received from 80 breeders in 11 countries: Australia, Belgium, Canada, Denmark, Finland, Great Britain, Germany, Italy, Norway, Sweden, and the United States. This high level of participation is quite remarkable for a program that is barely a year old, and makes it a truly international program. From 227 Korats tested as of May 1999, 38 G_{M1} carriers and 14 G_{M2} carriers have been detected. Therefore, the carrier frequency of both mutations in Korats is approximately 23 per cent. As shown in table 77-2, there are some variations in the number of carriers detected and the distribution of G_{M1} versus G_{M2} carriers. Only 1 country (United Kingdom) appears to be unaffected to date, and Australia has no G_{M1} carriers and only 1 G_{M2} carrier. This low frequency may result from the strict quarantine of animals entering these 2 countries, which restricts entrance of new breeding animals from other countries. Except for these 2 island nations, all other countries have G_{M1} carriers. Four European countries and Canada have no G_{M2} carriers. Norway and the United States appear to have a disproportionately high frequency of G_{M2} carriers. The possibility that 1 in every 5 breeding Korats is a carrier of one of the gangliosidoses is staggering! In addition to the very high carrier frequency for these mutations, these results provided additional surprises. First, G_{M2} gangliosidosis has been known as an inherited disease of Korats since at least 1985, although until now the actual magnitude of the problem had not been determined. Second, G_{M1} gangliosidosis was not known to exist in Korats until 1998, but it is clear from our sampling to date that G_{M1} appears to be far the more important of the 2 mutations, in terms of the breadth of family lines affected. Thus far, we have not found a single Korat that carries both mutations, which is very good news, because such "compound heterozygotes" would add materially to the complexity of the problem, and could slow elimination of these diseases.

What are the keys to the early success of a screening program such as this one? First and foremost, the strong leadership of the few breeders who took the initiative to start this program has been the foundation from which progress has been built. The Korat breed is relatively small, and enthusiasts communicate easily, facilitating the exchange of information about the
program and testing results. The example provided by the organizers leads directly to the remarkably high rate of participation experienced. Continued success of this program must be attributed to the 80 breeders who comprehend the gravity of this problem for the Korat breed, and who make the effort to determine which cats are affected, so that the continuing spread of these dangerous mutations can be stopped. Support of a program that defines the presence of inherited diseases is not embraced universally by breeders, and indeed there is not complete support for the Korat Gangliosidosis Screening Program. Some skepticism probably derives from the earlier limitations of the Korat $G_m2$ testing program, based on enzyme activity that was not reliable for unambiguous detection of carriers. It is also clear that the rapid success and international impact that this program has enjoyed would not have been possible without the Internet and nearly universal use of e-mail. Additionally, strong leadership was provided by breed associations and registries that emphasized the importance of testing and organized sample submissions. Plans are being developed by some breed associations to require confirmation of the gangliosidosis genotype of any Korat registered. When the Korat Gangliosidosis Screening Program first began, whole-blood samples were submitted by American and Canadian participants, and were found to be stable at ambient temperature for the usual 2 to 3 days in transit. However, DNA extraction was performed before shipment on many samples coming from other continents where speed of shipment was concern, because purified DNA is very stable at ambient temperature. DNA extraction from cat blood is sufficiently different from other species that most laboratories are not uniformly successful with the extraction process. As a result, we spent much time and effort trying to use some of the samples without success. It is clear now that overnigt delivery service provided by most carriers, and relatively easy compliance with US Department of Agriculture import requirements for cat blood (USDA Guidelines for Importing #1102), make it unnecessary to do any processing outside our labaratory. Whole blood may be submitted from any of the participating countries, even if transit time is 5 to 7 days. We continue to use direct genetic sequence analysis for detecting carriers. This method is laborious, timeconsuming, and expensive. Therefore, we are developing other methods that can be adapted more easily to high throughput processing, thus reducing processing time and expense. In consultation with several Korat breeders who helped launch this program, we developed an official certificate that verifies test results. Results are reported by e-mail, so that owners will have access to this information as soon as possible, but the certificate is recognized as the formal document for verifying the gangliosidosis status of any Korat. To facilitate processing official
Genetic Counseling of Breeders

How should a clinician advise a Korat owner who learns that his or her line carries the gangliosidosis disease traits? In the past, the recommendation not to breed carriers was the standard, if not completely satisfying advice. It is clear that in Korats, the very high carrier frequency, coupled with the limited gene pool of this small breed, will not allow simple removal of all carriers from the breeding pool because of the genetic bottleneck that could result. Consequently, breeders who wish to preserve the best phenotype of champion Korats should be offered assistance in developing controlled breeding programs. If an otherwise healthy, phenotypically superior carrier is selected for breeding, only genotypically normal cats should be selected as mates, and all kittens produced should be tested. As many as 50 per cent of kittens from these matings will be normal and available to perpetuate the best characteristics of that family line, whereas carriers should be neutered and placed as pets. The firm restrictions of this strategy are: (1) a known carrier must be mated only to a known normal to ensure that no diseased kittens are born, (2) all resulting kittens must be tested, and only normals can be returned to the gene pool as breeders, and (3) all carriers must be neutered. This option probably would not be available without the unambiguous determination of genotype that the molecular test provides. This strategy is being adopted by a few breeders, and although our experience to date is limited it appears to be working well. Figure 77–1 illustrates an actual pedigree resulting from a planned mating between 2 Korat queens known to be carriers of $G_{M1}$ gangliosidosis and a Korat tom, known to be normal for both $G_{M1}$ and $G_{M2}$ gangliosidosis. In this case the breeder had tested the breeding stock and wished to preserve the championship phenotype of the queens that tested positive of $G_{M1}$ carriers. Of the 8 kittens produced in these 2 litters, 6 were normal genotypically and may serve as replacement breeders. These results clearly demonstrate the benefits of molecular testing in selection of breeding stock and in identification of phenotypically normal prodigy, preserving a diverse and healthy gene pool for future generations of Korats. In this particular instance, litter number 2 had the expected number (50 per cent) of normals and carriers, but litter number 1 had no carriers. In addition, all of the carriers are male. This apparent disagreement with the expected distribution...
for an autosomal recessive trait most likely is the result of a small sample size. Without molecular testing, this powerful strategy and resulting benefits could not be offered to a breed with a small gene pool.

With pedigree analysis, testing even a relatively small number of Korats is likely to have a significant impact on identification of carriers and ultimately on elimination of the gangliosidoses from the breed. Using molecular test results, pedigree analysis serves as a powerful tool to identify the carrier status of parents, grandparents, and siblings. This is particularly applicable to the Korat breed, which requires pedigrees for all registered cats, tracing each individual back to an ancestor in their country of origin, Thailand. As the number of family lines being tested increases, pedigree analysis will become an even more powerful tool for expanding the genotypic data base.

The Future of Molecular Testing
What does the future hold? The Korat Gangliosidosis Screening Program is a historical event. Never before in veterinary medicine has molecular diagnosis been applied successfully worldwide, in an attempt to eliminate an inherited disease from a pure breed. If this program ultimately is successful, it will be the first time that inherited diseases have been controlled systematically or eliminated from a pure breed through an organized testing program. Based on the enthusiastic participation experienced to date, and the self-imposed, organized restrictions placed on breeding Korats for whom the gangliosidosis status is not known, it is possible to reduce the carrier frequency substantially or even eliminate these diseases from the Korat breed entirely. This historic "experiment" will be imitated many times in the decades ahead, and may become the standard procedure to control inherited diseases, in much the same way that vaccination now is the standard for controlling infectious diseases.
REFERENCES


