Chair: Mr. Didier Boussarie

Induction Reception of Mr. Gustavo AGUIRRE on February 1 , 2024

Mr. President of the French Veterinary Academy, Ladies and Gentlemen, Dear colleagues, Dear Gilles,

It is a pleasure to be here today and thank all of you for the honor of being inducted into the Research Section of the French Veterinary Academy. I have had a long and very rewarding association with French veterinary ophthalmology, and formalizing this connection through the French Veterinary Academy is most appropriate. With this in mind, a small history of this association is in order.

I first met Professor Bernard Clerc when he, unexpectedly at least to me, was waiting outside of the Ophthalmology Section office one hot summer day around 1975 for a planned 2-week visit. During this visit, he showed several of the section members his method of parotid duct transposition via the oral cavity. Impressive to say the least!! We soon became good friends. I visited Bernard several times, and in one of those early visits I met Prof. Francis Lescure who invited me to the Faculty in Toulouse to present talks and discuss ophthalmology topics of mutual interest; it was in Toulouse that I met Dr. Marc Simon. The three of us and my wife once went by car from Toulouse to Montpellier to attend an annual veterinary ophthalmology meeting scheduled in that city at the time. In Francis Lescure's Mercedes Benz model 500, the drive passed quickly as Francis was cruising at 220-240 Km/hr. My wife fell asleep as she often did when nervous and expecting a fatal crash, and Marc Simon kept referring to Francis as 'Speedy Gonzalez', the mouse cartoon character that was the "Fastest Mouse in all of Mexico'. This conversation was held in Spanish, so Francis was not aware that his driving was the subject. Very fond memories, and sadly both Bernard Clerc and Francis Lescure are no longer with us.

As noted by Gilles, my interest in ophthalmology started with summer research projects with Lonnie Rubin during my veterinary studies; at that time, he was studying the canine electroretinogram (ERG) during normal development of the retina and asked me to participate in the studies. The instruments available then were advanced for the time but antiquated compared to now. But with attention to using standardized protocols, it was possible to obtain reliable signals that could be easily interpreted. Sadly, the field has not advanced much in the last 60 years because the equipment, vastly improved over those in the 1960's, are often not used with standardized methods because of the incorrect assumption that great technology can give results even when short-cuts and non-physiologic methods are used. The beauty of ophthalmology, at least from the perspective of an aspiring clinician, was that the different structures of the eye could be visualized, and their function studied in the living animal, something that was difficult to do for internal organs.

At the time of my residency training and early years on the faculty, basic research activities were encouraged, particularly if they generated salary support and administrative overhead from Federal funding agencies, namely the National Institutes of Health. Then many veterinary ophthalmologists were involved in research, and participated on an equal footing with medical ophthalmologists in these activities. Now the organized ophthalmology Colleges, both ECVO and ACVO, have an ever-growing number of diplomates. Although a surprising number have PhD degrees, the number doing more basic and translational research is small. The emphasis has been on applied clinical research in university setting or clinical practice, and this, in my

opinion, is not good for the specialty as we need to emphasize both clinical and basic research to balance patient-relevant findings with advancing the science of ophthalmology.

My interest in research evolved from clinical studies. Early on in my residency training, a breeder of Norwegian elkhounds approached Lonnie Rubin because several of her dogs had developed retinal degeneration which was considered to be a form of progressive retinal atrophy (PRA). Initial studies on these dogs by David Cogan and Toichi Kuwabara (1965) at Harvard Medical School had stopped after showing that the disease was inherited, and retinal pathology was present early. The breeder had a number of affected dogs and wanted assistance in helping to find more about the disease. When you are the lowest and least important member of the section, that work falls right on your lap as no one else was interested. That was a fortuitous event as the breeder was very cooperative and bred several litters for me. The dogs followed me from PENN to my fellowship at Johns Hopkins Medical School. I raised 11 of these dogs in my home in rural Maryland in outdoor kennels I built with the help of another veterinary ophthalmologist, Dr. Charles Parshall, and I would transfer them from home to my Hopkins lab in cages in the backseat of the car. For very good reasons, this is something no longer allowed by regulatory agencies or University regulations.

Several studies on the Norwegian elkhound disease, which I called rod dysplasia (*rd*) because of the very specific and selective rod photoreceptor abnormality, were published over a 15-year period (1971, 1973, 1976, 1978, 1987). These detailed the structural and functional abnormalities of the disease, and showed, for the first time, that the ERG could be used for specific diagnosis of affected dogs at a time when the retina was normal on clinical examination. Sadly, the colony of affected dogs was not continued and the gene and mutation for this important and very interesting disease has not been identified.

The studies in the Norwegian elkhounds with *rd* soon expanded into studies of other retinal diseases, mainly in dogs, but also in cats. In dogs, studies of different forms of PRA that were breed-specific soon followed. These included rod-cone dysplasia 1 and 2 (*rcd1, rcd2*) in Irish setters and collies, respectively, progressive rod-cone degeneration (*prcd*) in miniature poodles, Labrador retrievers and English cocker spaniels among several other breeds, and early retinal degeneration (*erd*) once again in Norwegian elkhounds. These studies focused on genetics of the disorders, structural, functional and, where appropriate, biochemical basis of the diseases. But while the more we learned about the diseases was important and impressive, treatment of the diseases was never in the picture, The tools needed to develop treatments just were not there.

This soon changed, at least in cats. In studies done with Roy Bellhorn, one of the founding fathers of veterinary ophthalmology in the US, we characterized a cat retinal degenerative disease called Feline Central Retinal Degeneration (FCRD; 1974). In affected cats, fundus lesions were characterized by atrophic areas in the area centralis which were circular or oval and which progressed in some cats. Critical examination of these lesions showed striking similarities to an experimental retinal degeneration in cats caused by feeding taurine depleted casein-based diets. Fortuitously the 'light bulb' turned on and I directed studies in clinical cases to diets. I found that cats fed dog foods developed taurine deficiency retinopathy with lesions identical to FCRD (JAVMA, 1978). It turns out that most dog foods, particularly dry foods, are based on vegetable protein which does not contain taurine. For cats, taurine is an essential amino-sulfonic acid as it is not synthetized in the liver like in dogs or humans. Finally, I achieved success in 'treating' a retinal degeneration-by feeding taurine I could arrest the progression of the disease or prevent it altogether by ascertaining that diets fed to young kittens were taurine adequate.

The early 1990's was a period of great progress in the field of molecular genetics of the eye, particularly the retina. While several disease loci were mapped previously to specific human chromosomes by linkage mapping, it was in 1990 that a mutation in the rhodopsin gene was found to be causally associated with autosomal dominant retinitis pigmentosa. This was soon followed by identification of a large number of genes that were associated with different forms of retinal disease. At present, there are over 300 different retinal diseases mapped to specific chromosomal regions in man, and close to 300 genes identified.

For those of us working with canine models, we looked at this progress and wealth of genomic information with envy. In early to mid 1990, we had **no!!!** genomic resources to use in identifying causally involved genes and mutations. We were limited to using a candidate gene approach for these studies. Unaware then that there were at least 300+ genes that could cause retinal disease, we selected 'reasonable' potential disease candidates. These were cloned, polymorphisms identified, and these were used to test whether the candidate gene was linked or associated with the disease using straightforward linkage studies. If no linkage, then the gene was eliminated, and a new 'reasonable' potential disease candidate was selected, and process repeated. This often took ~6 months of lab work/gene exclusion. If associated, then the candidate gene, usually in the form of complementary DNA (cDNA) was sequenced in control and mutant dogs with the goal of identifying the sequence change that caused disease. In spite of these limitations, success was achieved in the finding in the 1990's of the *PDE6B* and *RPE65* genes and mutations that caused PRA in Irish setters and what we now call canine Leber congenital amaurosis in the Briard.

We recognized that to do modern molecular genetics in dogs we needed to build the tools and genomic resources to do this work. Greg Acland and I collaborated with Elaine Ostrander's group and created in 1997 the first linkage map as well as a panel of canine-rodent hybrid cell lines to partition the canine genome. Now, high-density SNP chips as well as targeted, exome, whole-genome, RNA sequencing and other methods are possible due to the sequencing of the canine genome. Genomic resources for doing cutting edge molecular genetic studies are now possible. The dog is no longer the unwanted 'orphan' species as in the past when many companies and even funding agencies would note: 'we accept samples from humans, rats, mice, but no dogs'. With these advances, we could now identify many new retinal diseases. The number is impressive, but this has added confusion as there has been no uniform way of naming these newly identified diseases. To this end, a small group of investigators, with the blessing of the ACVO and ECVO, has undertaken the renaming task. The group, led by Freya Mowat and including Simone Iwabe, Simon Petersen-Jones and Gustavo Aguirre, have written a paper (Consensus guidelines for nomenclature of companion animal inherited retinal disorders) that will be published in the *Veterinary Ophthalmology* journal in 2024.

In the past, most studies of retinal diseases in dogs involved detailed clinical, functional and structural analyses that complemented the genetic work to determine the mode of inheritance. Once the gene and mutation were found, then it was possible to understand the molecular mechanisms of the disease and the phenotype-genotype correlation. That is no longer the case as disease identification is done on clinical patients, and research colonies of dogs are almost non-existent. With the large array of genomic resources available, it is often the case that the gene and mutation for an inherited retinal disease is found after very superficial clinical examinations are done. This occurs before there is any information about the clinical phenotype or if the disorder is a rod-cone or cone-rod abnormality, or the influence of the mutation on disease progression or eventual findings. Often we know the causative gene and mutation before adequately understanding the disease.

Progress towards developing therapies for inherited retinal diseases has rapidly progressed since our first successful gene therapy in a Briard cross-bred dog affected with canine Leber congenital amaurosis. Again it was a 'light bulb' moment that required a great team effort. After all, we had the model and had identified the gene and mutation and several groups had been working on developing viral vectors to facilitate gene transfer to retinal cells. Of these, the adeno-associated viral vectors were optimal as they were non-replication and caused minimal to no disease. Together with Greg Acland, I asked Samuel Jacobson and Artur Cideciyan at Penn's Scheie Eye Institute and William Hauswirth at the University of Florida to participate in a collaborative project to treat and hopefully cure a blinding disease. On a sunny day in July, 2020 the results came in-subretinal delivery of a viral vector with the therapeutic *RPE65* cDNA resulted in restoration of retinal function and vision in the three dogs treated by this route of vector administration. The three dogs treated by intravitreal injection remained blind.

These results were published in *Nature Genetics*, 2021 and energized the field. This therapy is now commercialized in the USA and EU as well as in other countries. The success of gene therapy in dogs for other diseases, including *CNGB3* achromatopsia, *RPGR* X-linked retinitis pigmentosa, *RHO* autosomal dominant retinitis pigmentosa, *BEST1* vitelliform macular degeneration and *NPHP5* cone-rod dystrophy have been carried out at Penn mainly by Dr. William Beltran. In the dog they have shown efficacy and safety. Several have progressed to Phase 1/2 or Phase 3 clinical trials in man, and several are in the late phases on the investigational new drug (IND) development pathway to then be used in Phase 1/2 clinical trials in man.

Little did I anticipate the progress that veterinary ophthalmology would achieve when watching Professor Bernard Clerc doing a parotid duct transposition via the oral cavity in 1975. This progress has truly been remarkable.