

ANIRIDIA SYNDROME IS A GENETIC DISORDER RESULTING IN LOW VISION, BLINDNESS AND OTHER MEDICAL ISSUES FROM BIRTH THROUGHOUT LIFE

Eye on Aniridia



Aniridia Foundation
International

www.make-a-miracle.org

Fall 2016

Save the Date: Celebrate the 10th AFI “Make a Miracle” Conference and 15th Anniversary of AFI with us!

Please join us August 2-6, 2017 for the 10th Aniridia Foundation International “Make a Miracle Conference.” It will be held in the beautiful surroundings of Lake Junaluska, North Carolina (28 miles west of Asheville, NC). Airport transfers will be available through a third party.

The mission to advance Aniridia Syndrome research, provide support, and advance knowledge of this syndrome was started 15 years ago with the creation of Aniridia Foundation International (AFI). We could not have come this far without the support and dedication of our members, donors, and medical and scientific professionals. This conference will be an excellent opportunity for **all of us** to come together and become vested in a mission which will benefit those with Aniridia Syndrome for years to come through our educational yet relaxed atmosphere conference. As always, the AFI conference will have the latest in medical and scientific presentations; however, there will be more time for events to meet other members, and to become involved and facilitate more progress towards a cure. There will also be a structured and fun childcare/teen programs making lifetime friendships, and of course our “Make a Miracle Gala” celebrating 15 years of advancements.

Current members will receive invitations to the conference and more information will be sent as it becomes available, so make sure your contact information is up to date. This venue is cost effective and also conducive to adding a couple days before or after the conference to make it a vacation. You don’t need to choose between taking a family vacation or attending the conference, because now you can do both! **Registration will open early in 2017 and deadlines will be earlier than normal as there is limited space, so start planning your vacation time and bring the whole family.**

Celebrate with us, how far we have come in conquering the challenges of living with Aniridia Syndrome, catch up with old friends and make new ones, and learn the latest information in research and medical care. **Watch your E-mail and mail for your invitation and more details soon. We hope to see you this coming summer!**



Also in this Issue

Aniridia Fibrosis
Syndrome 2

Take our Hands, Walk with Us, Share Our Dreams, and Make a Miracle!

Aniridia Fibrosis Syndrome: An Infrequent But Potentially Very Serious Problem In Congenital Aniridia

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Congenital Aniridia patients, before they develop any aniridia-associated complications, are often legally blind at birth due to foveal hypoplasia, a malformation of the retina that prevents high resolution light detection. Since the part of the brain needed for vision requires high resolution light detection to form properly, this foveal hypoplasia can lead to amblyopia (“lazy eye”), strabismus (“crossed” or misaligned eyes) and nystagmus (repetitive, uncontrolled eye movements) as a baby grows. Fortunately, these conditions usually do not cause catastrophic vision loss (hand motion/light perception only vision, or complete blindness). However, as aniridia patients age, nearly all develop one or more additional ocular diseases which may include glaucoma (an increase in ocular pressure which eventually kills the cells that allow the eye to communicate with the brain), cataract (the clouding of the lens), and keratopathy (an abnormality in the surface of the cornea which eventually will prevent light from entering the eye). If any of these conditions are left untreated, vision can deteriorate significantly.

As aniridia patients get older, regular expert eye care is essential, as most will require eye treatments to maintain their current level of vision, and many will eventually require eye surgery. This can include cataract removal with implantation of an artificial lens (with or without an iris prosthesis), glaucoma surgery with or without placement of a tube shunt to reduce high pressure in the eye to slow the progression of glaucoma, and corneal surgery including corneal transplantation, transplantation of corneal stem cells (keratolimbal allograft stem cell transplantation), or removal of the damaged cornea and installation of an artificial one (a keratoprosthesis). These interventions often allow patients to preserve their baseline vision and can even restore lost vision in some instances.

Unfortunately, some congenital aniridia patients who undergo penetrating ocular surgery develop Aniridia Fibrosis Syndrome (AFS), the uncontrolled formation of scar tissue in the eye of a congenital aniridia patient following surgery. This scar tissue is very damaging to vision in that it is both not transparent and can damage other ocular structures leading to extremely poor visual outcomes- even loss of the eye altogether (Tsai et al, 2005).

However, at this point, we really do not know why some congenital aniridia patients develop AFS while others do not. First, congenital aniridia is a very rare condition so there are limited people available to study. Thus, we encourage you to enroll in the Aniridia Foundation Medical Registry and provide as much medical information as possible including any genetic results you may have had done. This can allow us to start to find common factors between those who develop AFS and those who do not. Second, as AFS has been only described for the past 10 years, not all ophthalmologists are aware of it, and many people with aniridia who may actually have AFS are instead told “their eye is full of scar tissue” or “they lost their vision to retinal detachment”. This would lead to some people with AFS not knowing that they have this condition. Lastly, as AFS appears to be an uncommon occurrence in aniridia patients (only 5% of congenital aniridia patients in the Cincinnati Eye Institute case series have been diagnosed with AFS), there are pretty small numbers of affected individuals. Thus, it is quite difficult to obtain enough patients to obtain validated, objective evidence on the causes of AFS. Nonetheless, clinical impressions are evolving and include:

Genetic risk factors:

- Congenital aniridia is not one disease, at least not genetically speaking. While it appears that most patients have mutations in the PAX6 gene, a significant minority do not. Currently, it appears that AFS only develops in those with



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The Eye on Aniridia is published by Aniridia Foundation International

AFI is a 501(c)3 non-profit organization dedicated to the support of people with aniridia, public awareness of aniridia, and aniridia-related research.

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c/o UVa Ophthalmology

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Eye on Aniridia

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PAX6 mutations. However, the PAX6 status of every aniridia patient is not known, so this is not 100% certain. Thus, we encourage all aniridia patients to be genotyped for the known genetic causes of aniridia, and provide this result to the Aniridia Foundation International Medical Registry. (Contact AFI for help with genetic testing and cost).

- Many different types of PAX6 gene mutations can cause aniridia. It is possible that each may play a different role in terms of defining AFS risk. Knowing the PAX6 status of many people who do and do not develop AFS will help us figure that out.
- People with the same PAX6 mutation, even in the same family, are known to exhibit very different severity of their aniridia, likely due to other “modifier” genes. While we do not know what these are as yet, the technology to study human genetics is rapidly evolving. You can help by participating in these genetic studies as they are begun.
- The phenotypic extent of aniridic disease in the eye may play a role – more severely affected patients may be at higher risk of AFS.

Surgical risk factors:

- Age at surgery may play a role – younger patients (who presumably have more severe or rapidly advancing aniridic disease) may have a higher risk of AFS.
- The extent of surgery may play a role – minor procedures may have a lower AFS risk than larger procedures.
- The associated inflammation during and after surgery may play a role – more inflammation may be associated with a higher risk of AFS.
- The experience of the surgeon in dealing with the complexities and nuances of operating on congenital aniridia patients may be associated with how “cleanly” the surgical procedure is performed. Cleaner surgery may be associated with a lower risk of AFS.

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- The extent to which the surgery impacts the root of the immature iris may play a role – particularly, during cataract surgery. “In the bag” implantation of all lens or iris prostheses devices may reduce AFS risk.
- The precise type of implanted hardware may correlate to AFS risk. Implantation of the Ophthec, Morcher, and Humanoptics custom flexible iris devices have been associated with a few cases of AFS, but have a reasonable track record over all in the United States (Doran, 2013). Multiple aniridia websites have reported that a Russian iris implant is associated with more cases of AFS or presumed AFS. However, it is entirely unclear if these perceived differences in clinical course are real or, if real, if they relate to the iris device, the implanting surgeons, the underlying disease state of the eye, or a combination of the above factors.

Unfortunately, the small number of people with congenital aniridia, and the wide variability in the severity of their eye disease makes it near impossible to perform a valid, randomized clinical study of these devices in congenital aniridia. Thus, decisions on whether to implant iris prostheses must be made on a case by case basis.

Consequences and treatment of AFS

The clinical significance of, and treatment for, AFS depends on the severity of the disease and the location of the scar tissue within the eye. If the AFS fibrotic membranes are limited to the front of the eye, surgical removal of this scar tissue, with or without device/hardware removal, can result in an excellent outcome. However, the inner lining of the cornea (corneal endothelium) can be damaged by the scar tissue itself and/or the surgeries to remove it, causing visual loss. This is particularly the case if the scar tissue has grown over and covered the corneal endothelium. Fortunately, corneal endothelial failure may be successfully treated by replacing this damaged tissue by transplant (endothelial keratoplasty).

However, if the AFS has progressed into back of the eye (the posterior segment), the prognosis becomes much more complicated.

- The ciliary body is the structure right behind the immature iris root that makes the fluid – aqueous humor – that keeps the eye pressurized and round. If AFS involves the ciliary body to a significant extent, the ciliary body can slow or stop its production of fluid causing low eye pressure (hypotony). Insufficient fluid secretion can result in the collapse of the eye and catastrophic visual loss. The only treatment is the removal of the AFS membranes from the ciliary body. This surgery, the epiciliary peel, is complex and technically challenging, usually requiring a pars-plana three-port vitrectomy, endoscopic visualization and may entail removal of all implanted hardware such as iris and lens prostheses. Even after successful surgical removal of AFS from the ciliary body, ciliary body function may not return to normal. In these instances, the eye may need to be filled with silicone oil long term, which may stabilize intraocular pressure enough to preserve vision.
- The retina – which is responsible for detecting the light entering the eye and transmission of this information to the brain – terminates about 3mm behind the ciliary body. If the AFS is severe and grows over the ciliary body and onto the retina, the outcome can be dire. AFS membranes, like all scar tissue, have the ability to contract since they contain large numbers of “muscle-like” cells called myofibroblasts. If the AFS contacts the retina and contracts, this can result in a tractional retinal detachment. Retinal detachments will cause catastrophic visual loss if not treated promptly because the retina begins to die once detached from the back wall of the eye. This, is especially the case when the retinal detachment is complicated by the above described AFS induced damage to the ciliary body and retina. The only treatment for this dire scenario is a complex and heroic surgery including membranectomy, retinal detachment repair, and usually, filling the eye with silicone oil to maintain both the retinal reattachment and

good ocular pressure. Many of these eyes have severe aniridic disease states, and already have an artificial cornea (Dohlman keratoprosthesis) in place, or end up with one shortly after the AFS surgery.

Surgical intervention is successful in preventing catastrophic loss of vision in some, but not all, eyes. This may be because the AFS associated damage to the eye was too great already prior to treatment, since the patient, who already suffers from low vision, may not have noticed further vision loss until it was severe. Thus, it is critical that aniridia patients undergo regular examination of the eye following any ocular surgery, with special attention paid to the iris root where AFS often starts. Also, in some patients, AFS recurs after treatment (which is an invasive ocular surgery itself), leading to further damage, and additional surgeries, each with the potential for a poor outcome.

Ongoing clinical research into the treatment of AFS directed by Dr. Riemann at Cincinnati Eye

AFS bears some clinical similarity to another, unrelated, ocular scarring condition which is well known to vitreo-retinal surgeons. Each year, approximately 1 in 10,000 Americans develop a retinal detachment subsequent to a retinal tear (rhegmatogenous retinal detachment; RRD). Known risk factors for RRD include, advanced age, family history, myopia, thin retinas (lattice degeneration), cataract surgery, and retinal breaks or holes. For reasons that are not completely understood, 5–20% of patients undergoing repair of a RRD develop severe fibrosis that contracts and re-detaches the retina, a condition called proliferative vitreoretinopathy (PVR). PVR is treated by a repeat surgical procedure to remove this scar tissue and reattach the retina, but less than half of these patients ever regain fine detailed vision. Thus, there is great interest in the development of drug therapies to prevent the formation of scar tissue after retinal reattachment surgery with a focus on blocking the pathways that encourage uncontrolled cell growth and associated fibrosis.

For instance, methotrexate is an anti-cancer drug that was first approved for use in 1948. It has

a very long history of safety and efficacy against cancer and some autoimmune diseases. It has been directly instilled in the eye since the 1960s for the successful treatment of certain eye cancers, ocular inflammatory conditions, and postsurgical epithelial ingrowth – another condition which shares some features of AFS. A recent study directed by one of us (Dr. Riemann) has shown that methotrexate may improve the visual outcome of some patients with PVR as it appears to block its recurrence in a retrospective study (Sedaka et al, 2016). Prospective clinical studies are underway to further elucidate the extent of any possible anti-PVR effect and we are (cautiously) optimistic about this line of research.

Currently, Dr. Riemann's group at the Cincinnati Eye Institute is beginning studies on the use of methotrexate both during and after surgery for AFS in order to slow AFS progression, and prevent recurrences after surgery, since this drug has the potential to block both the formation of new myofibroblasts after surgery and inflammatory processes that can induce fibrosis. However, whether methotrexate may benefit patients with AFS is not known, although we have used it in a congenital aniridia patient with PVR (Sadaka et al, 2016). We are currently treating our very first AFS patient with methotrexate at the Cincinnati Eye Institute. We believe this to be the first AFS patient worldwide to receive this treatment and are very hopeful that it will be effective.

Ongoing translational research into the basis and treatment of AFS at Dr. Duncan's laboratory at the University of Delaware funded by Aniridia Foundation International

As described above, we have little knowledge about the causes of AFS because it is an uncommon complication of an already very rare disease. At the onset of our studies two years ago, even the composition of the membranes seen in human AFS was in question. Thus, we began our studies by studying the membranes that were removed from the eyes of human patients during the treatment of AFS at the Cincinnati Eye Institute. This allowed us

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to show that AFS “membranes” are classical scar tissues comprised of myofibroblasts (the contractile cells shown in green in Figure 1), which produce an inflexible matrix composed of the proteins collagen type I and fibronectin (fibronectin is shown in red in Figure 1). This led us to start studying whether the mechanisms that we know are responsible for scar tissue formation in other parts of the body are active in AFS. Our studies have indicated that human AFS membranes may exhibit activation of two different known “scar formation” pathways, (ie transforming growth factor beta (TGFbeta) and canonical WNT signaling). This is critically important since TGFbeta and WNT signaling can synergize with each other to enhance scar tissue formation in other fibrotic diseases.

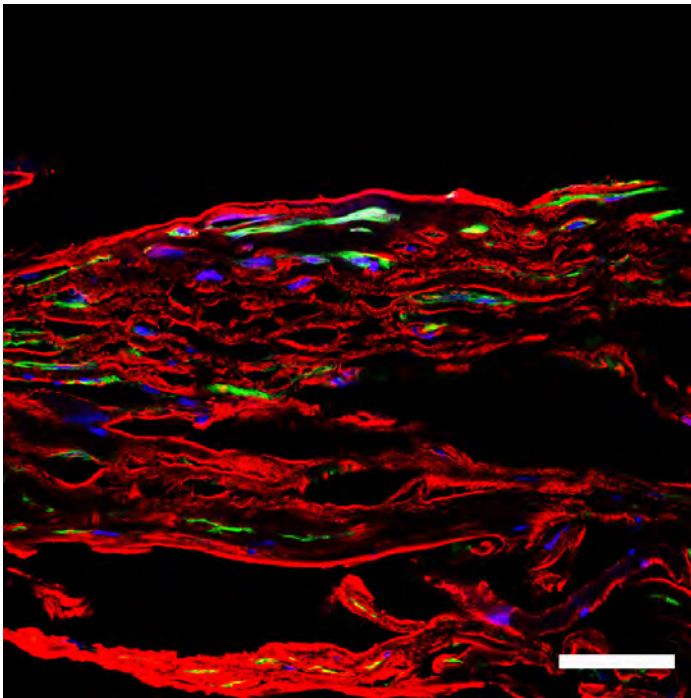


Figure 1: Under the microscope, human AFS membranes exhibit features of classical scar tissue including high expression of fibrotic proteins such as fibronectin (red) and the presence of myofibroblasts (green, which labels α -smooth muscle actin, α SMA, a biomarker of myofibroblasts). These myofibroblasts are cells that produce the proteins found in scars and can contract to cause damage to other eye structures. Blue-DNA, scale bar=62 μ m.



Figure 2: The external appearance of the wildtype and Pax6 mutant mice used in our study. The wildtype mouse has normally sized eyes with clear lenses and corneas, while the Pax6 mutant mouse has small eyes with cloudy corneas and lenses.

However, at this point, further studies on human tissue were not feasible since little AFS tissue is available and there is no way to obtain tissue from aniridia patients prior to the onset of AFS without compromising their vision. To overcome this hurdle and better understand the full mechanisms involved, we needed to conduct studies in animals that can model human congenital aniridia. Notably, there are many mouse strains available that have mutations in the Pax6 gene (the small eye mouse). The Pax6 mutant mice develop many of the same eye disorders as aniridia patients as well, including the lack of an iris, cataract and corneal damage (keratopathy) (Figure 2). Aniridia Foundation International, via support from generous donors, has provided us funds to use these mice to investigate the pathogenesis of AFS.

First, we found that Pax6 mutant mice have a tendency to develop small regions of scar tissue in their lenses and corneas even without surgery. This suggests that Pax6 mutations may sensitize the eye to develop scar tissue even without surgery. Following corneal surgery, Pax6 mutant eyes develop more scarring compared to that of normal mice, both at the site of the surgical wound, and at the base of the iris and ciliary body. Importantly,

this scar tissue is very similar to AFS membranes as it is comprised of myofibroblasts in a matrix of type I collagen and fibronectin (Figure 3). Further, like human AFS material, the fibrosis seen in the Pax6 mutant mice exhibits elevated transforming growth factor beta and WNT signaling. These observations show that we can induce AFS in mice that is very similar to that seen in humans so that we can figure out how and why AFS forms in order to figure out the best ways to either prevent or treat this condition.

Most prior scientific studies on Pax6 function in the eye have focused on how Pax6 controls the formation of the eye prior to birth. Notably these studies have shown that, among other things, Pax6 is important to turn off TGFbeta and WNT signaling in the early embryo, and this is apparently one reason that mice completely lacking both copies of the Pax6 gene do not develop eyes at all. We have also found that mice with one mutant copy of the Pax6 gene have more WNT and TGFbeta signaling than normal. We are now testing the hypothesis that adult eyes from mice carrying one mutant copy of Pax6 have too little of the molecules that should be keeping WNT and TGFbeta signaling in check. If this is the case, this could explain why congenital aniridia patients who have one mutant copy of the

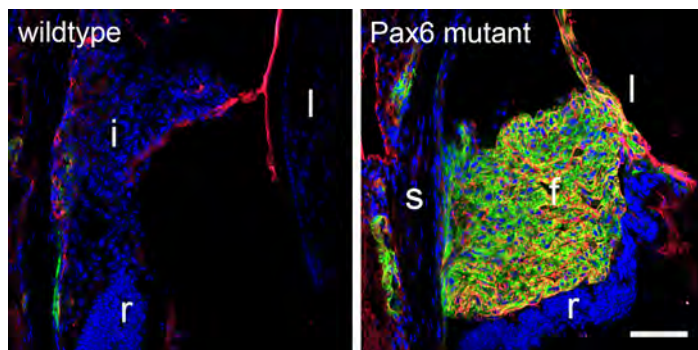


Figure 3: Five days after corneal surgery, Pax6 mutant mice develop obvious scar tissue at the base of the iris and ciliary body, which was absent in the wildtype. Under the microscope, this scar tissue has exactly the same structure as seen in human AFS membranes, including the presence of fibronectin (red) and myofibroblasts (green; which labels α -smooth muscle actin, α SMA, a biomarker of myofibroblasts). Blue-DNA, f-fibrotic plaque (scar tissue), i-iris, l-lens, r-retina, s-sclera, scale bar=62 μ m.

Pax6 gene are more susceptible to severe scarring (ie AFS) following ocular surgery. Overall, this study is the first investigation ever into the causes of AFS and has already provided information that is critically needed in order to develop rational ways to prevent this condition. We hope to finish this initial study over the next few months and submit it for publication in the scientific literature early in 2017.

Future opportunities for translational research into the causes and treatments of AFS

After the completion of the current study, Dr. Duncan's group would like to test whether inhibitors of the WNT and TGFbeta signaling pathways, that have been used previously to treat other fibrotic diseases as well as cancer, are safe for installation in the eye and can block the robust scar tissue formation that forms in mice that carry a mutant Pax6 gene. We hope that a combination of these approaches can greatly improve the long term prognosis for both congenital aniridia patients who develop AFS as well as other blinding conditions caused by uncontrolled ocular scarring such as PVR.

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Read more details on page 1. We hope to see you this summer!

