

# Progressive, Irreversible Loss of Vision: Gyrate Atrophy of the Choroid and Retina

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## Summary

**In our highly visual society, a genetic disease that gradually dims one's vision must be seen as a cruel and debilitating disease. One such disease is the gyrate atrophy of the choroid and retina (GA), an autosomal recessive disease caused by mutations in the gene encoding the mitochondrial enzyme ornithine aminotransferase (OAT). GA is characterized by early onset myopia and night-blindness followed by cataracts and progressive loss of peripheral vision culminating in complete blindness. More than 60 mutations in the gene coding for OAT cause the 150 known cases of GA by an unknown mechanism. Clinical genetic tests are available and should be carried out if there is a family history of the disease, as early dietary restriction of arginine and clinical doses of vitamin B6 slow its progression. Experiments in cultured epidermal keratinocytes show promise towards gene therapy for GA. Future research should be geared towards understanding the molecular basis of GA and finding a permanent cure.**

## Introduction

Gyrate atrophy of the choroid and retina (GA) is a rare, mostly Finnish, autosomal recessive inborn error of metabolism disease caused by a mutation in the nuclear gene which codes for the mitochondrial protein ornithine aminotransferase (OAT) (Valle and Kaiser-Kupfer, 1982). The symptoms of GA include myopia early in the first decade of the patient's life followed by night-blindness, cataracts, reduced peripheral vision, and progressive narrowing of the visual field (tunnel vision) due to the death of cells in the choroid and retinal regions of the eye in sharply demarcated circular regions. The disease culminates in total loss of vision by the age of 30 or 40, as the regions of gyrate atrophy progressively increase and consolidate (Valle and Kaiser-Kupfer, 1982).

Biochemically, GA is characterized by elevated plasma ornithine concentrations (hyperornithinemia), and lowered proline and creatine concentrations not accompanied by hyperammonemia (Valle & Simell, 2001). This distinction serves to distinguish GA from other urea cycle diseases like hyperammonemias, as well as from hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, which is the other hyperornithinemia.

## Impact of GA

Our eyes enable us to distinguish friend from foe, recognize foods and avoid things that cause us harm; they provide an extraordinary evolutionary advantage in

our world. This is apparent from the fact that different types of eyes have evolved independently multiple times in the animal kingdom.

We are a very visual society. Our attention to television, art and architecture attest to that, as do phrases like 'seeing is believing.' The development of a written language and the prominence of the internet have cemented the importance of vision in our life. The biological and cultural significance of sight makes an inherited disease like GA an important one to study.

I am personally attracted to the study of this disease because it initially affects night vision. I find that the night makes most things appear more sublime and serene. It is my favorite time, and I would hate to lose my ability to appreciate the beauty of the night. Our society is also becoming more night oriented since we are no longer dependant on sunlight, which further increases the burden on GA patients.

Also, I think of my world in a very visual way—I learn things by writing and reading them, I love photography, the movies and architecture—and therefore I consider our sense of sight to be more important than our senses of touch, taste, and hearing.

## The Aberrant Macromolecule: OAT

Ornithine aminotransferase (OAT) is a nuclear encoded mitochondrial matrix enzyme that is the primary catabolic enzyme for the non-protein amino acid ornithine (Inana et al, 1989). It catabolizes ornithine to pyrroline-5-carboxylate (PC) with the help of the coenzyme pyridoxal phosphate and thence to glutamate or proline (see figure 1).

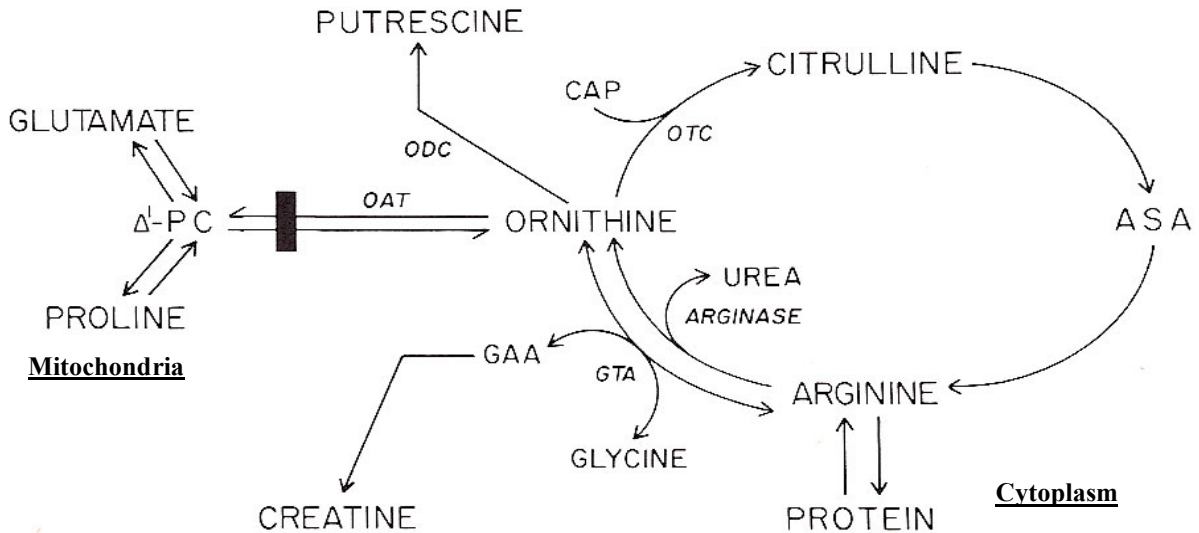
Ornithine is an important component of the urea cycle, which is responsible for reducing the nitrogen burden in the body by removing the toxic ammonia—formed as a result of amino acid catabolism—from the body by converting it to urea. Ornithine is the primary acceptor of ammonia in the urea cycle and, since the urea cycle occurs partly in the cytoplasm and partly in the mitochondrial matrix, ornithine along with citrulline are essential for the complete turn of the urea cycle.

OAT is translated in the cytoplasm as a 48 kDa precursor containing a leader sequence that allows its transport to the mitochondrion (Inana et al, 1989). This leader sequence is cleaved in the mitochondrial matrix to give rise to the 45 kDa mature protein. Four to six of the proteins come together to form the active quaternary structure of the enzyme (Inana et al, 1989).

There are more than 60 known mutations in the gene coding for OAT that lead to GA (Wang et al. 2000). Each mutation has a different effect on the gene product and thus might give rise to a slightly different form of GA. There are many known nonsense codon mutations that lead to reduced OAT mRNA (Mahima et al., 1992), and microdeletions that lead to splicing defects (McClatchey et al., 1990), as well as many point mutations including those that lead to the loss of the initiator codon (Mitchell et al., 1988).

It is remarkable that such a large number of mutations are known to cause this rare disease with only about 150 biochemically-documented cases (Valle & Simell, 2001). This suggests that OAT could be a very sensitive protein, and that changing its primary

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**Figure 1.** The Urea Cycle, showing the role of ornithine and OAT. CAP is carbamyl phosphate, the source of fixed ammonia in the mitochondria. The figure is modified from Valle and Kaiser-Kupfer (1982).

structure even slightly affects its ability to form the functional homo-tetramer. This allelic heterogeneity could also imply that the nature of the gene is such that it is more susceptible to mutations.

A small subset of these cases are of a form of GA called pyridoxine (vitamin B<sub>6</sub>) responsive GA since the progress of the disease can be stemmed by pharmacological doses of vitamin B<sub>6</sub> (Valle, & Simell, 2001). This type of GA is caused by mutations in regions of the gene coding for OAT that are responsible for its binding to its coenzyme pyridoxal phosphate. This form of the disease is rarer, and known to be caused by 3 mutations; V332M, A226V, and E318K (Valle & Simell, 2001, and Michaud et al, 1995).

### Molecular Basis of GA

The molecular basis of GA and its primary targeting of cells of the choroid and retina are the disease's greatest mysteries (Valle, & Simell, 2001). The complexity in determining the molecular model of the disease lies in the fact that this model must explain the hyperornithinemia without an accompanying hyperammonemia. It must also explain the limited involvement of other organ systems, and elucidate the reason for the progressive degeneration seen in this disease.

Three possible models are described by Weleber, et al. (2003). The first supposes a direct toxic effect of ornithine on the pigmented epithelium. According to this model, high levels of ornithine have a direct toxic influence by some unknown mechanism. Supporters of this model cite as evidence for this model the fact that reduction of ornithine levels is sufficient to treat GA (Wang, et al., 2000). This evidence cannot rule out the second hypothesized model.

The second model states that the suppression of proline synthesis (due to the inhibitory effect of accumulated ornithine) is the molecular cause of the disease. Proline can be synthesized in the body from PC, which is the product of ornithine catabolism by OAT. This is the major or only pathway for proline synthesis in many tissues in the body (Valle & Simell,

2001). Therefore it is possible that high levels of ornithine have an indirect toxic effect in the body due to the lowering of proline levels. This model is supported by an experiment conducted by Ueda et al, in 1998 in human retinal pigmented epithelial cells where they used an irreversible inhibitor of OAT to cause ornithine cytotoxicity. This toxicity was prevented by supplying the cells with proline.

The third disease model identifies the suppression of the synthesis of creatine and creatine phosphate in the retina as the culprit in GA. High levels of ornithine inhibit the working of glyceraldehyde transaminidase, which is the first enzyme in creatine synthesis (Valle and Kaiser-Kupfer, 1982). Treatment of GA by supplying high levels of creatine helps alleviate some muscle abnormalities seen in GA patients but does not stop the ocular progression of the disease suggesting that creatine does not play a major role in the chorio-retinal atrophy (Valle and Kaiser-Kupfer, 1982).

An additional level of complexity arises due to the rarity and heterogeneity of the disease, the fact that it is not known which type of cell in the retina is affected first, and also due to the fact that cells in the retina are highly interdependent (Valle & Simell, 2001) and may die due to pressures from neighboring cells.

### The OAT Gene

Mitchell et al. and O'Donnell et al. (1988) found that the OAT gene was located on the human chromosome 10. Mitchell et al. (1988) showed that the gene is 21 kilobase pairs in length, and contains 11 exons with exon 2 being almost always absent from all mRNA transcripts. Interestingly, the translated region for the gene begins in exon 3 as opposed to the first two exons.

Low stringency southern blots of human DNA probed with OAT cDNA indicate the presence of multiple OAT pseudogenes on the short arm of the X chromosome (Valle & Simell, 2001). Pseudogenes are sequences in the genome that are evolutionarily related to a gene, no longer code for a protein, but may play a role in regulating the gene expression (Gibbs, W. W.,

2003). The OAT pseudogenes, however, appear to be non-functional (Valle & Simell, 2001).

OAT is expressed in hepatocytes, renal tubular cells, brain, neural retina, retinal pigment epithelium, and greatly expressed in neonatal intestinal cells (Wang, et al., 1995). This widespread expression requires an explanation for the fact that GA affects primarily the retina.

### Model Organisms

Wang et al (1995) created the first knockout mice homozygously lacking OAT. Interestingly, the lack of OAT was fatal to these mice, and they exhibited an unexpected hypoorithinaemia. This shed light on the different role of OAT in neonatal and adult mice. In neonatal mice, OAT is required to synthesize ornithine from glutamate. This ornithine is then used to synthesize arginine, which is needed by the quickly growing mouse but is not available in required amounts in its diet. At an older age, when the need for arginine is fulfilled, the role of OAT is reversed: it becomes involved in breaking down ornithine and giving rise to glutamate or proline. Thus, mice lacking OAT develop hyperornithinemia and the symptoms of GA. The hypoorithinaemia in neonatal mice encouraged researchers to look at human infants who had GA (this was not previously possible since GA symptoms don't manifest themselves until well within the first decade of patients' lives). They found that human infants also exhibited hypoorithinaemia. Thus, mice were established as a good model organism for the study of GA and gave great insight into the role of OAT from the beginning.

In 1996, Wang et al. used the mouse model to follow up on the initial observations from the previous study and provided good evidence that, in mice, the retinal pigment epithelial (RPE) cells are the initial site of attack because they were the first to show signs of pathological changes in two month old mice.

The mouse model was also used to provide evidence in support of the idea that ornithine itself has a direct toxic effect in GA (Wang et al., 2000). Mice deficient in OAT and expressing hyperornithinemia were maintained on an arginine restricted diet at the age of three weeks, which led to a reduction of ornithine in their system to normal levels. This reduction of ornithine accumulation correlated with a prevention of retinal degeneration suggesting that the reduction of ornithine accumulation is sufficient to treat GA (Wang et al., 2000).

### Genetic Tests and Treatment

Clinical genetic tests for OAT deficiency are available in a lab in Finland (EDDNAL, 2002) and six labs in the United States (NHLBI, 2004). If there is a family history of GA, the test should be carried out because finding the disorder early allows the disease to be treated and the condition stabilized.

The three main GA treatments are ornithine reduction by restriction of arginine in the diet and augmentation of renal loss, creatine supplementation since creatine levels are lower in GA patients, and administration of pharmacological doses of pyridoxine (for pyridoxine-responsive GA). Of these treatments, dietary arginine restriction has been shown to be most successful (Valle, & Simell, 2001). These diets are often supplemented by synthetic sources of amino acids, which are quite expensive; however, Santinelli et

al. (2004) showed in a 26 year follow-up study that a natural low-protein diet is sufficient to reduce ornithine accumulation in the body and thus can be used to retard GA progress.

Sullivan et al. (1997) devised a novel approach to reduce ornithine accumulation in the body. Their approach involves setting up a metabolic sink to reduce ornithine levels. In a proof of concept paper, they over-expressed OAT in keratinocytes cultured from a GA patient by transducing the cell using an adenovirus vector. They showed that these cells remove ornithine from the media they are bathed in much faster than normal keratinocytes. In addition, Christensen et al. prepared a 2002 clinical trial at the NIH in which a small patch of keratinocytes were grafted onto the thigh of a GA patient to check the efficacy of this treatment. Results of this study remain to be evaluated. If successful, this treatment would provide an easier and more permanent alternative to current treatment methods requiring precise and ongoing dietary restrictions.

None of the current treatments repair damage caused by the disease; rather, they attempt to stabilize the patient's condition. Eye transplants could serve as a short-term solution for the disease, but the high levels of ornithine in the body will eventually cause transplanted organs to deteriorate. If applied early enough, the metabolite sink method could prove to be a suitable treatment for GA. However, more permanent cures need to be found that will halt the disease early in the life of the patient. Gene therapy, despite its present shortcomings, may one day prove quite valuable for GA treatment.

### Looking to the Future

Research in the field of GA has done a lot to explain the role of OAT in the body and has demonstrated methods to prevent the progress of the disease. However, many questions still remain unanswered. The molecular model of the disease must be elucidated, and further studies should be conducted to determine why it primarily targets the choroid and the retina. Understanding the mechanism of the disease is essential to finding a permanent cure for it.

Also, there is much evidence and argument that GA might not just be a disease of the eye, but is rather a systemic disease involving the central and peripheral nervous system (Peltola, et al. 2002). This may explain why none of the treatments are effective one hundred percent of the time, and it is an important concept to investigate in the future.

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