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### **Aspartylglucosaminuria (AGU)**

Aspartylglucosaminuria (AGU) is a lysosomal storage disorder that is characterized mainly by progressive mental retardation. AGU patients are born seemingly normal, but within the first years of life, the symptoms of the disease manifest with progressive loss of mental capabilities such as speech. In their early adulthood, most AGU patients are severely mentally retarded, but upon good medical care, the life expectancy is relatively long. AGU is a rare disease, with an unknown prevalence in most countries. However, due to the peculiar population history of Finland, the disease is enriched in the Finnish population.

### **Aspartylglucosaminidase (AGA)**

AGU is caused by recessive mutations in the gene coding for a lysosomal enzyme, aspartylglucosaminidase (AGA). This enzyme participates in one of the final steps of lysosomal degradation of proteins that are N-glycosylated, that is: decorated with specific sugar chains. In the absence of AGA activity, the substrates of AGA, the so-called glycoasparagines, accumulate in the lysosomes and are even excreted in the urine, which can be used as a diagnostic measure for AGU.

The AGA enzyme is synthesized as a single polypeptide chain, but enzyme activity is only observed after two precursors soon after their synthesis join together and become cleaved into two subunits called  $\alpha$  and  $\beta$ . Thus, the active enzyme is a heterotetramer consisting of two  $\alpha$  and two  $\beta$  subunits.

### **Effect of AGU mutations on AGA**

The most common AGU mutation worldwide is the AGU<sub>Fin-major</sub> mutation found in the vast majority of Finnish AGU patients. This mutation is actually a combination of two amino acid substitutions, an exchange of cysteine 163 to serine and arginine 161 to glutamine. However, only the Cys163Ser exchange is pathologically relevant, as it results in destabilization of the AGA enzyme structure and thus prevents the processing of the precursor into the active form. A second typical AGU mutation, a deletion called AGU<sub>Fin-minor</sub>, is present in some Finnish patients. Patients from elsewhere in the world primarily have their individual gene defects that range from point mutations to deletions and insertions.

The three-dimensional structure of the human AGA enzyme at atomic resolution has been solved. This has facilitated a detailed analysis of the consequences of AGU mutations on the AGA enzyme structure. Therefore, we are now able to understand in which way the AGU mutations impair the function of the enzyme. Some of the mutations result in a profound lack of any AGA protein at all, since a part of the gene is missing (deletion), or the protein synthesis is prematurely interrupted (so-called nonsense mutations). However, some mutations, including AGU<sub>Fin-major</sub>, alter the enzyme structure, but the amount of the AGA protein is not severely reduced. In most of these cases, the activation of the AGA precursor is impaired and only a very low degree of residual AGA activity is detected in the cells of the affected patients. Depending on where in the enzyme structure these mutations reside, the degree of functional impairment may be relatively moderate, and it may even be possible to correct or reactivate these mutated enzymes.

## Animal models and therapy for AGU

Two different AGU mouse models were created in the 1990s, both of which lack AGA expression due to disruption of the *AGA* gene. Adult mice show massive accumulation of glycoasparagines and very similar pathological changes as found in human AGU patients. No mouse strain carrying a specific disease-causing mutation, such as AGU<sub>Fin-major</sub>, has been created yet. The existing AGU mice have been used to test different treatment options, e.g. gene therapy and enzyme replacement therapy (ERT). The gene therapy trials in which a virus carrying the *AGA* gene injected into the brain of adult AGU mice gave promising results. The amount of storage material in the lysosomes was clearly reduced and the AGA enzyme was even found in cells that had not been infected with the virus, indicating that the AGA protein can be transported from one cell to another. However, for the gene therapy to become available as a treatment option for AGU, more time is needed to test the safety and to establish this method for use in patients.

In further studies, AGU mice were subjected to ERT. For this, AGA protein purified from AGA overexpressing cells was injected into AGU mice of different age. In all studies, the level of enzyme uptake was tissue-dependent. In general, non-neuronal tissues, especially liver and spleen, gained high AGA activity and showed a profound (> 60%) reduction in intracellularly stored glycoasparagines. However, in the brain, ERT was less successful in adult mice, but promising in neonatal mice, stressing that early start of ERT is important to prevent the progression of AGU. Despite the beneficial effects of ERT in mice, no human trials have been performed yet. This is due to the fact that it has been difficult to produce sufficient amounts of AGA protein for ERT in patients. So far, no pharmaceutical company has launched this therapy option for clinical use.

In the 1990s, seven AGU<sub>Fin-major</sub> patients (1-10 years of age) were subjected to bone marrow transplantation (BMT). Only in 5 patients, the procedure was successful and initially led to promising results. Transplanted patients had increased AGA activity in blood cells and improved brain scan data. However, the patients suffered from different post-transplant complications and had to endure long hospital stays. Unfortunately, after 4-7 years of follow-up, no mental improvement was noted, and the patients still experienced AGU-specific health problems. However, further progression of the disease appeared to be slowed down. Altogether, BMT was not recommended as a treatment option due to the complications associated with the therapy and the relatively benign therapeutic effect.

Recently, our group has developed a pharmacological chaperone therapy (PCT) as a putative treatment option for AGU. Pharmacological chaperones in general are small molecules that directly bind and stabilize their target protein. By doing so, they may help to stabilize the mutated enzymes and thus increase their activity. Our group could show that the small molecule betaine (a component of beet root) and the amino acid glycine were able to reactivate AGU<sub>Fin-major</sub> and another mutated form of AGA with a single amino acid exchange. The PCT can only be effective in patients who exhibit mutations that do not affect the active site of the enzyme, but rather produce milder defects in the structure of the AGA enzyme and thus affect its processing into subunits. However, this treatment option will not work for nonsense mutations, splicing mutations or deletions.

Currently, no curative therapy is available for AGU patients. However, we have decided to convert our promising findings on PCT for AGU into clinics. A clinical trial using Cystadane (trade name of betaine) with 21 AGU patients who are homozygous for the AGU<sub>Fin-major</sub> mutation has started in January 2018 in Finland (EU Clinical Trial number 2017-000645-48).

AGU patients with a nonsense mutation in their AGA gene, i.e. a mutation that results in a premature stop codon and therefore synthesis of either truncated AGA proteins or no protein at all, may benefit from treatment with Amlexanox. Recently, our group was able to show that this substance is able to stabilize the mutated mRNA, which would normally be degraded by a process called nonsense-mediated decay (NMD). Furthermore, Amlexanox induces a translational read-through at the nonsense codon, which allows the synthesis of a full-length, functional AGA enzyme. In some countries, Amlexanox already is an approved drug for e.g. asthma, and it is currently being tested in clinical trials for Diabetes Type II and obesity.

*Recommended reading:*

- Arvio & Mononen <https://www.ncbi.nlm.nih.gov/pubmed/27906067>
- Banning et al. 2016 <https://www.ncbi.nlm.nih.gov/pubmed/27876883>
- Banning et al. 2017 <https://www.ncbi.nlm.nih.gov/pubmed/29247835>
- Kaartinen et al. 1996 <https://www.ncbi.nlm.nih.gov/pubmed/8946839>
- Jalanko et al. 1998 <https://www.ncbi.nlm.nih.gov/pubmed/9425233>
- Virta et al. 2006 <https://www.ncbi.nlm.nih.gov/pubmed/16518877>
- Peltola et al. 1998 <https://www.ncbi.nlm.nih.gov/pubmed/9930336>
- Kelo et al. 2005 <https://www.ncbi.nlm.nih.gov/pubmed/15342551>
- Dunder et al. 2010 <https://www.ncbi.nlm.nih.gov/pubmed/20607610>
- Laitinen et al. 1997 <https://www.ncbi.nlm.nih.gov/pubmed/9137882>
- Arvio et al. 2001 <https://www.ncbi.nlm.nih.gov/pubmed/11174635>
- Malm et al. 2004 <https://www.ncbi.nlm.nih.gov/pubmed/15316370>
- Autti et al. 1999 <https://www.ncbi.nlm.nih.gov/pubmed/10706021>