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An Overview on C-Terminal Agrin Fragment

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Abstract:

Agrin is a large heparan sulfate proteoglycan predominantly expressed in the nervous system and at the neuromuscular junction, where it plays a pivotal role in synapse formation and stabilization. The C-terminal agrin fragment (CAF) is a cleavage product generated by the proteolytic activity of neurotrypsin, a serine protease that cuts agrin at specific sites, producing fragments including the 22-kDa C-terminal portion known as CAF22. This fragment has gained attention as a potential circulating biomarker for muscle wasting, particularly in conditions such as sarcopenia, frailty, and chronic diseases including chronic kidney disease and heart failure. Elevated serum levels of CAF22 are believed to reflect increased muscle catabolism and neuromuscular degradation, correlating with physical decline and functional impairment. Its role as a non-invasive biomarker offers significant clinical relevance for early detection, risk stratification, and monitoring of therapeutic interventions in muscle-wasting disorders. Furthermore, research into CAF may contribute to a deeper understanding of the neuromuscular junction integrity and its role in systemic diseases.

Key words: C-terminal agrin fragment (CAF), Agrin, Muscle wasting.

Introduction:

Agrin is a multidomain heparan sulfate proteoglycan that plays a vital role in the formation and maintenance of the neuromuscular junction (NMJ). It is predominantly expressed in motor neurons and skeletal muscle, where it facilitates the clustering of acetylcholine receptors, ensuring efficient neuromuscular transmission. The biological activity of agrin is tightly regulated through proteolytic cleavage by the enzyme neurotrypsin, which generates distinct fragments, including the 22-kDa C-terminal agrin fragment (CAF22) **(1).**

The C-terminal agrin fragment, particularly CAF22, has emerged as a potential circulating biomarker for muscle wasting and neuromuscular degradation. Elevated levels of CAF22 in the bloodstream are associated with aging, sarcopenia, chronic kidney disease, and frailty, making it a candidate for the early detection and monitoring of neuromuscular dysfunction in both elderly and clinical populations **(2, 3).** CAF measurement offers a non-invasive approach to assess muscle integrity and functional decline, particularly in patients with limited physical performance.

Current research is focused on validating CAF as a reliable marker for clinical application and understanding its role in neuromuscular pathophysiology. By exploring CAF dynamics in health and disease, researchers aim to develop diagnostic tools and potential therapeutic targets for managing conditions related to muscle atrophy and motor impairment **(4).**

Agrin is a large proteoglycan which has a role in the development of the neuromuscular junction during embryogenesis. Agrin is named based on its involvement in the aggregation of acetylcholine receptors during synaptogenesis.

In humans, this protein is encoded by the AGRN gene. It is a major proteoglycan component in the glomerular basement membrane and may play a role in the renal filtration and cell-matrix interactions **(5).**

Agrin was first identified by the U.J. McMahan laboratory, Stanford University. Agrin was initially isolated from basal lamina extracts of Torpedo californica (electric ray) and shown to have the ability to induce differentiation of the postsynaptic membrane of muscle cells **(6).**

Agrin is a large, multidomain protein, approximately 220–240 kDa in size, with several functional regions, including: **N-terminal**: Contains a domain involved in heparan sulfate proteoglycan binding, which helps anchor agrin in the extracellular matrix, **Central region**: Includes a laminin G-like domain that is involved in receptor binding and a repeating structure similar to the thrombospondin type 1 repeats, **C-terminal**: This region contains the fragment that gets cleaved, and the structural features here are particularly important for its activity in neuromuscular junction development and function **(7).**

The transcript of the Agrin gene can be differentially spliced to produce different isoforms of the protein, which determine its localization and function.

Alternative splicing at the amino terminus produces either a type II transmembrane protein (TM-Agrin) or a secreted protein (SS-Agrin) **(8).**

SS Agrin also contains a laminin-binding domain immediately following the secretion sequence, which anchors the secreted form to the extracellular matrix.

There are at least two additional splicing sites close to the carboxyl terminus of the protein know as A/y and B/z **(9).**

Expression of AGRIN:

Agrin is highly expressed in the brain, where its function has been linked to proper synaptic transmission of excitatory but not inhibitory synapses in the cerebral cortex. Agrin exists in several splice variants and can be expressed as a secreted protein, containing the N-terminal agrin (NtA) domain, which is the most abundant form of agrin and the predominant form expressed in motor neurons. It is produced in the soma of the neurons, transported down the axon and released from the axon ending of the motor nerve into the synaptic cleft of the NMJ **(10).**

Here it acts as an agonist of low-density lipoprotein receptor related protein 4(LDLR4) and may also become a component of the basal lamina. In the central nervous system (CNS), Agrin is expressed as a type-II transmembrane protein by alternative splicing at the N-terminus lacking the N-terminal NtA domain.

Among non neuronal tissues, Agrin is highly expressed in the kidney, where it substantially contributes to the formation of the glomerular basement membrane possibly linking it to podocytes. These cells are part of the glomerulus, which is the organelle filtering small molecules and small proteins from the blood **(11).**

The glomerulus consists of capillaries with fenestrated endothelium and the mesangial cells. These cells are modified smooth muscle cells that lie between the capillaries and the glomerulus. The purpose of these cells is to regulate blood flow and to secrete extracellular matrix to build up the glomerular basement membrane, prostaglandins, and cytokines **(12).**

Podocytes are surrounding the capillaries by creating a large cellular surface due to lots of foot processes (pedicels). These pedicels express nephrins, which are membrane bound proteins capable as a filtration barrier between the blood stream and the primary urine in the Bowman's capsule **(13).**

The characteristic of this filtration barrier is that large proteins or negatively charged proteins cannot permeate and remain in the blood stream, Low molecular weight proteins or metabolites are able to permeate and are transferred into the primary urine. A part of these substances is recycled in the nephron or degraded by cells of the tubular system or remains in the urine **(14).**

Cleavage of AGRIN:

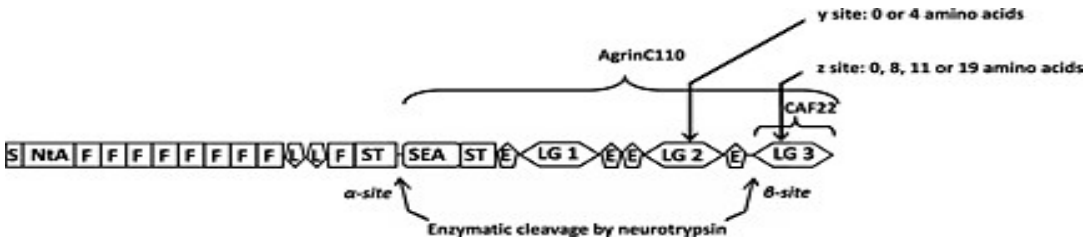

Figure (1): Cleavage of AGRIN.

Cleavage Mechanism:

1. **Proteases Involved**: The primary protease involved in agrin cleavage is **neurotrypsin**, an enzyme expressed in neurons, but other proteases, such as **matrix metalloproteinases (MMPs)**, can also contribute. These enzymes break the agrin protein at specific cleavage sites, resulting in the release of various agrin fragments.
2. **C-terminal Fragment**: The **C-terminal fragment** is one of the cleaved products. This fragment retains key functional domains, such as:

Laminin G-like domains: Important for receptor binding and neuromuscular junction signaling.

Heparan sulfate proteoglycan-binding domains: Help in attachment to the extracellular matrix (**15**).

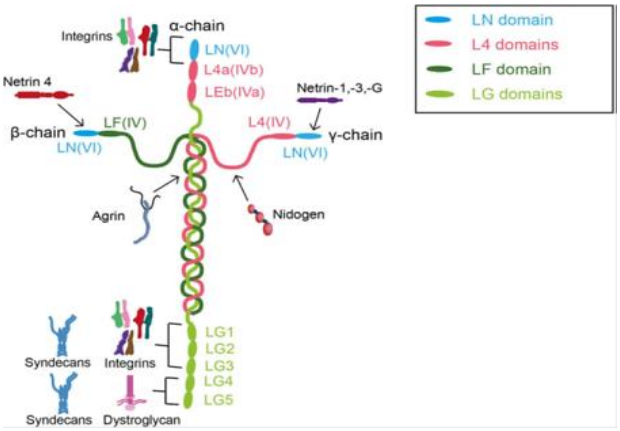

Figure (2): Association of AGRIN with other proteins.

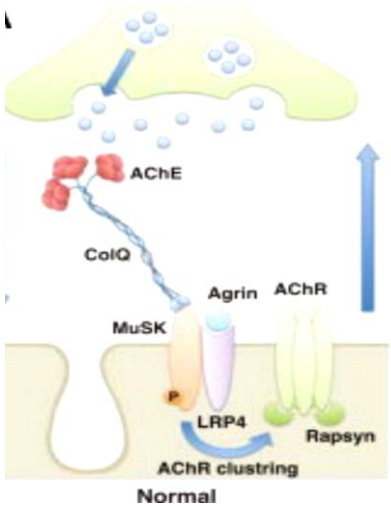

Figure (3): Mechanism of action of AGRIN.

Role of agrin in muscle loss:

As we age or in certain diseases, the integrity of the NMJ declines. This increased cleavage of agrin produces more CAF, which is released into the blood. Therefore, a higher serum CAF level indicates increased NMJ breakdown and is a strong predictor of declining muscle strength and mass so serum C-TAF level is not a marker for a single disease but rather a **biomarker of neuromuscular junction integrity and muscle metabolism.**

Its main diagnostic utilities are:

- **Diagnosing Sarcopenia:** Identifying age-related muscle loss before it becomes clinically severe.
- **Stratifying Risk:** Predicting functional decline and frailty in the elderly.
- **Monitoring Progression:** Tracking muscle wasting in chronic diseases like CKD, ALS, and critical illness.
- **Differential Diagnosis:** Helping to distinguish between different types of muscle-wasting diseases.

It is important to note that while highly promising, CAF measurement is still primarily a **research tool** and is not yet part of routine clinical diagnostics. However, it is one of the most compelling biomarkers on the horizon for managing sarcopenia and related conditions**(15).**

Role of agrin in kidney:

Agrin is found to be a major HSPG expressed in the glomerular basement membrane (GBM) of kidneys **(15).**

It is proposed that the high anionic content of HSPGs in GBM is a critical factor controlling glomerular permeability and the observed reduction of heparan sulphate levels in various renal inflammatory diseases, was associated with the increased

In this study, serum CAF is estimated in patients with CKD who are not on regular hemodialysis.

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