Possible new explanation for ALS: Researchers discover RNA-binding proteins play important role

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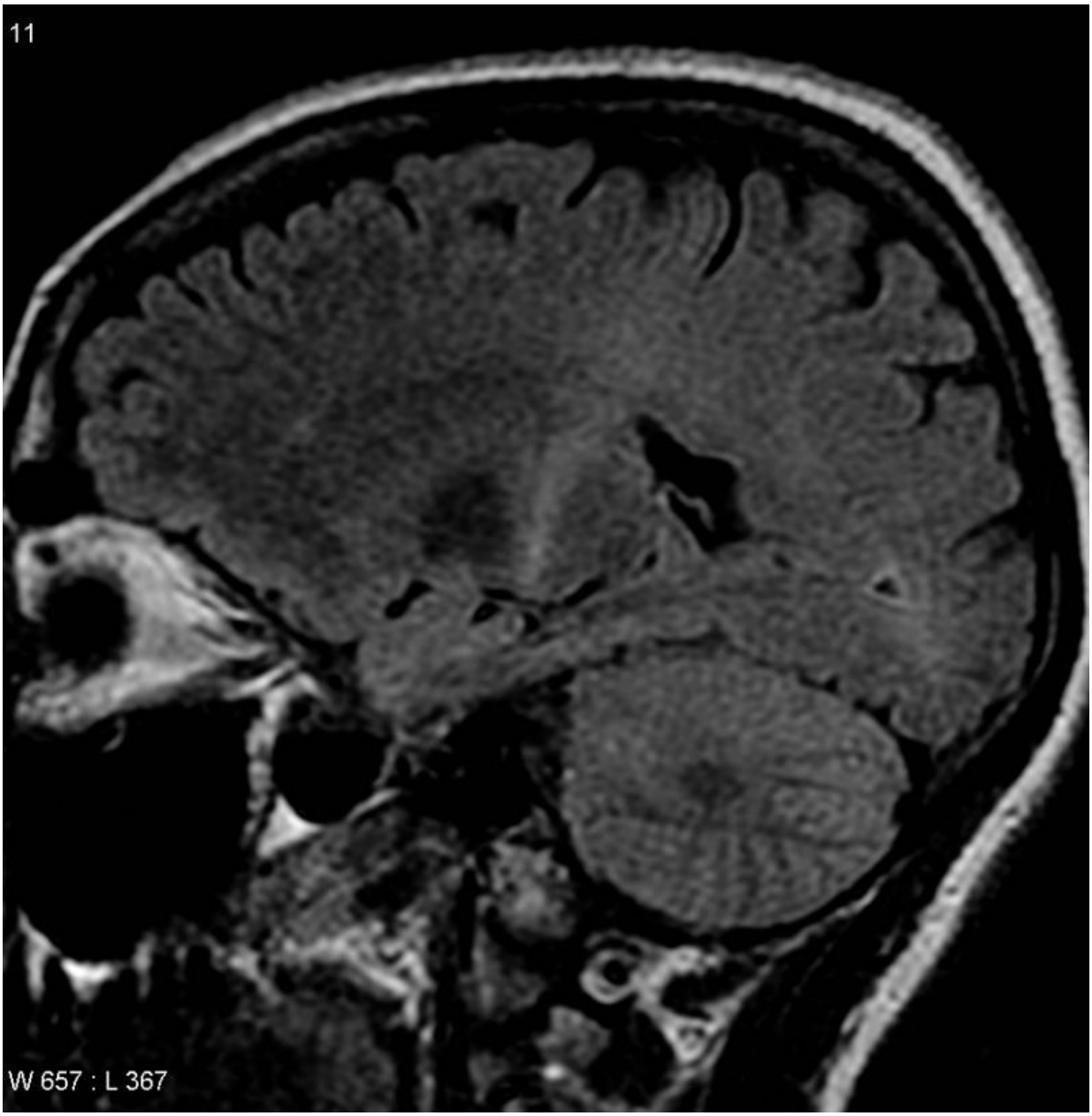

An MRI with increased signal in the posterior part of the internal capsule which can be tracked to the motor cortex consistent with the diagnosis of ALS. Credit: Frank Gaillard/Wikipedia

Scientists at the University of Cambridge have identified a new property of essential proteins which, when it malfunctions, can cause the build up,

or 'aggregation', of misshaped proteins and lead to serious diseases.

A common characteristic of neurodegenerative diseases - such as Alzheimer's, Parkinson's and Huntington's disease - is the build-up of 'misfolded' proteins, which cause irreversible damage to the brain. For example, Alzheimer's disease sees the build-up of beta-amyloid 'plaques' and tau 'tangles'.

In the case of some forms of motor neurone disease (also known as amyotrophic lateral sclerosis, or ALS) and frontotemporal dementia, it is the build up of 'assemblies' of misshapen FUS protein and several other RNA-binding proteins that is associated with disease. However, the assembly of these RNA binding proteins has several differences to conventional protein aggregates seen in Alzheimer's disease and Parkinson's disease and as a result, the significance of the build-up of these proteins and how it occurs has until now been unclear.

FUS is an RNA-binding protein, which has a number of important functions in regulating RNA transcription (the first step in DNA expression) and splicing in the nucleus of cells. FUS also has functions in the cytoplasm of cells involved in regulating the translation of RNA into proteins. There are several other similar RNA binding proteins: a common feature of all of them is that in addition to having domains to bind RNA they also have domains where the protein appears to be unfolded or unstructured.

In a study published today in the journal *Neuron*, scientists at the University of Cambridge examined FUS's physical properties to demonstrate how the protein's unfolded domain enables it to undergo reversible 'phase transitions'. In other words, it can change back and forth from a fully soluble 'monomer' form into distinct localised accumulations that resemble liquid droplets and then further condense into jelly-like structures that are known as hydrogels. During these

changes, the protein 'assemblies' capture and release RNA and other proteins. In essence this process allows cellular machinery for RNA transcription and translation to be condensed in high concentrations within restricted three-dimensional space without requiring a limiting membrane, thereby helping to easily regulate these vital cellular processes.

Using the nematode worm C. elegans as a model of ALS and frontotemporal dementia, the team was then able to also show that this process can become irreversible. Mutated FUS proteins cause the condensation process to go too far, forming thick gels that are unable to return to their soluble state. As a result, these irreversible gel-like assemblies trap other important proteins, preventing them carrying out their usual functions. One consequence is that it affects the synthesis of new proteins in nerve cell axons (the trunk of a nerve cell).

Importantly, the researchers also showed that by disrupting the formation of these irreversible assemblies (for example, by targeting with particular small molecules), it is possible to rescue the impaired motility and prolong the worm's lifespan.

Like jelly on a plate

The behaviour of FUS can be likened to that of a jelly, explains Professor Peter St George Hyslop from the Cambridge Institute for Medical Research.

When first made, jelly is runny, like a liquid. As it cools the fridge, it begins to set, initially becoming slightly thicker than water, but still runny as the gelatin molecules forms into longer, fibre-like chains known as fibrils. If you dropped a droplet of this nearly-set jelly into water, it would (at least briefly) remain distinct from the surrounding water - a 'liquid droplet' within a liquid.

As the jelly cools further in the fridge, the gelatin fibres condense more, and it eventually becomes a firmly set jelly that can be flipped out of the mould onto a plate. This set jelly is a 'hydrogel', a loose meshwork of protein (gelatin) fibrils that is dense enough to hold the water inside the spaces between its fibres. The set jelly holds the water in a constrained 3D space - and depending on the recipe, there may be some other 'cargo' suspended within the jelly, such as bits of fruit (in the case of FUS this 'cargo' might be ribosomes, other proteins, enzymes or RNA, for example).

When the jelly is stored in a cool room, the fruit is retained in the jelly. This means the fruit (or ribosomes, etc) can be moved around the house and eventually put on the dinner table (or in the case of FUS, be transported to parts of a cell with unique protein synthesis requirements).

If the jelly is re-warmed, it melts and releases its fruit, which then float off?. But if the liquid molten jelly is put back in the fridge and re-cooled, it re-makes a firm hydrogel again, and the fruit is once again trapped. In theory, this cycle of gel-melt-gel-melt can be repeated endlessly.

However, if the jelly is left out, the water will slowly evaporate, and the jelly condenses down, changing from a soft, easily-melted jelly to a thick, rubbery jelly. (In fact, jelly is often sold as a dense cube like this.) In this condensed jelly, the meshwork of protein fibrils are much closer together and it becomes increasingly difficult to get the condensed jelly to melt (you would have to pour boiling water on it to get it to melt). Because the condensed jelly is not easily meltable when it gets to this state, any cargo (fruit, ribosomes, etc.) within the jelly essentially becomes irreversibly trapped.

In the case of FUS and other RNA binding proteins, the 'healthy' proteins only very rarely spontaneously over-condense. However, disease-

causing mutations make these proteins much more prone to spontaneously condense down into thick fibrous gels, trapping their cargo (in this case the ribosomes, etc), which then become unavailable for use.

So essentially, this new research shows that the ability of some proteins to self-assemble into liquid droplets and (slightly more viscous) jellies/hydrogel is a useful property that allows cells to transiently concentrate cellular machinery into a constrained 3D space in order to perform key tasks, and then disassemble and disperse the machinery when not needed. It is probably faster and less energy-costly than doing the same thing inside intracellular membrane-bound vesicles - but that same property can go too far, leading to disease.

Professor St George Hyslop says: "We've shown that a particular group of proteins can regulate vital cellular processes by their distinct ability to transition between different states. But this essential property also makes them vulnerable to forming more fixed structures if mutated, disrupting their normal function and causing disease.

"The same principles are likely to be at play in other more common forms of these diseases due to mutation in other related binding proteins. Understanding what is in these assemblies should provide further targets for disease treatments.

"Our approach shows the importance of considering the mechanisms of diseases as not just biological, but also physical processes. By bringing together people from the biological and physical sciences, we've been able to better understand how misshapen proteins build up and cause disease."

More information: Murakami, T et al. ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into

irreversible hydrogels impairs RNP granule function. *Neuron*; 29 Oct 2015