

BDSRA research update - May 2015:

A greater understanding of how altered cell calcium handling contributes to impaired brain cell function in Batten disease.

Alexandra Grubman and Anthony White

Department of Pathology, University of Melbourne

Although the cause of Batten disease is still unknown, the different forms of the disease all share in common abnormal function of the lysosome in brain cells. The lysosome is a compartment within all cells that acts like a waste disposal depot, taking in cell 'junk', degrading it, sorting it, sending the good stuff to be recycled and putting the bad stuff in barrels (vesicles) where it can be expelled from the cell and body or kept out of harms way. However, in Batten disease the lysosome is often overwhelmed and large amounts of material accumulate, most likely with toxic effects on the brain cell, leading to its death. Another cell process closely related to lysosome function is known as autophagy. This is when the cell engulfs material inside the cell to be degraded, and when these garbage collection units are formed, they are called autophagosomes. This process is closely linked to the lysosome and they work together to clear unwanted material in the cell. This is especially important in neurons, which are cells that live for a long time and through normal processes can accumulate a lot of unwanted bits and pieces like fats, proteins and sugars. The process of autophagy is much like a garbage truck collecting waste to take it to the waste depot. In Batten disease, this process seems to be overactive but faulty and now researchers in the US (and Australia) are starting to gain important insights into why.

In a recent scientific paper published in *Journal of Biological Chemistry* by Chandrachud and colleagues in the laboratory of **Susan Cotman** (Harvard Medical School), it was shown that cells from CLN3 form of Batten disease have impaired handling of the important element, calcium (Ca^{2+}). We are all familiar with the essential role calcium has in keeping our bones healthy, but calcium has many other very important functions. In brain cells, calcium is shuttled carefully around the cell by calcium pumps (called calcium ATPases), where the element controls the action of many processes including autophagy and lysosomal function, the very processes that are compromised in Batten disease. Lysosomes need to be acidic to effectively break down proteins, whereas in CLN3 Batten disease, researchers have observed a loss of lysosomal acidity. Loss of normal CLN3 protein function also results in fewer and smaller lysosomes in neurons, which may further overwhelm the degradation machinery of the cells. A loss of lysosome number and function may in turn over-activate autophagy most likely in an attempt to deal with the accumulating garbage. This recent research indicates that lysosomal changes and autophagy seem to be closely tied to loss of normal handling of calcium in the cell.

As the waste disposal machinery is defective in CLN3 Batten disease cells, the authors were interested in drugs that would affect this process. Initially the authors screened a library of known biologically active compounds in neural stem cells from CLN3 mutant mice that were engineered to have fluorescent autophagy components. They found that of over 300 compounds tested, almost half of the compounds that affected autophagy specifically in CLN3 mutant cells, were drugs that control calcium shuttling. They then verified (using neural stem cells from both CLN3 mutant mice and CLN3 Batten disease patients) that a drug (thapsigargin) that controls calcium ATPase (important calcium pump) impaired autophagy

much more strongly in CLN3 brain cells than in normally functioning cells. This means that brain cells that lack normal CLN3 protein can't regulate autophagy properly, and this is related to abnormal calcium movement around the cell. The consequence is obvious. If you can't remove the garbage properly, it will soon pile up and cause all sorts of problems. But the authors then went further to try and understand this process in more detail. When the authors bound all the cell's freely available calcium, the effects of thapsigargin were reduced, showing that autophagy was specifically controlled by calcium in CLN3 cells. The authors then investigated how calcium is shuttled within CLN3 mutant cells. They found that CLN3 mutant cells can't maintain stable calcium concentrations, and often saw damaging spikes in cellular calcium concentrations. The CLN3 mutant cells also stored calcium in the wrong compartments: for example they stored too much calcium in the lysosomes at the expense of other calcium-requiring sites such as the cell's energy production factories (mitochondria).

Recent research from **University of Melbourne** published in the journal *Chemical Science* suggests that incorrect calcium storage is present in other genetic forms of Batten disease. These studies using imaging techniques at the Australian Synchrotron show changes to the location and cellular shuttling of both zinc and calcium stores within brain cells from mice with naturally occurring CLN6 Batten disease. Zinc has long been known to be an important metal for brain function, as among its many functions, it controls transmission of signals between neurons that allow us to think and move. The research group in Melbourne, led by **Assoc. Prof. Anthony White** and **Dr Alexandra Grubman**, have also found changes to calcium and zinc concentrations in the brains and eyes of mice and the brains of sheep that have naturally occurring CLN6 Batten disease. Importantly, a zinc-complex, which delivered zinc to the cells was able to restore the relationship between subcellular zinc and calcium stores to that in healthy control cells. Zinc delivery also reduced the number of calcium aggregates in CLN6 mutant cells. The drug is currently being tested in young CLN6 mutant mice to see if it can also stabilise the impaired calcium and zinc stores in the eyes and improve vision in the mice.

The research from the US and Australian teams is continuing to advance our understanding of the relationship between elements such as calcium and zinc and the breakdown in cellular processes that lead to neuronal degeneration in Batten disease. It is hoped that this will allow us to find new ways to target therapeutics to treat the disease.

References:

Chandrachud U, Walker MW, Simas AM, Heetveld S, Petcherski A, Klein M, Oh H, Wolf P, Zhao WN, Norton S, Haggarty SJ, Lloyd-Evans E, Cotman SL. Unbiased Cell-Based Screening in a Neuronal Cell Model of Batten Disease Highlights an Interaction Between Ca²⁺ Homeostasis, Autophagy, and CLN3 Function. *Journal of Biological Chemistry* 2015 Apr 15. pii: jbc.M114.621706.

Grubman A, James SA, James J, Duncan C, Volitakis I, Hickey JL, Crouch PJ, Donnelly PS, Kanninen KM, Liddell JR, Cotman SL, de Jonge, White AR. X-ray fluorescence imaging reveals subcellular biometal disturbances in a childhood neurodegenerative disorder. *Chemical Science* 2014 Jun;5(6):2503-2516.