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Notes from the Or.S.A Angelman Syndrome Conference 2013

By Katie Cunnea

On 11th October 2013 I attended the Italian AS international conference (Or.S.A) in Rome. I have written some notes from most of the scientific talks. This is certainly not an exhaustive description of everything that was said. There are also other scientists working on AS who didn't attend the conference, but I learnt a lot, and I have done my best to explain this as simply as possible. Over the next year I hope to gradually gather more and more information from these and other scientists, so that we can all stay up to date with what is happening in terms of scientific research into Angelman Syndrome.

Katie Cunnea (ASSERT Science & Research Trustee)

UBE3A/E6AP: Not Just an ubiquitin-protein ligase?



Martin Scheffner

<http://cms.uni-konstanz.de/scheffner/lab-members/scheffner-lab/prof-dr-martin-scheffner/>

About Martin

Martin is a biochemist based at Universitat Konstanz in Germany. His work focuses on proteins involved in a process called the ubiquitin-conjugation system. The gene that people with Angelman Syndrome are missing normally enables a protein called Ube3A to be made, which we now know is involved in the ubiquitin-conjugation system. This is why Martin's laboratory is interested in Angelman Syndrome.

Ubiquitin is an incredibly important protein and plays a very important role in the body. Most proteins in our body are modified by ubiquitin. A major job of ubiquitin is to signal which proteins should be broken down and removed from the body. It does this by binding (sticking) to them. However, it can have other roles, including changing the activity of the protein, or affecting the protein's ability to interact with other molecules.

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There are many proteins involved in the ubiquitin system. In fact there are about 1000 genes involved (4-5%) of human genome. So deregulation of this system results in many different diseases, including some neurological disorders like Angelman Syndrome.

Ube3A

Scientists do not actually know very much about the Ube3A protein. We do know that it is an ubiquitin ligase. This means that it is a protein that recognises other proteins and earmarks these for removal from the body by attaching ubiquitin.

Ube3A is just one ubiquitin ligase, there are many others too. They will all recognise different proteins for alteration/removal from the body. One of the main things we don't know is which proteins Ube3A specifically recognises.

Martin's research group is interested in finding which proteins Ube3A works on.

Interestingly Ube3A was originally discovered as a protein that is recognised by a Human Papilloma Virus protein, E6. Certain types of human papilloma viruses cause cervical cancer, and it is these viruses that we vaccinate teenage girls against. While this doesn't directly relate to Angelman Syndrome it does mean that in recent years there are more scientists interested in finding out how Ube3A works. So indirectly this could benefit Angelman Syndrome because more scientists have heard of Ube3A and are looking at how it works.

Ube3A and AS

Scientists know that loss of Ube3A results in Angelman syndrome. They also assume that too much Ube3A results in some autism spectrum disorders. So it is very important that the amount of Ube3A in the body is very carefully controlled. How the body controls the amount of Ube3A, is one thing that Martin's group is interested in discovering. Alongside this they need to work out the best ways to identify which cells Ube3A is working in.

He is also trying to identify which molecules Ube3A recognises and targets for modification.

Another area of interest is that Ube3A might not be active on its own. It might be inhibited from working or activated to work, by other molecules. A protein called HERC2 is one such molecule that has been identified.

The gene for HERC2 is found on the same chromosome, close to the gene for Ube3A. HERC2 is like Ube3A because it is an ubiquitin ligase. HERC2 is deleted in 70% of patients with AS. It is not known

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whether this additional deletion of HERC2 has an additional clinical effect on an individual with Angelman Syndrome.

Martin's group looked at whether HERC2 destroys Ube3A or the other way around. They found that neither molecule destroys the other. Instead they believe that when HERC2 interacts with Ube3A it activates Ube3A (Kühnle et al. J Biol Chem. 2011 Jun 3;286(22):19410-6. doi: 10.1074/jbc.M110.205211).

Evidence for this theory has been provided in a collaborative effort with the group of Andrew Crosby (a human geneticist), London, and published by Harlalka et al, 2013. <http://jmg.bmj.com/content/50/2/65.abstract>

This scientific paper describes the clinical features of people who lack HERC2 but still have Ube3A. They found that patients have similar characteristics to people with Angelman Syndrome, but in a much milder form. These observations make sense if HERC2 is an activator of Ube3A, because Ube3A is still present in the body, but it is not as active.

Martin stressed that it is important to realise that Ube3A interacts with other proteins. There is likely to be a complex chain of events that result in the different clinical features of AS. If we can understand these processes and the molecules that are involved it will give us a better insight into how we might correct processes that do not happen properly in people with AS. We might find that it is difficult to replace Ube3A, but easier to correct processes that occur in a later chain of events.

Some molecules that have been proposed to interact with Ube3A include Ring1b and ARC. It is unclear what the role of Ring1b in AS is. ARC is an important molecule for synaptic plasticity. So this could cause problems in synaptic transmission.

Martin has tried to create a set of rules to define how we identify which molecules Ube3A targets for removal from the body's cells. Martin is not sure that Ube3A interacts directly with ARC and that Ube3A removes ARC by attaching ubiquitin to it. Instead he thinks that Ube3A affects nuclear hormone receptors, and these in turn affect ARC levels. So Ube3A indirectly regulates ARC by regulating the ARC gene.

Summary:

- Ube3A does more than target proteins for degradation.
- There are other factors involved in Ube3A's regulation.

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Investigating the impact of rare CNVs on the clinical heterogeneity of Angelman patients carrying deletion. Silvia Russo

Silvia Russo is a geneticist who works with patients. She is based in the Molecular geneticslaboratori, in the Istituto Auzologico Italiano, Milano Italy.

Silvia has studied whether larger deletions in the region of the chromosome that causes Angelman Syndrome, result in patients with more severe disabilities.

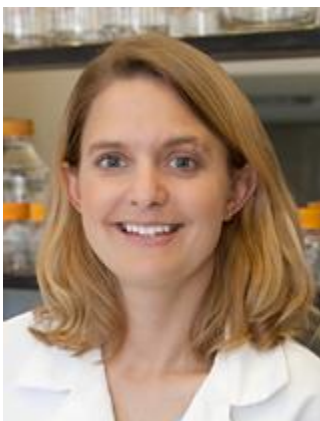
She identified two places on DNA where a deletion might begin and called these class I and class II. She then looked at the patients clinical features compared to the amount of DNA deleted.

Silvia noted that a similar thing was described in a paper, Valente 2013. However, she felt that this study was done on too few patients, and so could potentially be misleading.

She looked at 47 patients who were deletion positive. She did not find any significant difference between patients with the larger deletion verses the smaller deletion. However, she did note that patients with a larger deletion did appear to have worse epilepsy, were slower to learn to walk, and more likely to be wheelchair bound.

Silvia also tried to identify any additional genes in the regions that are deleted. One gene that she felt was important was *CHRNA7*. This is a candidate for causing severe epilepsy in AS (according to their study).

Lessons learned from induced pluripotent stem cell models of Angelman syndrome



Stormy J. Chamberlain

<http://facultydirectory.uchc.edu/profile?profileId=Chamberlain-Stormy>

Stormy is in charge of a laboratory at the University of Connecticut.

Her specialty is in the creation of human stem cells. Stem cells are the cells that are first created when a sperm fertilizes and egg. These stem cells then go on to become all sorts of different types of cells. There are a couple of ways you can make human stem cells artificially in the laboratory. One is to take discarded human embryos (from IVF

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treatment). Stormy does not feel this is something she would want to do from an ethical point of view, and so uses an alternative method.

Instead she takes skin or blood samples from patients and reprograms them to become stem cells. She then uses another technique to turn these stem cells into neurons (this takes about 10 weeks). She thinks the resulting neurons are the equivalent of neurons in a foetus of about 14 weeks. These cells are not designed to be put into humans. They are created so that we can model what happens in human brain cells. This means we have a way of testing chemicals, including potential drugs, on human brain cells.

Angelman Syndrome

Stormy described how the chromosome we inherit from our father is switched off in Angelman Syndrome compared to the one we inherit from our mother.

She has found that the Ube3a antisense RNA associated with AS in humans is a bit different to the equivalent RNA in mice. In humans there is a much longer stretch of Ube3a antisense RNA that stops in a different place than the same RNA in mouse. This stretch of RNA becomes even longer in neurons, and prevents the Ube3A gene from being switched on from the father's chromosome.

She has grown cells that only contain chromosome 15 from a father, and cells that only contain chromosome 15 from a mother.

Stormy has taken cells from individuals with Angelman Syndrome and using the technique described above, made and grown brain cells in a plastic dish in the laboratory.

The next step is to check that these neurons (brain cells) are working and that they have Angelman Syndrome. This is obviously important to check so that she knows her results are valid.

When she checked the activity of the neurons to make sure they are functional, she found it takes 10 weeks to grow functional brain cells.

Stormy then took the neurons (brain cells) with Angelman Syndrome and tried to work out how the gene from our fathers is switched off.

Comparing DNA from patients with Prada Willi Syndrome and Angelman Syndrome provided some clues.

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Her results indicated that the chromosome from our fathers is folded up into a shape that acts as a physical barrier to stop the Ube3a antisense RNA in non-neurons. This Ube3a antisense RNA prevents Ube3a from being produced from the fathers' chromosome in neurons.

Stormy also looked at whether non-brain cells express Ube3A in the same way as brain cells. She found that normally they don't, but if you remove some elements that regulate the gene, you can make non-brain cells silence the father's copy of Ube3A.

Mouse and humans regulate Ube3a slightly differently, so it is important to understand how humans do it so we can understand how well the mouse models Angelman syndrome.

Preventing and reversing synaptic and memory deficits in Angelman syndrome

Eric Klann

<http://www.cns.nyu.edu/corefaculty/Klann.php>

Eric Klann leads a research group at New York University. He is a molecular neuro-scientist, interested in brain disorders.

He recently returned from a year abroad where he was working in association with a pharmaceutical company with an interest in AS.



To look at the brain and how it is affected by AS, their group worked out how to breed mice with AS.

They then wanted to identify molecules that caused the problems in the brain of mice with AS.

They looked at multiple molecules and their potential role in AS. This is quite complex but to summarise some findings:

- They found a drug called PD158780 that can reverse the memory deficits in AS mice. He tried to work out the way that this worked.
- They found some structural differences in the brain cells of mice with AS compared to those that did not have AS.
- They found that the amounts of certain molecules were altered between the two brains.

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- They tried to breed problems out of the AS mouse to prove their hypothesis.
- They think that the synapses are there but not working properly ... so if we can find factors that improve the way synapses work we might be able to help them to work better.

Understanding the molecular mechanisms underlying the neurological deficits of AS



Ype Elgersma

<http://beta.neuro.nl/people/y.elgersma.html>

Neuroscience Institute, Erasmus University, Rotterdam, The Netherlands.

Ype is interested in looking at how we might switch on the Ube3A gene that we inherit from our fathers to correct symptoms of AS.

He also wants to understand the role of Ube3A in the synapse and identify targets for developing drugs.

He is looking at indirect therapeutics. So it might not be easy to replace Ube3A, but this molecule is likely to trigger a chain of events, and we might find drugs that act on molecules later in this chain.

They have discovered some novel proteins that interact with Ube3A. They then looked at whether they were targets for Ube3A? A substrate for ubiquitination?

They did some molecular modelling and saw similar amino acids in two molecules that interact with Ube3A.

Summary

1. His group wants to know if AS is reversible in adults. Is there a critical age, above which we can no longer correct AS?
2. His results so far have not found anything positive so far, however they are not conclusive. Much more work needs to be done.
3. Activating the Ube3A gene in adult mice does not rescue the selected behavioural deficits that they looked at in mice.

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4. Activation of the Ube3A gene at 3 weeks (but this could be equivalent to an older child/teenager in humans) rescues the rotarod deficits. More research is needed to see which behaviours can be rescued at which age.

Pharmacogenetic insights into AS

Ben Philpot

<https://www.med.unc.edu/physiolo/faculty/philpot>

Ben is a professor at University of North Carolina School of Medicine. He leads a research group which is interested in Angelman Syndrome; Autism Spectrum Disorders; Neurodevelopmental Disorders; Experience-Dependent Synaptic Plasticity; Learning and Memory; and Restoration of Plasticity in Neurological Disorders.



A new angle on AS therapeutics

Deletions or mutations of the maternal *UBE3A* allele are the main cause of Angelman Syndrome. It is also known that increased genes dosage of *UBE3A* can be found in common forms of autism. It is therefore clear that it is very important to accurately control UBE3A protein levels. This is obviously important for scientists to consider if they are trying to work out how we might go about replacing missing UBE3A protein in people with AS.

Ben has looked at how the paternal *Ube3A* allele is switched off, and how this switch can be turned back on. He works with mice that lack maternal Ube3a, and thus model AS, to carry out experiments.

We know that UBE3A protein is expressed throughout the brain and that a deletion on the chromosome we inherit from our mothers stops this happening.

Ben is trying to discover therapeutics for AS. To help achieve this he has formed collaboration with Mark Zylka and Bryan Roth, both at the University of North Carolina. Mark Zylka is renowned for neurogenetic and molecular biology research, while Bryan Roth is world renowned in the field of drug discovery, as he leads the NIMH Psychoactive Drug Screening Program. This is a very exciting collaboration for both Ben's team and the AS community.

Ben's research team is studying how the paternal Ube3a allele can be switched on.

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To test whether the gene is on or off he has taken advantage of a fluorescent reporter mice developed by Scott Dindot while he was in Art Beaudet's lab. This mouse has a fluorescent tag to see if the *Ube3A* gene is active. He took neurons that have the fluorescent reporter on the paternal *Ube3a* allele, and then added drugs to see if they could switch on the gene as indicated by an increase in fluorescence.

His research found one compound that increased Ube3A levels in cells, called irinotecan. This is a drug called a topoisomerase inhibitor, currently used to treat cancer.

So they looked at other similar drugs to see if they could find a drug that would increase levels of protein made from the paternal copy of *Ube3A* gene. They found that a drug called Topotecan did.

So next they needed to see if it would work in a living animal – e.g. a mouse. So first they injected the drug into the spinal column of mice. They used this method of getting the drug into the body because this is the way that the drug is already administered to children to treat leukaemia. This has been done for many months with few side effects.

He needed to see if Ube3A is produced in the same way in the spinal cord, as it is in the brain.

Then they injected mice with topotecan. When they did this they saw an increase in Ube3A. However, Ben stressed that there are limitations to this research. For example they found smaller amounts of Ube3A than you would expect in a normal cell.

They wanted to find out if the increase in Ube3A was long lasting, or whether you would have to keep giving injections of the drug all the time to keep producing Ube3A? So they injected a group of mice for two weeks, and then compared the levels of Ube3A, immediately, after 4, 12 etc weeks upto 1 year, and compared the mice. They found that even a year after treatment Ube3A was still being produced.

However, they then did the same experiment in cultured cortical neurons and found the production of Ube3A could be initiated but it was lost within about 48 hours. So very different to spinal cord in vivo. The differences in these two findings could be because of the type of cell, but it could also be because one study looked at what was happening inside mice, while the other was on cells grown in a dish outside the body. There are other possible reasons for these differences, but it is possible it is simply different in the spinal column versus brain cells.

They have asked the question...Will topoisomerase inhibitors switch on Ube3A on in human neurons with AS?

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So they are now working on patient derived cell lines from Stormy Chamberlain and they have found that you can, indeed, turn on the paternal copy in human genes.

What is the mechanism of turning on the Ube3A gene we inherited from our fathers?

One thing they have realised is that the DNA strand needed to make the Ube3A-antisense, which silences the paternal Ube3a allele, is very long.

Topotecan inhibits expression of long genes, and interestingly many long genes are associated with Autism related genes.

It is a concern for the group, that if they tried to treat AS very early, would they accidentally induce autism? So they feel it will be very important to determine when the critical period for treating someone is?

They found that the production of Ube3A induced by Topotecan doesn't happen throughout the brain. So the group is now trying to identify additional topoisomerase inhibitor like compounds which can penetrate into the brain better.

They are also aware they need to

- Determine critical periods for treatment of AS.
- Identify the best way to deliver the drugs into the body to increase therapeutic benefits while minimizing potential adverse consequences.
- Determine the mechanism of action of these drugs to guide other attempts to turn the cell on to produce Ube3A.
- Carry out additional unbiased drug screens.
- Work out the right amount of topotecan needed for it to function in an optimal way.

Mark Cushman and Yves Pommier are creating topoisomerases as drugs for treating cancer. So they are looking at topoisomerases that cross the blood-brain barrier better, which of course could be good news for AS too.

Ben told us that he has just signed a contract with a pharmaceutical company to test a new drug. He will be looking at its ability to treat AS, to see if it works better than the topoisomerases he is currently investigating. He is unable to give any specific details because he is bound by a confidentiality agreement. But said he is very excited by the collaboration. Ben finished by saying that, increased basic science discoveries, increased Ube3A research, a number of therapeutics in the pipeline and engagement of Pharmaceutical companies hold promise for future treatments.

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