A recent study has discovered a strong association between increased methylation of the CpG islands, which regulate the expression of oxytocin receptor gene and autism.

Possible epigenetic cause for oxytocin insensitivity and autism

Headed by Prof. Jessica Connelly (University of Virginia, VA, USA) and Prof. Simon Gregory (Duke University, NC, USA), a recent study into the causes of autism has broken new ground by demonstrating an epigenetic influence on the development of this disorder. From their published paper, "for the first time, [these data] implicate the epigenetic regulation of the oxytocin receptor in the development of [autism]".

Cases of autism, a neural developmental disorder, have been reported as being on the rise for many years, and although this increase in prevalence is thought to be owing to improvements in monitoring and diagnosis, environmental factors cannot yet be ruled out. Regardless of whether any such factors are to be discovered, it is clear that there is a strong genetic element to the disorder, and it is highly heritable. At present, the exact mechanism of heritability is unclear; many different genes and interactions have been examined for their role in autism, and while minor effects have been observed, no conclusive explanation has yet been put forth.

These previous attempts to seek a genetic basis for autism may have been unsuccessful owing to a failure to consider the additional epigenetic dimension recently uncovered. As Prof. Connelly commented "the epigenetic link to autism … argues [for] the importance of exploring epigenetic markers in complex disease".

The hormone oxytocin is thought to be involved in a variety of sexual and social behaviors. It has previously been investigated in relation to autism, its connection with trust and pair-bonding established in several different studies, making it a prime candidate for research into social disorders. While studies have suggested that administering oxytocin can partially mitigate some symptoms of autism, trials are still ongoing in this area.

> *"the epigenetic link to autism … argues [for] the importance of exploring epigenetic markers in complex disease."*

Rather than examining the hormone itself, this latest study focused mainly on oxytocin receptor expression in those diagnosed with autism.

While genomic deletion was also examined, the most notable observation was that the methylation of certain nucleotides in a CpG island believed to regulate the expression of the oxytocin receptor gene was significantly different between individuals with autism and those without. Autistic individuals were more likely to show methylation at those points, leading to diminished expression of the oxytocin receptor gene. "In both blood samples and brain tissue, the methylation status of specific nucleotides in the oxytocin receptor gene is significantly higher in someone with autism", announced Gregory.

This discovery demonstrates that methylation testing may be a useful aid in the complicated process of diagnosing autism in the short term, and may point to a potential therapy in the long term, in the form of drugs to specifically modify methylation status.

Source: Gregory SG, Connelly JJ, Towers AJ et al.*: Genomic and epigenetic evidence for oxytocin receptor deficiency in autism.* BMC Med. *7, 62 (2009).*

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First complete maps of the human methylome published

The first genome-wide, single-base-resolution maps of methylated cytosines in a mammalian genome has recently been published in a paper by *Nature*. These maps were generated from both human embryonic stem cells and fetal fibroblasts. They also include the comparative analysis of mRNA and small interfering RNA components of the transcriptome, several histone modifications, as well as sites of DNA–protein interaction for several key regulatory factors. A group of investigators from the Genomic Analysis Laboratory at the Salk Institute at La Jolla (CA, USA) were responsible for carrying out the important research.

"Being able to study the epigenome in its entirety will lead to a better understanding of how genome function is regulated in health and disease but also how gene expression is influenced by diet and the environment", commented Dr Joseph Ecker, professor and director of the Genomic Analysis Laboratory at the Salk Institute and a member of the San Diego Epigenome Center (CA, USA).

In their study, Ecker and colleagues compared the epigenomes of human embryonic stem cells and differentiated connective lung cells or fibroblasts. They discovered from their analysis that there is a tightly controlled but dynamic template of methyl-groups that are responsible for turning gene expression on or off. Interestingly, they also found that stem cells appeared to have a particular DNA methylation pattern, which they hypothesize may explain how stem cells are able to establish and maintain their pluripotent state.

"We believe this knowledge will be extremely valuable for understanding diseases such as cancer and possibly even mental disorders. Right now we just don't know how the epigenome changes during the aging process or how the epigenome is impacted by our environment or diet", concluded Ecker.

The team's next project is to look at how the human epigenome changes during normal development and various disease states.

This study is part of the 5-year Roadmap Epigenomics Program funded by the NIH.

Source: Lister R, Pelizzola M, Dowen RH et al.*: Human DNA methylomes at base resolution show widespread epigenomic differences.* Nature *(2009) (Epub ahead of print).*

Epigenetic variation in humans shown to be influenced by aging and the environment

Research investigating the causes and extent of tissue-specific interindividual variation found in human epigenomes is scarce and thus this variation among humans is poorly characterized. Therefore, a team of researchers from Brown University (RI, USA) and their collaborators have recently led an effort to map the variations found in the epigenomic structure of more than 200 human tissue samples.

Their research, which was recently published by *PLos Genetics*, surveyed over 200 carefully annotated human tissue samples for methylation alterations that may be related to clinically important epigenomic alterations. The researchers found that aging and environmental exposures such as tobacco smoking was significantly related to variation in methylation and contributed to an individual's increased susceptibility to several diseases.

"Scientists have already found out that it is critical to look at genetic variation to diagnose disease", explained Brock Christensen, a postdoctoral research associate at Brown University's Department of Pathology and Laboratory Medicine. "What we are trying to do is complement that by looking at what is normal and how much variation in epigenetics exists".

In their paper, the researchers advocate that additional research is necessary to define the mechanisms controlling epigenomic variation. Christensen explained that "more tissue samples and data are needed to allow for a thorough mapping of epigenetic variability in cells".

They concluded: "We have begun to lay the foundations for essential normal tissue controls for comparison to diseased tissue, which will allow the identification of the most crucial disease-related alterations and provide more robust targets for novel treatments".

Source: Christensen BC, Houseman EA, Marsit CJ et al.*: Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context.* PLoS Genet. *5(8), E1000602 (2009).*

About the News and Views

The News and Views highlights some of the most important events and research in epigenomics. If you have newsworthy information, please contact: Tarryn Greenberg, Launch Editor, *Epigenomics*, Future Medicine Ltd, Unitec House, 2 Albert Place, London, N3 1QB, UK; Tel.: +44 (0)20 8371 6090; Fax: +44 (0)20 8343 2313; t.greenberg@futuremedicine.com

An application for unmethylated CpG DNA in Alzheimer's disease?

Japanese researchers have discovered that the activation of CNS immune cells with unmethylated CpG DNA can promote the clearance of oligomeric amyloid-β (oAβ), the protein thought to cause Alzheimer's disease.

Alzheimer's disease is a terminal, degenerative disease of the brain, estimated to affect more than 35 million people worldwide according to the organization, Alzheimer's Disease International. At present there is no cure, which makes the recent research by Prof. Suzumura and his group (Nagoya University, Nagoya, Japan) potentially very important.

> *"CpG may be an effective therapeutic strategy for limiting oA1-42 neurotoxicity in [Alzheimer's disease]."*

Unmethylated CpG oligodeoxynucleotides are small, single-stranded DNA molecules with the 'CG' motif. They are considered to be pathogen-associated molecular patterns, relatively abundant in the genomes of microbes compared with those of vertebrates, and are immunostimulatory via Toll-like receptor 9 (TLR9). Different classes of CpG DNA exist, distinguished by their effects on immune response, which are in turn thought to be determined by differing structural characteristics, such as their length, arrangement of motifs and backbone modifications. For the purposes of this study, CpG classes B and C were proved to be most useful.

Long considered to be a completely immunologically privileged site, the brain was subsequently discovered to have its own forms of immune response under certain conditions, and hence its own autoimmune problems. Glial and microglial cells are the immune cells for the CNS, and it was while investigating the coordination of their activity that Suzumara's group made this latest discovery.

Although they have been observed to gather at the sites of the amyloid-β plaques, which is a distinctive sign of Alzheimer's disease pathology, it is uncertain exactly what contribution microglia make towards oAβ toxicity. While this remains unclear, progress has been made in identifying the causative agent for neurotoxicity – oligomeric rather than fibrillar Aβ – and the means by which this toxicity can be reduced. The introduction of unmethylated type B and C CpG DNA was shown to reduce oAβ-related neurotoxicity *in vitro* and to increase the clearance of the oAβ protein by microglia, presumably by binding to TLR9. When exposed to CpG DNA, the microglia were found to increase their production of heme oxygenase-1, an antioxidant enzyme, without the additional production of the molecules nitric oxide and glutamate associated with activated microglial cells and neurotoxicity. As the research group put it, "among subclasses of CpGs, class B and class C activated microglia to promote neuroprotection".

Moving beyond *in vitro* testing, the application of CpG DNA directly to the brain of a mouse model via intracerebroventricular injection produced encouraging results, reducing the negative effects of Alzheimer's disease on learning, memory and cognition. This more practical demonstration of the theoretical association indicated by the *in vitro* neuron–microglial co-culture lends strong support to a conclusion from the paper: "CpG may be an effective therapeutic strategy for limiting oA1-42 neurotoxicity in [Alzheimer's disease]."

Source: Doi Y, Mizuno T, Maki Y et al.*: Microglia activated with the Toll-like receptor 9 ligand CpG attenuate oligomeric amyloid-β neurotoxicity in* in vitro *and* in vivo *models of Alzheimer's Disease.* Am. J. Path. *175(5), 2121–2132 (2009).*