

How to deal with paternal mitochondria. By the Oocyte

Inherited mitochondrial disorders are passed mainly from the maternal side. Of course, this broad statement ignores the increasing awareness of the role of autosomal encoded genes and proteins in many conditions attributed to mitochondrial dysfunction that we neurologists encounter in our clinics. This aside, we know and accept that mitochondria and their DNA are almost exclusively maternal. But why and how is this so?

Until recently, maternal inheritance of mitochondria was believed to be simply a consequence of the dilution of the spermatozoan mitochondria within the larger fertilised oocyte. However, recent work, both published in the same edition of *Science*, suggest that the process of paternal exclusion is far more active. Al Rawi et al. and Sato and Sato describe their work using the nematode *Caenorhabditis elegans* to study the events surrounding oocyte fertilisation. Independently, they both show that shortly after fertilisation, both the paternal mitochondria and associated mitochondrial DNA from the sperm disappear. This process was found to be due to the activation of autophagy in the oocyte, a process by which a double-membraned structure engulfs cytoplasmic contents for transportation to the lysosome for degradation. By disrupting autophagy, paternal mitochondria and DNA persist, leading to a situation known as heteroplasmy where two different mitochondrial genomes exist. Interestingly, 95% of worms missing a key component of the autophagy machinery die before the larval stage, although it is not clear whether this is due to the persistence of paternal structures per se or a general consequence of disturbed protein clearance. While these findings are interesting with respect to worm development, importantly, Al Rawi et al. also show that autophagy appears to be activated in mouse zygotes suggesting a conserved mechanism in higher organisms, probably extending to humans.

Why does the oocyte actively discard paternal factors? One suggestion is that a basic mechanism identifying the sperm mitochondria and associated DNA as foreign intruders is activated, similarly to invading bacteria, and cleared using a conserved mechanism of degradation. However, this does not address why maternal mitochondria and DNA are preferentially inherited, sometimes with deleterious consequences when harbouring mutations leading to disease. Another possible explanation proposed is that after such a long journey to meet the oocyte, the paternal mitochondria are exhausted and susceptible to reactive oxygen species damage posing a risk to the developing organism if not cleared. Whatever the reason, identifying these mechanisms may allow specific manipulation of the underlying processes and provide possible therapeutic targets as we improve our understanding of mitochondrial diseases.

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Degradation of Paternal Mitochondria by Fertilization-Triggered Autophagy in *C. elegans* Embryos. Sato M, and Sato K. *SCIENCE* 2011;334:1144.

Postfertilization Autophagy of Sperm Organelles Prevents Paternal Mitochondrial DNA

Transmission. Al Rawi S. et al. *SCIENCE* 2011;334:1144-7.

Myasthenia Gravis with antibodies to MuSK – potential pathogenic mechanism

Antibodies against Muscle Specific Tyrosine Kinase (MuSK) are present in 40-60% of myasthenic patients that test negative for acetylcholine receptor (AChR) antibodies. These patients often have a distinct phenotype with selective involvement of bulbar and facial muscles and typically respond well to immunosuppressive agents and plasma exchange (Guptill et al *Muscle Nerve* 2011).

It has been ten years since the first description of myasthenia caused by MuSK antibodies but the pathophysiology of MuSK MG remains poorly understood (Hoch et al 2001). MuSK is a transmembrane end plate protein. It is essential for the formation and maintenance of the neuromuscular junction (NMJ) via a complex intracellular signalling pathway involving nerve secreted agrin and other post synaptic proteins including Lrp4, Dok7 and rapsyn. A second function of MuSK is to anchor acetylcholinesterase (AChE) to the basal lamina. At the NMJ AChE is linked to a collagenic subunit-ColQ- the C terminal of which binds to MuSK (Cartuad et al *J Cell Biol* 2004).

In this current paper, Kawakami et al suggest via three sets of experiments that MuSK IgG may exert a pathological effect by disrupting the interaction between MuSK and ColQ. The investigators firstly showed that MuSK antibodies disrupt the binding of ColQ tailed AChE to the neuromuscular junction by way of an *in vitro* overlay assay on ColQ^{-/-} muscle preparations. Secondly, an *in vitro* plate binding assay demonstrated that MuSK IgG decreases the binding of ColQ tailed AChE to MuSK in a dose dependent fashion whereas binding of Lrp4 to MuSK was unaffected. Finally, passive transfer experiments were performed to show that MuSK IgG significantly reduced the expression of ColQ and AChE at the neuromuscular junction whereas AChR and MuSK expression were only moderately affected.

This study is significant as it compellingly suggests a novel mechanism of action of MuSK IgG. However, there are discrepancies between human and animal data that leave important questions unanswered. The findings of this study suggest that patients with MuSK MG should have end plate AChE deficiency. However, there has been no evidence of this from patients' intercostal muscle biopsies.

Moreover, there are discrepancies between this study and previously described passive transfer experiments. Kawakami et al showed that AChR expression was only moderately reduced by MuSK IgG whereas previous investigators have shown evidence of significant AChR loss in passive transfer models (Cole et al *J Physiol* 2010, Punga et al *Exp Neurol* 2011).

One possible explanation for these disparities is that individual muscles have different levels of expression of MuSK and ColQ tailed AChE as well as different twitch properties. These differences seem to render some muscles more vulnerable than others to the effects of MuSK IgG. In light of the recent finding that ColQ participates in controlling AChR clustering via its interaction with MuSK, the authors also suggest that even a partial disruption of the ColQ/MuSK interaction may be sufficient to cause post synaptic destabilisation (Sigoillot et al *J Neurosci* 2010). Further research is required to fully outline the reasons for these discordant results. Nevertheless, the current study is an exciting development in that it sheds new light on the pathogenesis of this complex autoimmune disease.

– Jennifer Spillane, Clinical Research Associate, Institute of Neurology, Queen Square.

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Immunogenic VGKC-complex antibodies: in mice and men

A recent fascinating paper has elegantly correlated a set of human clinical and serological observations with an experimental animal model. The clinical observation was the development of a monophasic, sensory-predominant, inflammatory polyradiculopathy in swine abattoir workers (Lachance DH, et al). Those working near to the point of the brain extraction/aerosol emission were preferentially

affected. In all patients, the authors observed a pattern of patient serum-IgG binding to mouse brain sections ('signature-IgG'). Now, they build on these clinical findings with refinements of the patient IgG-specificities, and report some remarkable clinical and serological correlations in mice exposed intranasally to liquefied brain tissue (Meeusen JW, et al.)

Seventy-nine percent of affected abattoir workers had VGKC-complex antibodies whose concentrations correlated well with the concentrations of signature-IgG. However, the frequent presence of antibodies against VGCCs and myelin basic protein (MBP), and the absence of VGKC-complex antibodies in 21%, show that polyclonal specificities were also generated by immunisation. This was also the case in mice that were intranasally challenged with brain tissue: all developed signature-IgG and specific antibodies against the VGKC-complex, VGCC and MBP. Neither patients nor mice had antibodies against the NMDA-receptor, aquaporin-4 or amphiphysin. In both the mice and patients, MRI showed swollen nerve roots and neural histology was often demyelinating. In mice, anaesthesia induction produced marked hyperactivity which is characteristic of VGKC-dysfunction. Subsequent removal of the murine intranasal antigenic stimulus, was followed by a fall in the VGKC-complex IgG levels.

The most striking aspect of this study is the generation of VGKC-complex antibodies in mice and patients who were intranasally exposed to brain antigens. While polyclonal antibody specificities were also present, this strongly suggests there is something innately immunogenic about the VGKC complex proteins – but which protein in particular? It is now established that the VGKC-complex proteins (LG11, CASPR2 and Contactin-2), not the VGKC itself, are the major targets of the patient antibodies (Irani SR, et al.). Only around 10% of the VGKC-complex antibodies in mice and abattoir workers were directed against CASPR2, and none targeted LG11. In humans, CASPR2-antibodies are often associated with neuromyotonia, but many patients with neuromyotonia have VGKC-complex antibodies without LG11, CASPR2 or Contactin-2 antibodies. Therefore, it is possible that an immunogenic component of the VGKC-complex involved in peripheral nerve function is yet to be established in mice and men.

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From alpha to omega: a paradox is unraveling in hypokalaemic periodic paralysis

Attacks of weakness in periodic paralysis occur when a proportion of muscle fibres flip to an abnormally depolarised resting potential that inactivates sodium channels and renders the cells inexcitable. Strangely, the trigger in hypokalaemic periodic paralysis (HypoPP) is a fall in external potassium. These cells appear to be making a rather basic physiological gaff; the textbook says that increasing the potassium gradient by reducing external potassium enhances potassium efflux and leads to hyperpolarisation. Curiously, paradoxical depolarisation in low external potassium occurs in normal muscle too, but only in extreme hypokalaemia (<1 mmolar external potassium). In HypoPP an abnormal inward current predisposes to depolarisation in the context of mild hypokalaemia. The origin of this current was mysterious for a long time. Francis and colleagues provide important support for a fascinating idea that has come to dominate thinking about the pathomechanism of HypoPP – the 'gating pore hypothesis'.

Nav1.4, the skeletal muscle sodium channel, is associated with a spectrum of phenotypes from pure myotonia, through paramyotonia and hyperkalaemic periodic paralysis to HypoPP. But unlike the other phenotypes, HypoPP mutations almost invariably neutralise a positive charge in one of the four voltage sensing S4 helices of the channel alpha subunit. The same is true for HypoPP mutations in Cav1.1, which has a homologous structure. This loss of positive charge creates an accessory pathway through the alpha subunit past the mutant S4 helix, completely independent of the main pore. The inward leak of cations through the accessory pathway, known as ω -current or gating pore current, could well be the critical abnormality in HypoPP.

While virtually all HypoPP is associated with neutralisation of a positive S4 arginine residue, not all such mutations cause HypoPP; one of them (R1448C in Nav1.4) leads to Paramyotonia Congenita. The gating pore hypothesis holds that the fundamental difference between HypoPP and other sodium channelopathy phenotypes is the presence of an ω -current in former and not in the latter. So the R1448C paramyotonia mutation is a fly in the hypothetical ointment. Francis and colleagues tested both R1448C and a previously untested HypoPP mutation of the sodium channel, R1132Q. Only the HypoPP mutation supported an ω -current. The ointment is clean. All 6 of the HypoPP mutations tested so far support the ω -current.

The gating pore hypothesis is the first mechanistic theory that satisfactorily links the clustering of HypoPP mutations on voltage sensing channel segments with the muscle phenotype. But it has not explained everything (yet). Several HypoPP mutations are substitutions of

an arginine to a histidine, but the accessory pathway created is predicted to be permeable only to protons – in muscle this proton leak is somehow converted into a leak of sodium and other ions. Furthermore HypoPP muscle displays a number of ion channel abnormalities that, while they may be secondary, are nevertheless potentially important for the development of the phenotype. Lastly, the origin of the extreme and dangerous hypokalaemia that frequently accompanies attacks of HypoPP remains unclear. The story is not over yet.

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Francis DG, Rybalchenko V, Struyk A, Cannon SC. Leaky sodium channels from voltage sensor mutations in periodic paralysis, but not paramyotonia. NEUROLOGY 2011;76(19):1635-41.

Another shocking revelation for the distal hereditary motor neuropathies

Distal hereditary motor neuropathies (dHMN) are rare diseases that attract attention due to the insights they provide into other motor neuron disorders such as ALS. To date, 11 causative genes have been discovered accounting for only 20% of all cases. The recent discovery by Blumen et al. that homozygous mutations in the heat shock protein (HSP), Homo-Sapiens J-domain protein 1 (HSJ1) are a cause of dHMN is therefore welcome (Blumen, et al., In press). They report a family of Moroccan Jewish ancestry with an aggressive form of autosomal recessive dHMN.

HSPs are a family of molecular chaperones that prevent protein aggregation and target misfolded proteins to the proteasome. Mutations in the small HSPs, HSPB1, HSPB8 and HSPB3 are known to cause autosomal dominant dHMN by a presumed toxic gain of function. HSJ1, on the other hand, is a member of the J domain class of HSPs and is the first to be identified as a cause of a motor neuropathy. In the paper by Blumen et al, the reported mutation affects a donor splice site leading to the translation of a truncated protein implying that loss of HSJ1 function leads to motor neuron death. This is intriguing as pharmacological up-regulation of HSPs has been shown to be an effective treatment in the SOD1 mouse model of ALS (Kieran, et al., 2004). Exactly which HSPs are responsible for this therapeutic effect is unknown. HSJ1 now appears to be a promising candidate for targeted pharmacological up-regulation in motor neuron diseases generally.

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Visualising the problem in Parkinson's Disease

Parkinson's disease patients experience an array of visual symptoms throughout the course of their disease but their neural basis and prognostic significance is not well understood. Archibald et al studied these symptoms in patients with PD (n=64) and PD dementia (n=26) using a variety of methods (questionnaires, semi-structured interview, ophthalmological assessment), comparing the findings to an elderly control group (n=32). Complex visual hallucinations (i.e. specific and well-defined images, often involving animals or people) and minor hallucinatory experiences (including illusions, feelings of presence and feelings of passage) were more common in PD patients, especially when responses were confined to the month prior to questioning. With the exception of passage hallucinations, each was even more common in patients with PD dementia. Complex visual hallucinations – present in 38% of the total PD and PD dementia cohort – were also associated with depression and impaired visual acuity, suggesting a multifactorial aetiology. Excessive daytime sleepiness and REM sleep-behaviour disorder contributed to models predictive of illusions and presence, hinting at a more brain-stem origin. Reduced visual acuity and loss of contrast sensitivity were more common in PD patients (with or without dementia) compared to controls, and disease-related risk factors included age and higher UPDRS III score rather than cognitive status. Diplopia was reported by 38% of the total PD and PD dementia cohort and logistic regression pointed towards longer PD duration, excessive daytime sleepiness, abnormal ocular alignment and hypometric saccades as causative factors. There was no difference in the frequency of floaters, simple visual hallucinations (e.g. brief flashes of light) or migrainous visual aura between PD patients and controls.

By grouping all visual hallucinations together, Gallagher et al from Queen Square also uncovered a multitude of risk factors in their PD cohort (n=94). Several of these survived multivariate analysis – presence of REM sleep-behaviour disorder, autonomic dysfunction (SCOPA autonomic scale), executive cognitive deficits (SCOPA-COG executive domain) and impaired higher visuo-perceptive function (Birmingham Object Recognition Battery). Unlike Archibald et al, they failed to find any ophthalmic factors contributing towards visual hallucinations, prompting them to suggest that it is cortical

pathology in visual pathways that drives visual hallucinations in the majority of PD patients. They supported this hypothesis by presenting neuropathological data demonstrating higher Lewy body density – particularly in temporal and frontal cortical areas – in patients with visual hallucinations on retrospective case note examination.

These two studies highlight the range and extent of visual symptoms in PD, and help us to understand the pathophysiology underpinning these symptoms.

– **David P Breen, Clinical Research Fellow in Neurology, Cambridge Centre for Brain Repair.**
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Sig-1R: haloperidol for ALS?

With the mapping of the human genome, homozygosity mapping rapidly developed as a powerful way of identifying recessive mutations. It relies on the simple fact that offspring of consanguineous parents will possess many regions of homozygosity, including at disease-causing loci. The power of this technique is clear in the recent study of a family with atypical ALS (Al-Saif et al 2011). Using the ubiquitous whole-genome SNP microarray they were able to identify a single, tiny linked locus on chromosome 9p in a Saudi family with a recessive, slowly progressive, juvenile onset, predominantly upper motor neurone disorder (depicted nicely in their figure 1). The DNA of only four affected individuals in a single generation was needed and only 9 genes were in the locus. A missense mutation was subsequently found in SIGMAR1 (encoding sigma non-opioid intracellular receptor 1, Sig-1R). Sig-1R has been shown to suppress apoptosis induced by endoplasmic reticulum stress. Al-Saif et al used NSC34 cells (a hybrid cell line produced by fusion of motor neuron enriched, embryonic mouse spinal cord cells with mouse neuroblastoma) to show that the E102Q Sig-1R mutation appeared to reduce this protective capacity. The E102Q mutation occurs in a predicted transmembrane domain and subcellular fractionation suggested that the mutation caused the protein to shift to lower density membrane fractions where it formed detergent-resistant complexes.

The normal roles of Sig-1R are not entirely clear, though it regulates K⁺ channels and is involved in Ca²⁺ signalling through IP3R. It is a receptor for a variety of ligands including steroids, psychostimulants and haloperidol. It also has chaperone activities in the ER, which further implicates the unfolded protein response in motor neurone disease. Interestingly, variants in the 3' untranslated region of SIGMAR1 were recently linked with autosomal dominant ALS-FTLD (Luty et al

2010). One variant appeared to increase Sig-1R expression, while two others decreased expression. Pathological studies demonstrated, uniquely, the presence of both TDP-43 and FUS inclusions in different cells (rather than one or the other). *In vitro*, mutant Sig-1R appeared to force TDP-43 into the cytoplasm, an effect reduced by Sig-1R ligands (Luty et al 2010).

Pathological studies of the Saudi cases would be interesting to identify inclusions, though it seems the extended survival of patients may preclude this for the time being. No animal studies of these mutations have yet been conducted, but a recent study in mice showed that the native Sig-1R protein is located exclusively in motor neurones, and that knockout causes motility problems (Mavlyutov et al 2010). Further pathological studies of these mice are needed, and it is necessary to create transgenic animals carrying the disease-linked dominant and recessive mutations to help delineate the underlying pathogenesis. Perhaps there is a loss of function with the recessive mutation and a dominant negative effect with the 3' UTR mutation? Could haloperidol be used as a treatment for ALS?!

– **Jemeen Sreedharan, King's College, London.**
Amr Al-Saif, Futwan Al-Mohanna and Saeed Bohlega. A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. ANNALS OF NEUROLOGY 2011;70(6):913-9. DOI: 10.1002/ana.22534
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