

EDITOR'S CHOICE

Neurogenetics: Making a diagnosis by whole-genome sequencing

As neurologists, at least in the UK, we tend to approach the patient with a probable inherited neurological disease in a fairly structured way. Following a careful history and examination, the search for the precise genetic abnormality begins. For certain monogenic neurological conditions, the likelihood of achieving a genetic diagnosis is favourable; for example, Huntington's disease in a patient with chorea, dementia and a relevant family history. However, we now know that many neurological conditions can present with identical phenotypes but be caused by a wide variety of underlying genetic defects. Charcot-Marie-Tooth disease (CMT), the commonest inherited neuromuscular disorder, is a familiar example. While a number of excellent clinical and neurophysiological algorithms exist to help request the appropriate gene test in CMT, logistical reasons dictate that most patients will have the commonest mutations tested first, leaving many without a precise underlying genetic diagnosis. This may change following the publication of a paper by Lupski et al. describing their approach to genetic diagnosis in a patient with demyelinating CMT, deemed to be autosomal recessive.

Instead of taking the traditional 'one gene at a time' approach, Lupski et al. decided to take advantage of the advances seen in DNA sequencing in the past 10 years and sequence the whole genome of their patient and extended family members. This included both coding and non-coding regions of DNA. Using reference genome sequences and the sequences obtained from unaffected family members, the precise genetic mutations in the known CMT-associated gene,

SH3TC2, were identified. While one could argue that the clinical details, family history and neurophysiological data available would have pointed towards the *SH3TC2* gene using the traditional approach to genetic diagnosis, Lupski et al. show that whole-genome sequencing is now not only possible, but can also be applied to the clinical setting. The impressive advance that whole-genome sequencing provides is its ability to identify all DNA changes in each haplotype (exons, introns and copy-number variants), making it a potential powerful tool when applied to diseases with complex genetic aetiologies.

Nevertheless, there remain a number of cautionary notes. First, the diagnosis was possible due to the fact that the *SH3TC2* gene was already known and characterised. Second, the accuracy of the genetic disease databases is questioned as the patient also displayed a mutation in the *ABCD1* gene reported to cause X-linked adrenoleukodystrophy, without showing any clinical features of this disease. And the price? Lupski et al. estimate that their whole experiment would now cost less than \$50,000. In comparison, a clinical-testing panel looking at the copy number variant that commonly causes CMT along with mutations in 15 other genes associated with CMT currently costs \$15,000. We should not put away our tendon hammers and tuning forks just yet.

– Rhys Roberts, Cambridge University.

Lupski JR, et al. Whole-Genome Sequencing in a Patient with Charcot-Marie-Tooth Neuropathy.

THE NEW ENGLAND JOURNAL OF MEDICINE

2010 Apr 1;362(13):1181-91. Epub 2010 Mar 10.

FTLD genetics: progress through collaborative splitting

The frontotemporal lobar dementias (FTLDs) are a clinically, pathologically and genetically heterogeneous group of diseases. Although frequently described as "the second commonest cause of presenile dementia", FTLD remains an uncommon disease (incidence 3.5, prevalence 4-15, cases per 100,000 in 45-64 year olds; Mercy et al 2008). It has therefore taken a large collaborative study using samples from North America, Europe and Australia to identify the first association of common genetic variants with FTLD. Van Deerlin et al identified association with single nucleotide polymorphisms on chr 7p21 surrounding the *TMEM106B* gene, which encodes an uncharacterised 274 amino acid transmembrane protein. Functional studies suggested that risk alleles could result in increased brain expression of *TMEM106B* and a more aggressive disease course.

Genome-wide association studies (GWAS) in general are used to identify association in common diseases, such as diabetes, and require many thousands of cases to generate the power to determine true associations. Nevertheless, Van Deerlin et al appear to have succeeded with an n of just 515. Their success stems partly from preparation of a homogeneous group of cases made up only of patients with TDP-43 pathology (FTLD-TDP). By excluding those with tau inclusions and other less common pathological subtypes of FTLD, they appear to have distilled the

genetic pool under scrutiny, reducing the chances that a true association would be lost. It must be noted, however, that the majority of the chr 7p21 association appears to be due to a sub-population of their cohort already identified to have autosomal dominant FTLD due to *GRN* mutations. A further interesting observation is that the most significant SNPs Van Deerlin et al identified on chromosome 9 are in a region of great interest in another 'TDP-43 proteinopathy', amyotrophic lateral sclerosis (Van Es et al 2009, Vance et al 2006).

– Jemeen Sreedharan, Guy's and St Thomas'

NHS Trust, London.

Van Deerlin VM et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions.

NATURE GENETICS 2010 Mar;42(3):234-9. Epub 2010 Feb 14.

Mercy L, et al. Incidence of early-onset dementias in Cambridgeshire, United Kingdom.

NEUROLOGY 2008 Nov 4;71(19):1496-9.

van Es MA, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis.

NAT GENET. 2009 Oct;41(10):1083-7.

Vance C, et al. Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. BRAIN 2006;129(Pt 4):868-76.

Memory: A name for forgetting

When considering memory it is easy to neglect forgetting. Over the centuries, forgetting has continued to represent the dark side of memory, often portrayed as a passive process of memory fading, or an 'overwriting' by competing memories. Modern science too has dwelled more on the mechanisms of memory than on those of forgetting. The authors in this study now take steps to redress this deficiency by directly examining the molecular mechanisms of forgetting. They define a molecular pathway which, when activated, causes memories to fade faster; conversely, inhibiting the pathway makes memories more lasting and more resistant to change. The study uses fruit flies expressing mutated versions of a molecule called Rac in defined adult neurones, analysing them with standard behavioural memory tests. The authors firstly demonstrate that memory fades more rapidly in the presence of a constitutively active version of Rac, and less rapidly if an inactive version is used (crucially, they also confirm that the differences are not because of an alteration in the strength of the initial memory). They go on to look at reversal learning, where the conditioned stimulus in a learning task is reversed on a second trial, forcing the flies to relearn; the reversal turns out to be easier in the presence of active Rac, showing that the initial memory is now less durable. Conversely, in the presence of inactive Rac the flies stick more stubbornly to their original memory, relearning the new association less readily. Finally, the authors show in wild type flies that levels of active Rac fall as learning is consolidated by repeated trials, but are raised in reversal learning paradigms, correlating with an increased need to 'remove' or overwrite inappropriate associations.

That active Rac seems to promote forgetting comes as something of a surprise. Conventional understanding has it that memories, and molecular correlates such as long-term potentiation, rely on glutamate receptor clustering and increases in polymerised actin within strengthened synapses; both of these changes are driven by active Rac (and its downstream target PAK1). On this background, the idea that Rac activity promotes forgetting seems counter-intuitive. One solution to the paradox may be that Rac is key to a forgetting mechanism that is effectively integral, 'built in as standard', to each new memory; and which wanes as the memory matures with repeat exposure. Rac activity then becomes a marker of the recency of memories, with newer memories being more susceptible to forgetting than consolidated ones – a useful feature in case fresh memories need to be overwritten before they become too established. Such insights are clearly relevant to diseases such as Alzheimer's, where forgetting is the rule, and where dysregulation of Rac pathways may well be involved. These questions are not directly addressed in this paper, but a central message stands out: forgetting has a mechanism, and it needs to be understood.

– **Philip Buttery, Cambridge Centre for Brain Repair and Queen Elizabeth Hospital, King's Lynn.**

Shuai et al. Forgetting Is Regulated through Rac Activity in Drosophila. CELL 2010;140(4):579-89.

Stimulating the brainstem in Parkinson's Disease – a step in the right direction?

The use of deep brain stimulation (DBS) to treat patients with movement disorders is now well established and this is perhaps most obvious in advanced Parkinson's Disease (PD) with DBS of the subthalamic nuclei. However, whilst this treatment works well for many features of PD, it does not improve all aspects of the disorder, especially those that involve more axial features and this includes gait. As a result modifications have been sought that can help this disabling aspect of PD, and one approach has been to target brainstem nuclei involved with locomotion such as the pedunculopontine nucleus (PPN).

The PPN is a small structure within the brainstem that has widespread connections throughout the CNS, receives from the basal ganglia output nuclei, and has been thought for many years to be a vital relay station in the initiation and generation of gait. It has been found to be affected in PD, having pathology itself as well as being in receipt of a disordered basal ganglia output. As such it became the target for the stimulating electrode of curious neurosurgeons! The initial open label studies sug-

gested that benefits could be seen using low frequency stimulation of the PPN in patients with PD. However, such studies are hard to interpret given the placebo problems inherent in small open label studies and the different criteria used to select patients for this treatment in these trials. Thus better controlled studies are needed although what constitutes a good control arm is difficult when the treatment under scrutiny involves invasive neurosurgery. Nevertheless with DBS, one does have the opportunity to switch on or off DBS without the patient knowing the activation status of their stimulator. This strategy has now been exploited in two recent studies reported in Brain.

First, Ferraye et al took 6 patients with PD and severe freezing of gait that was unresponsive to L-dopa or DBS of the subthalamic nucleus. These patients all had bilateral electrodes placed in the PPN with the primary outcome being the improvement in gait, freezing episodes and falls at 1 year. There was a period between 4-6 months when a double blind cross over study was done with the stimulator being on or off. The main finding was that at one year the number of freezing episodes and falls was reduced, although none of the other outcomes were improved nor was there any clear consistent effect seen in the double blind period. In the second study by Moro et al, 6 patients with advanced PD and significant gait and postural abnormalities were recruited and treated using a unilateral PPN stimulator (the side chosen for stimulation being contralateral to the most severely affected side of the body). In this case the patients had to have failed medical therapy but had not had DBS of the subthalamic nucleus. These patients were then subject to a double blind treatment with the stimulator being either switched on or off, and again some benefit was seen with respect to falls but no other measures using subsections of the UPDRS motor examination.

So what are we to make of all this? I think the studies have suggested that the PPN may be a useful target in helping treat axial features of PD – especially issues of gait and falls. This is important because these features are often resistant to L-dopa therapies and carry significant morbidities and impact on quality of life. Thus being able to offer something useful in this domain is worth pursuing, but bigger studies are needed to know the extent to which this stimulating approach really works and benefits patients with PD.

– **Roger Barker**

Ferraye MU et al. Effects of pedunculopontine nucleus area stimulation on gait disorders in Parkinson's disease. BRAIN 2010; 133: 205-214.

Moro E et al. Unilateral pedunculopontine stimulation improves falls in Parkinson's disease. BRAIN 2010; 133:215-224.

Parkinson's Disease: earliest diagnosis

Neurologists strive to differentiate early idiopathic Parkinson's disease (PD) from its mimics in order to properly advise patients about prognosis and treatment options. Clinical diagnosis alone will get it wrong about 15% of the time, and accuracy improves as disease progresses. Tang and colleagues at The Feinstein Institute for Medical Research in New York used fluorine-18-labelled-fluorodeoxyglucose-PET to differentiate idiopathic PD, multiple system atrophy and progressive supranuclear palsy in 167 patients with parkinsonism of unknown cause. They used an automated voxel-based classification procedure to map characteristic pattern abnormalities in these three conditions. The patients were scanned at an early stage of their disease, and followed-up for a mean of 2.6 years by a blinded movement disorder specialist to ascertain the final diagnosis. When the accuracy of the initial image-based classification was compared with the final diagnosis, the positive predictive value was greater than 90% for each condition. The imaging categorisation was reproducible on repeat scanning and confirmed in nine patients on post-mortem examination.

The fact that 32 patients had to be excluded at the outset because the final clinical diagnosis was unclear should not be forgotten, as this was the gold standard in the study. Further, patients with structural brain abnormalities that could potentially account for their symptoms (including white matter and ischaemic lesions which are commonly seen in the clinic) were excluded. The utility and cost-effectiveness of PET in the early differential diagnosis of parkinsonism needs to be proven further, but the desire to identify suitable candidates for novel drug trials and sur-

gical procedures (such as stem cell transplantation) means that there is bound to be considerable interest in this approach.

– **David Breen, Cambridge University Centre for Brain Repair.**
Tang CC et al. Differential diagnosis of parkinsonism: a metabolic imaging study using pattern analysis. LANCET NEUROLOGY 2010;9(2):149-58.

Parkinson's Disease: Reckless Generosity

Here is another phenomenon to add to the growing collection of impulse control disorders (ICDs) in Parkinson's disease. O'Sullivan et al describe three cases of "excessive and inappropriate philanthropy". All three were taking dopamine agonists and the behaviour improved or ceased when the agonist was reduced or discontinued.

The authors speculate as to the pathophysiological basis in dysfunctional dopaminergic reward pathway stimulation, an interaction between increased oxytocin release and dopaminergic reward systems, and impaired decision making, with an insensitivity to the negative consequences of a particular action. As with all impulse control disorders, their presence should be screened at each clinic visit, and patients warned. As one can imagine, the behaviours may be associated with profound consequences for the patients and carers, both financially and socially.

– **Wendy Phillips, Norfolk and Norwich University Hospitals NHS Trust**
O'Sullivan S et al. Reckless generosity in Parkinson's disease. MOVEMENT DISORDERS 2010;9999.

Rehabilitation: the importance of prior depression in surgical outcome

Although the relationship between disability, depression and pain is a complex one, is there a case to be made for attempting to optimise psychological as well as physical health prior to complex surgical procedures? The existence of the term "failed back syndrome" to describe patients who have had poor outcomes from spinal surgery illustrates the chronic and disabling consequences of adverse results for this intervention. It is obvious that optimising general health improves outcome, and high risk patients will be assessed from an anaesthetic perspective well in advance to minimise their physical co-morbidities. This paper looks specifically at outcomes following lumbar spinal stenosis surgery in relation to depressive symptoms in the pre-operative and post-operative period. Rather than focussing solely on pre-operative depressive symptoms, the authors have looked at "depressive burden" across the pre-operative, post-operative and late (3 and 6 months) periods. Depressive symptoms were evaluated with the Beck Depression Inventory and disability was assessed by walking capacity, back and leg pain, and the Oswestry Disability Index. Perhaps not surprisingly, late depression had a strong association with pre-operative depression although depressive symptoms generally improved following surgery. There is a clear relationship between pre-operative depression and post-operative outcome, in terms of disability, however. This is true for both younger and older patients. There are serious clinical, economic and social consequences of a "failed back" and whether these could be attenuated by rigorous pre-operative screening and intervention for psychological issues remains to be seen.

– **Lloyd Bradley, Western Sussex Hospitals Trust**
Sinikallo S et al. Depressive symptoms predict postoperative disability among patients with lumbar spinal stenosis: A two-year prospective study comparing two age groups. DISABILITY AND REHABILITATION 2010;32(6):426-68.

Dementia: motor predictions

Mild parkinsonian signs (MPS) are common in the elderly, and two previous studies (including the Washington Heights Inwood Columbia Ageing Project, WHICAP) have suggested that MPS is a risk factor for developing dementia. The authors of this paper have studied a new cohort from 1999-2001, an extension of the original WHICAP cohort (1992-1996). Again, these prospective population based data show that MPS in elderly people

(>65 years) is associated with a greater than two-fold risk of developing dementia, mainly (86.4%) Alzheimer's disease (AD). A cohort of 1851 randomly sampled elderly people underwent a neurological examination, abbreviated unified Parkinson's disease rating scale (UPDRS) evaluation and a standard neuropsychological battery; mean follow up was 3.7 years. MPS was defined as ≥ 2 UPDRS rating of 1 or one rating of ≥ 2 (PD was defined as ≥ 2 UPDRS rating of ≥ 2). People with MPS were more than twice as likely to develop dementia as those without MPS, particularly in the domains of language and memory. Furthermore, with each point increase in the UPDRS score, the risk of dementia increased by 15%. Patients with MPS were older and more had diabetes and stroke - but the increased risk of dementia still held after adjusting for these confounders (hazard ratio, HR = 1.98), and also when the 408 participants with mild cognitive impairment (MCI) were removed from the analysis. Those with axial dysfunction and tremor (rather than rigidity) were more likely to develop dementia. The risk of dementia with MPS was not compounded by other known risk factors (including education, family history, stroke, apoE). There was no interaction between MCI and MPS - so MPS cannot be used as a predictor of MCI progression.

MPS have been thought to be relatively benign, but this study underlines a substantial risk of dementia. Although the majority of patients developed AD, it would have been interesting to know what other dementias were diagnosed - particularly PD dementia, vascular dementia and Lewy body disease; and, the progression of MPS to frank Parkinsonism. This is particularly relevant given that the distinction between MPS and PD is relatively subjective (only one point on the UPDRS could make the difference). The basis for MPS as a risk factor for dementia is unknown but one possibility is that MPS represents basal ganglia tangles (and patients with AD often have MPS). It is perhaps surprising that those with tremor, rather than rigidity, were more likely to develop dementia given that tremulous PD is relatively benign. Neurologists do not tend to see the very frail elderly, so it is helpful to have a reminder that MPS are very common among this population, and that MPS are not necessarily 'normal for age'.

– **Wendy Phillips, Norfolk and Norwich University Hospitals NHS Trust**
Louis E et al. Mild parkinsonian signs are associated with increased risk of dementia in a prospective, population-based study of elders. MOVEMENT DISORDERS 2010;25:172.

Cognition: finding where we keep track of when

It has been established for a long time that temporal perception, the ability to perceive the passage of time, is influenced by attention, and thus the neural systems underlying temporal perception and attention might include similar structures. This study used repetitive transcranial magnetic stimulation (rTMS) to test the a priori hypothesis that the right parietal lobe plays an important role in both orientation of attention and temporal perception. Previous studies using rTMS have established the role of the angular gyrus (AG) in temporal perception but the role of the supra marginal gyrus (SMG) had not previously been tested. A two stage experiment was designed in which healthy volunteers had to compare the duration of a visual stimulus presented in different durations with and without rTMS to three brain areas (Right SMG, left SMG and vertex). The difference between the two stages of the experiment was in rTMS timing. In the first stage, rTMS was delivered during presentation of the first sensory stimulus whereas in the second stage this happened during presentation of the second stimulus. This was to establish whether memory and decision making processes, which are more prominent in the latter part of the test, impose any significant effect on the temporal perception. Analysis of the results revealed that rTMS delivery to the right SMG consistently led to increased temporal perception compared to the left SMG, vertex stimulation and no rTMS delivery. These findings reinforce the existing theories regarding importance of the right parietal lobe in temporal perception, and for the first time highlight the particular role of right SMG in this process.

– **Seyed Sajjadi, Herchel Smith building and Neurology Unit, Addenbrooke's Hospital.**
Wiener et al. Fast Forward: Supramarginal Gyrus Stimulation Alters Time Measurement. J COG NEUROSCI. 2010 Jan