Defining neurogenetic phenotypes (or how to compare needles in haystacks)

Accurate diagnosis is the foundation of clinical neurology, providing both the physician and the patient with a guide to the future, hopefully leading to a treatment, and sometimes pointing towards a cure. Unlike many other clinical disciplines, the day-to-day practice of neurology involves a pantheon of symptoms and long lists of differential diagnoses. The challenge is to spot the rarities, especially if this is going to influence clinical management. This should be much easier in 2010, given recent advances in the routine diagnostic armamentarium; but paradoxically it seems more difficult, as we continue to dissect sub-categories of common disorders and overlapping clinical spectrums expand. So what are the distinguishing features of a particular disorder, and how can we reliably define them? This is particularly difficult if they are exceptionally rare ‘once in a lifetime’ diagnoses.

Two papers in this edition of Brain tackle this issue for two important genetic neurometabolic disorders (Jinnah et al., 2010; Leen et al., 2010), illustrating an emerging approach to this thorny topic.

In 1964, the medical student Michael Lesch and the paediatrician Bill Nyhan published the classic description of two brothers, aged 4 and 8, with a new familial disorder of uric acid metabolism and central nervous system dysfunction (Lesch and Nyhan, 1964). The primary biochemical defect was defined within a few years, affecting the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) (Seegmiller et al., 1967), ultimately leading to the identification of recessive mutations in the X-chromosomal disease gene HPRT1. After a normal pregnancy and birth, Lesch–Nyhan disease typically presents with developmental delay in the first year of life, most noticeably affecting the motor system, and leading to delayed sitting, crawling and walking. Involuntary movements develop before school age, with dystonia and choreoathetosis, leading on to spasticity that prevents affected boys from walking independently (Nyhan et al., 2005). Self-injurious behaviour is a defining feature, particularly affecting the fingers, hands, lips and cheeks; although more aggressive head or limb trauma can occur. A high serum uric acid is the key diagnostic clue, but is neither sensitive nor specific; so if there is a high index of suspicion, the diagnosis should be confirmed by the biochemical measurement of HPRT enzyme activity (in blood or cultured fibroblasts) and subsequent molecular genetic analysis. However, even in the late 1960s it became clear that some family members had a milder phenotype (Kelley et al., 1969), with progressively milder forms being described in case reports over the subsequent years (Puig et al., 2001).

Now, more than three decades after the original description, a multi-national consortium, including Bill Nyhan, set out to define these ‘attenuated variants of Lesch-Nyhan disease’ by studying 46 patients, most of whom were examined by Hyder Jinnah (Jinnah et al., 2010). Patients with overt self-injurious behaviour were excluded from the analysis, thus restricting the observations to non-classical cases, and the results were compared to 127 atypical cases indentified from a systematic literature review. Using this approach, a consistent pattern has emerged, with a spectrum of disease severity spanning from ‘almost classical’ Lesch–Nyhan disease through to mild adult onset cases with no neurological involvement (in <7% of examined cases). Neurological abnormalities were the presenting feature in ~2/3, usually in early childhood, but ~1/3 presented with the complications of hyperuricaemia (with overt gout in <1/5). The majority of patients examined had motor delay and displayed a range of movement disorders including dystonia, chorea, ballism and dystonic myoclonus. In some the movement disorder was subtle, including mild dystonic overflow and action-specific dystonias, clumsy hands or an awkward gait. Parkinsonian features were present in <10%. Hyper-reflexia was common, and some may have had subtle cerebellar signs. These features developed in childhood and were non-progressive in a substantial proportion, presumably leading to misdiagnoses that included cerebral palsy. Most had a disturbance of speech and gait. Intriguingly, the cognitive and behavioural disorder, which would alert the clinician to the diagnosis in classical cases, was mild or absent in a large proportion. This is, of course, partly related to the study exclusion criteria, but the key point is that intelligence may be above average and that the behavioural changes easily could go unmissed. Severe onychoophagia was present in 13%, suggesting that this behaviour lies on the self-mutilatory spectrum—a salutary reminder to those of us who have the occasional nail-nibble in front of the computer screen!
Significantly, the motor, cognitive and behavioural features were closely correlated across the study group, suggesting that these are all a direct consequence of the low HPRT activity. This contrasted with corticospinal tract involvement, which was equally present in classical and (milder) atypical Lesch–Nyhan disease. The authors invoke new mechanisms to explain this discrepancy, but it could simply reflect the exquisite sensitivity of the pyramidal system to low HPRT activity, yielding physical signs even in the mildest cases.

Although a completely different neurogenetic metabolic disease, these findings mirror those of the second study (Leen et al., 2010). Wilhelmina Leen and colleagues from the Departments of Neurological Disorders and Human Genetics in Nijmegen have been investigating suspected cases of glucose transporter-1 (GLUT-1) deficiency since 2004. Having identified 57 new patients, they present a comprehensive clinical, biochemical and genetic description of the disorder that was first described in 1991 (De Vivo et al., 1991).

GLUT-1 deficiency classically presents in infancy with drug-resistant epilepsy leading to developmental delay and acquired microcephaly, associated with a low fasting glucose level in the cerebrospinal fluid, despite a normal blood glucose (Klepper and Leendecker, 2007). Unlike most neurometabolic disorders, GLUT-1 deficiency displays autosomal dominant inheritance due to haplo-insufficiency mutations in the SLC2A1 gene on chromosome 1p35-31.3 causing a loss of protein function (Seidner et al., 1998). The transport of glucose into the brain is totally dependent on GLUT-1 function, and differentiation from other causes of hypoglycorrhachia is usually possible based on the clinical phenotype and low lactate levels in the cerebrospinal fluid. Over 100 patients with SLC2A1 mutations have been identified and, as with Lesch–Nyhan disease, the spectrum of phenotypes has broadened. For GLUT-1, this now includes a complex neurological phenotype with spasticity, ataxia and movement disorders as presenting features without seizures, but the full spectrum of disease had not been formally defined (Brockmann, 2009).

In the current study (Leen et al., 2010), detailed clinical characteristics were available for 55 of the 57 new patients. The majority (84%) had the ‘classical’ GLUT-1 deficiency phenotype, presenting either early in life (<2 years) or later in childhood. Early onset classical cases were more likely to develop moderate or severe mental retardation, whereas later-onset classical cases were more likely to develop a complex movement disorder. A wide range of epilepsy phenotypes were observed, from paroxysmal eye movements to tonic-clonic generalized seizures. Non-classical phenotypes included mental retardation without a movement disorder and ataxia.

Molecular genetic analysis revealed 37 different mutations in the 57 patients, with the vast majority being de novo, explaining the lack of a supporting family history in many cases. Clear autosomal dominant transmission was demonstrated in some, with a range of phenotypes in affected mutation carriers from the same family. This included the near-normal mother of one affected child, both of whom harboured the same mutation. Both multiple exon deletions and point mutations were identified throughout SLC2A1, confirming the previously held view that all domains of this highly conserved gene are important for the maintenance of brain glucose levels. Despite the clinical heterogeneity, there appeared to be a direct correlation between different types of SLC2A1 mutation, the CSF glucose level and the severity of the phenotype. Intriguingly, the patients with the non-classical (milder) phenotype presented at approximately the same age, implying that particular brain regions or systems are susceptible to the mildest of biochemical defects, and thus even the mildest mutations lead to a phenotype at the same age. In contrast, some brain regions are more resilient and are not involved in non-classical cases—or only at a later stage in life.

These two papers are a welcome contribution to a growing literature that is re-writing the neurology textbooks. The authors each took on a seemingly insurmountable task—to find and compare exceptionally rare diseases (the needles) in the whole population (the haystack). In order to compare many ‘needles’, and thus define the phenotypic spectrum, both increased the number and the size of the haystacks: studies of this sort are only possible through a large multi-national, indeed inter-continental, effort. Leen and colleagues take this to the extreme, with 57 patients accurately described by 53 authors from 45 departments in Europe and North America. A second strategy was to find many ‘needles’ using a very simple but sensitive and specific tool (akin to a large magnet used to collect iron needles). For Jinnah and colleagues, serum uric acid and blood HPRT was the key. Leen et al. (2010) used the low cerebrospinal fluid glucose, with the added ‘magnetic Tesla’ generated by offering an efficient and comprehensive molecular genetic diagnostic service to the international community. Of course, these tools have their drawbacks, leading to self-fulfilling prophecies. It therefore comes as no surprise that Leen et al. report a cerebrospinal fluid/blood glucose ratio of <0.5 in all but one of their patients, and that only mild or moderate cognitive phenotypes were identified by Jinnah and colleagues.

With more relaxed testing criteria, we will inevitably see a further expansion of the phenotype. This has happened, for example, with MECP2 analysis—originally thought specifically to cause classical cases of childhood-onset Rett syndrome (Amir et al., 1999), MECP2 mutations have now been described in a widely diverse phenotype group presenting at all ages in the neurology clinic (Percy et al., 2007).

Descriptive clinical work is often perceived to be unexciting, lack lustre and perhaps dull in some instances—why should we bother looking for needles in a neurogenetic haystack, either from a research perspective or in our routine general neurology clinics? However, the work of Jinnah, Leen and colleagues can be directly translated into clinical practice—without time consuming pre-clinical development or bureaucratic regulatory approval. Their work will have immediate benefits for patient care. Identifying ‘forme frustes’ of Lesch–Nyhan disease will enable the early treatment of pre-symptomatic hyperuricaemia which, unchecked, leads to soft tissue and renal damage and thus causes significant morbidity. Likewise, the diagnosis of GLUT-1 deficiency will initiate a ketogenic diet, replacing the primary brain energy source with ketone bodies that readily cross the
blood–brain barrier without the help of GLUT-1. This treatment improves many of the clinical features described by Leen and colleagues (2010).

So, we can breathe a sigh of relief—the precision of molecular genetics allows the clinician to provide the ultimate diagnosis, and the clinical clues provided in papers such as these will allow neurologists to find all of the patients with ease. Unfortunately, nothing could be further from the truth. The identification of oligo-phenotypic cases, presenting in an unusual way and at an atypical age, means that we need to consider these rarities with increasing frequency and in many different contexts. While it is appealing that there appears to be a close relationship between genotype and the biochemical phenotype for these disorders, it is not clear why different members of the same family can have such discordant clinical phenotypes. This invokes additional environmental or genetic explanations that further complicate the situation, but understanding this phenotypic variability is likely to provide the key to developing a cure.

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**‘Looks like epilepsy to me!’**

‘Looks like epilepsy to me!’ The late Professor Bryan Matthews did not get much wrong, but in the early 1980s he and I did not know about ‘limb shaking’ transient ischaemic attacks when I asked him to have a look at a patient under my care at the Radcliffe Infirmary in Oxford. Indeed, it was only in the 1980s that good descriptions were emerging of this unusual type of transient ischaemic attack, mostly in the US literature, and the fact that it almost only ever occurred in patients with very severe carotid disease in their neck—bilateral occlusion or severe stenosis, or unilateral occlusion with contralateral severe stenosis. What put me off focal epilepsy was that this patient also had severe bilateral carotid disease, a fact that I had not revealed to Professor Matthews, to avoid prejudicing his comments on what we were actually looking at—something that seemed on the surface very like focal motor seizures. But then focal epilepsy would not be expected to respond to revascularizing the brain, which, when it can be achieved, we now know is what may happen.

We also knew fairly soon after they were described that ‘limb shaking’ transient ischaemic attacks had a tendency to be brought on by things which might drop the systemic blood pressure such as standing up, a hot bath, coughing, exercise and starting or increasing hypotensive therapy. This fall in blood pressure might then cause a fall in cerebral perfusion in focal areas of critically impaired cerebrovascular reactivity, but only where autoregulation of blood flow was preserved. Where autoregulation was lost, focal motor seizures were more likely to occur.

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## References


