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The impact of molecular biology on clinical neurology

分子生物學對臨床神經病學的影響

Advances in molecular biology have increased our understanding of both inherited and sporadic forms of neurological disease. In this review, the impact of these advances is discussed in relation to specific neurological conditions. These include the hereditary neuropathies and ataxias, Huntington's disease, and the muscular dystrophies, as well as Alzheimer's disease, Parkinson's disease, and motor neuron disease. Genetic channelopathies, such as familial hemiplegic migraine, are also described. Although knowledge in this area overall is still relatively scant, current advances in molecular biology have helped in the reclassification of some neurological disorders, thereby providing a further step towards the development of rational therapies to treat these conditions.

分子生物學的進展增加了我們對神經學疾病的遺傳和偶發兩種形式的了解。本文討論這些進展涉及到特殊的神經學條件造成的影響，包括遺傳神經病和共濟失調，漢丁頓病和肌肉營養失調，以及阿爾茨海默病，帕金森病和運動原神經細胞病等。對諸如家族偏癱偏頭痛之類的遺傳管道病也進行了描述。儘管本文總覽的認識依然相當有限，分子生物學目前進展已經協助一些神經疾病重新分類，而且進一步走向處置這些病因之合理治療方法的發展。

Introduction

The 1990s have been described as the 'decade of the brain', to reflect the fact that major achievements in various aspects of neuroscience, in particular molecular biology, were made during that decade. These advances have increased our understanding of the basic pathophysiological mechanisms from which neurological disorders develop. In addition to providing insights into inherited conditions, molecular biology has instigated entirely new approaches to the many 'sporadic' neurological diseases. Practice and treatment in clinical neurology is still largely based on phenotypic features—that is, symptoms and signs—although this trend is set to change. Increased understanding of the pathophysiological mechanisms that underlie neurological disorders will allow for a more rationalised approach to their diagnosis, classification, and, perhaps, subsequent therapy. A complete account of the molecular advances in neuroscience is beyond the scope of this article. Instead, this review will discuss specific neurological disorders and illustrate the impact of molecular biology on their management in clinical practice.

Hereditary neuropathies

Charcot-Marie-Tooth (CMT) disease or hereditary motor and sensory neuropathy (HMSN) is conventionally diagnosed and classified by clinical

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features, mode of inheritance, electrophysiological findings, and, in selected cases, histopathology. Hereditary motor and sensory neuropathy I and II are mostly autosomal dominant conditions. Hereditary motor and sensory neuropathy I is a generalised demyelinating neuropathy, whereas HMSN II is an axonal degenerative neuropathy. Hereditary motor and sensory neuropathy III, or Dejerine-Sottas disease (DSD), is a rare autosomal recessive hypodemyelinating neuropathy of early onset, which results in severe disability during childhood. Other non-DSD autosomal recessive HMSNs also cause severe neuropathies. Hereditary motor and sensory neuropathy X is of X-linked dominant inheritance. It was thought to be a primary axonopathy with secondary demyelination. Electrophysiological abnormalities may be intermediate and difficult to distinguish from those of HMSN I or II. Another autosomal dominant disorder is hereditary neuropathy with liability to pressure palsies (HNPP). Hereditary neuropathy manifests as recurrent entrapment neuropathies and a progressive generalised axonal neuropathy of variable severity, and has typical histopathological findings.

Based on newer findings in molecular genetics, the classification of HSMN has been revised (Table). Hereditary motor and sensory neuropathy I is subdivided into CMT 1A to 1C; HMSN II into CMT 2A to 2D, HMSN III to DSD A, B, and autosomal dominant DSD, and HMSN X to CMT X1 and X2. Charcot-Marie-Tooth disease 4 specifically indicates autosomal recessive inheritance other than DSD, and is subdivided into CMT 4A to 4C and HMSN-Lom. The underlying genetic defects and probable pathogenic mechanisms

of many myelinopathies are now known. Peripheral myelin protein 22 (PMP22) and myelin protein zero (P0) are components of peripheral nerve myelin. The former is encoded by the *PMP22* gene at chromosome locus 17p11.2, and the latter by *P0* gene at 1q22-q23. Mutations of *PMP22* are associated with CMT 1A, HNPP, and DSD A.¹⁻⁴ Mutations of *P0* are found in CMT 1B, DSD B, and congenital hypomyelination neuropathy (CH).⁵⁻⁸ Some researchers now consider CMT 1 and DSD to be a different spectrum of the same disease. Mutations of the *Cx32* gene at chromosome locus Xq13.1, which encodes the gap-junction protein connexin 32, are found in some CMT X1 cases.^{9,10} Mutations of the *EGR2* gene at 10q21-22, which encodes a transcription factor that regulates gene expression, are associated with CMT 1C, DSD, and CH.^{11,12} No single gene has so far been associated with CMT 2 and 4, but some linkages have been reported—CMT 2A to 1p35-36,^{13,14} 2B to 3q13-22,¹⁵ 2D to 7p14,¹⁶ 4A to 8q12-21,¹⁷ or 5q23-q33,¹⁸ 4B to 11q23.1,¹⁹ and HMSN-Lom to 8q24.²⁰ Unlike the myelinopathies, the pathogenic implications of these linkages are still not understood.

Hereditary motor and sensory neuropathies have no specific treatment. For the purposes of genetic counselling, diagnosis can be made in most cases by using conventional techniques. Nevertheless, genetic testing can be helpful in some of these disorders. The most useful molecular test is perhaps the detection of *Cx32*-related abnormalities in suspected HMSN X, because electrodiagnostic parameters may overlap with those of HMSN I or II. A negative result for a genetic test, however,

Table. Molecular basis for differentiation and diagnosis of some hereditary neuropathies

Disease	Locus	Gene	Mutation
<i>Myelinopathies</i>			
CMT 1A	17p11.2	<i>PMP22</i>	Duplications, point mutations
CMT 1B	1q22-23	<i>P0</i>	Point mutations, small deletions
CMT 1C	10q21-22	<i>ERG2</i>	Point mutations
CMT X1	Xq13.1	<i>Cx32</i>	Point mutations, small and large deletions, frameshift mutations
CMT X2	?	?	
HNPP	17p11.2	<i>PMP22</i>	Deletion, frameshift-nonsense mutations
DSD A	17p11.2	<i>PMP22</i>	Point mutations
DSD B	1q22-23	<i>P0</i>	Point mutations, small deletions, frameshift mutations
DSD AD	10q21-22	<i>ERG2</i>	Point mutations
	8q23-24	?	
CH	1q22-23	<i>P0</i>	Point mutations
	10q21-22	<i>ERG2</i>	Point mutations
CMT 4A	8q12-21.1	?	
	5q23-33	?	
CMT 4B	11q23.1	?	
HMSN-Lom	8q24	?	
<i>Axonal neuropathies</i>			
CMT 2A	1p35-36	?	
CMT 2B	13q13-22	?	
CMT 2C	?	?	
CMT 2D	7p14	?	

does not exclude the diagnosis. Detection of *PMP22*-related abnormalities is helpful in the assessment of families with HNPP. There may be considerable phenotypic heterogeneity within the same family such that conventional methods may not detect all affected members. Nerve biopsy is invasive and cannot be justified in asymptomatic cases.

Hereditary ataxias

Friedreich's ataxia (FA) is the most common form of hereditary ataxia in Caucasians and it is inherited as an autosomal recessive condition. It manifests with progressive gait and limb ataxia, areflexia, pyramidal signs, optic atrophy, deafness, cardiomyopathy, and an increased risk of diabetes mellitus. In the early 1980s, Harding²¹ revised the classification of hereditary ataxias according to clinical features and mode of inheritance, and proposed the diagnostic criteria for FA. In 1996, the mutation in FA was identified as a GAA trinucleotide expansion in the *FRDA* gene at chromosome 9.^{22,23} The molecular definition of FA based on GAA expansion mutation is broader than the previous phenotype-based definition, because the same deficit can be found in ataxic syndromes that are not included in Harding's criteria.²⁴ There is a positive correlation between the size of the GAA expansion and the severity of the clinical disease. Small expansions are associated with a later age of onset and atypical clinical features. The *FRDA* gene encodes the protein, frataxin. The function of frataxin is still not fully understood, but evidence suggests that it may be a mitochondrial protein.²⁵ Deficiency in frataxin is associated with mitochondrial iron accumulation and cell death from oxidative stress. The expression of frataxin is high in the heart and pancreas, and relatively high in the spinal cord. Reduced frataxin levels in these structures is the probable cause of cardiomyopathy, diabetes mellitus, and neuronal loss.

A confident diagnosis of FA can usually be made clinically. The main indication for genetic analysis in FA is in diagnosing atypical cases.²⁶ The role of genetic counselling in FA appears limited. Firstly, many couples would have completed their families before the index case manifests, usually at the time of puberty, such that the genetic confirmation is usually too late to influence their decisions to have subsequent offspring. Secondly, because the frequency of the heterozygous mutation in the general population is low (1 in 85 among Caucasians), the chance of disease transmission by healthy siblings to their offspring is estimated to be less than 1 in 300, even if they are carriers. Genetic screening in siblings of the index case is, thus

not generally recommended. As with HMSN, there is currently no specific treatment available for FA.

Harding's classification of the autosomal dominant cerebellar ataxias (ADCA) was based on phenotypic abnormalities. Autosomal dominant cerebellar ataxia I is associated with ophthalmoplegia, optic atrophy, dementia, or extrapyramidal features. Autosomal dominant cerebellar ataxia II is characterised by pigmented retinopathy. Autosomal dominant cerebellar ataxia III presents with pure ataxia of late onset, whereas ADCA IV—which includes additional features of deafness or neuropathy—probably represents a mitochondrial disorder. The genetic heterogeneity underlying ADCA is now recognised. Autosomal dominant cerebellar ataxias are subdivided into spinocerebellar ataxia (SCA) types 1 to 8, although there is considerable phenotypic overlap among them. Five genotypes are associated with the CAG trinucleotide repeat: SCA 1 with gene locus at 6p23,²⁷ SCA 2 at 12q24,²⁸ SCA 3 at 14q24.3-q31,²⁹ SCA 6 at 19p13,³⁰ and ADCA II or SCA 7 at 3p12-21.1.³¹⁻³³ Apart from SCA 6, in which the gene locus encodes for the α_{1A} -subunit of the P/Q-type voltage-gated calcium channel, loci of the other SCA types encode their respective ataxins, the functions of which are still unknown. Spinocerebellar ataxia 8 is associated with CTG expansion at 13q21.³⁴ Autosomal dominant cerebellar ataxia III has been renamed SCA 5 and its gene locus lies on chromosome 11.³⁵

At present, the pathogenic basis of these genotypic abnormalities is still poorly understood, so that the impact of this reclassification on patient management is not significant. Occasionally, ADCA may present with prominent extrapyramidal features that are partially levodopa responsive. This presentation is more common in patients with SCA 3, formerly known as Machado-Joseph disease. This association might have a therapeutic implication, although the decision to use levodopa is based on clinical judgement.

Other trinucleotide repeat disorders

Apart from FA and SCA 1, 2, 3, 6, 7, and 8, other trinucleotide repeat disorders include myotonic dystrophy (MyoD), Huntington's disease, dentatorubropallidoluysian atrophy (DRPLA), X-linked spinobulbar muscular atrophy, and fragile X syndromes. These trinucleotide repeat disorders have their own distinctive neurological features.

Myotonic dystrophy is the first disease to be recognised as showing anticipation—a phenomenon of

increasing disease severity and earlier age of onset in successive generations. The mutation underlying MyoD is an unstable expansion of the CTG repeat in the *DMPK* gene on chromosome 19,³⁶⁻³⁹ and anticipation is caused by transmission of an increased number of trinucleotide repeats. The age of onset and clinical features of MyoD correlate with the size of the trinucleotide expansion. The size of the expansion can also increase with time, which accounts for the mid-life onset in classical MyoD and its progressive course.⁴⁰ Allelic mosaicism is present within an individual, thus resulting in variable repeat sizes between different organs and even among the same type of somatic cells. Despite identification of the underlying genetic defect, the pathogenic mechanism of MyoD is still poorly understood.

Muscular dystrophies and the principles of gene therapy

In the 1950s, Walton and his colleagues classified muscular dystrophies into Duchenne muscular dystrophy (DMD), facioscapulohumeral dystrophy, and MyoD according to their clinical patterns. A fourth type, limb girdle muscular dystrophy (LGMD), was added to include all dystrophies other than the three already mentioned. By grouping different phenotypes into one entity, the Walton classification system did not account for the heterogeneity within LGMD. Subsequently, immunohistochemical techniques have enabled misclassified conditions, such as metabolic myopathies, mitochondrial myopathies, and various storage disorders, to be distinguished from LGMD. Specific deficiencies of membrane protein components, including dystrophin and sarcoglycan, were also discovered. Their identification enabled a histological classification of some of the dystrophies into dystrophinopathy and sarcoglycanopathy, the former including Duchenne and Becker dystrophies, and the latter some of the LGMDs.

Duchenne and Becker dystrophies are both X-linked diseases. Existing evidence suggests that they are the same disease entity at different ends of a continuous spectrum. Patients with DMD become wheelchair-dependent at about the age of 12 years, whereas those with Becker dystrophy are ambulant beyond the age of 15 years. There is an intermediate variant, denoted 'outliers', in which the ability to walk is retained after the age of 12 years but not beyond 15 years. In DMD, staining for dystrophin shows none or only a few positive fibres. In Becker dystrophy, the deficiency is partial or, more often, related to reduced amounts of smaller molecular weight proteins. Cloning of the

dystrophin gene in 1987 enabled a new understanding of the dystrophinopathies.⁴¹ A deletion of this gene of variable size, encoding for dystrophin, is demonstrated in most cases of DMD and Becker dystrophy. Out-of-frame mutations, which create a translational stop codon downstream from the distal breakpoint and thereby disrupting the open reading frame, account for the DMD phenotype. In contrast, the reading frame is maintained in Becker dystrophy so that a semi-functional, truncated protein can still be produced. The reading frame explanation accounts for the phenotypic variations seen in most cases.

A definite diagnosis of DMD can be based on the clinical picture, gross elevation of serum creatine kinase, and muscle biopsy findings. Dystrophin antibody for histological staining is commercially available. Since the dystrophin gene is very large (more than 2000 kb of genomic DNA), it is prone to spontaneous mutations. Approximately one third of DMD cases are sporadic. It is important to determine the carrier status of the mother and female siblings in isolated cases during genetic counselling. A genetic diagnosis of DMD carrier status can be made in cases of gene deletion or duplication. The mother of a patient with sporadic DMD will still have a 14% risk of having another affected child, despite a negative DNA analysis, however.⁴² This is because of germline mosaicism, in which the mother may harbour a mutation in the oocytes but not in the tested leukocytes. Thus, genetic tests cannot totally exclude carrier status in the mother of a patient with sporadic DMD, and female offspring should be investigated independently.

Genetic analysis has also refined the classification of LGMD disorders. The prevalence of LGMD is approximately 1 in 100 000. Most LGMD phenotypes demonstrate weakness in a limb-girdle distribution, with sparing of the facial, extra-ocular, and pharyngeal muscles. The onset is usually around puberty. A genotype-phenotype correlation can be difficult because the onset and progress are variable even with the same mutation. About 10% of LGMD disorders are autosomal dominant (type 1), with 1A linked to 5q,⁴³ 1B to 1q11-21,⁴⁴ and 1C to the *CAV3* gene at 3p25, which encodes caveolin-3.⁴⁵ For autosomal recessive LGMD (type 2) disorders, 2A is linked to the *CAPN3* gene at 15q15, encoding for calpain-3,⁴⁶⁻⁴⁸ 2B to the *FER-1* gene at 2p13 for dysferlin,^{49,50} 2C to the *SGCC* gene at 13q12 for γ -sarcoglycan,⁵¹⁻⁵³ 2D to the *SGCA* gene at 17q21 for α -sarcoglycan,⁵⁴⁻⁵⁶ 2E to the *SGCD* gene at 4q12 for β -sarcoglycan,⁵⁷⁻⁵⁹ 2F to *SGCD* at 5q33 for δ -sarcoglycan,^{60,61} 2G to 17q11-12,⁶² and 2H to 9q31-33.⁶³

It is now understood that histopathologically defined sarcoglycanopathy actually encompasses at least four disease entities. The sarcoglycan complex consists of five units— α -, β -, γ -, δ -, and ϵ -sarcoglycans, with muscular dystrophies being associated with the first four. The integrity of the entire sarcoglycan complex is interdependent on each of its components. If one of the sarcoglycans represents the primary deficit, the membrane staining for any of the others will also be affected. Genetic testing is required to differentiate between the sarcoglycanopathies, although it is of limited clinical relevance in the absence of specific therapies.

The pathogenesis of muscular dystrophies is still not entirely known. Dystrophin and sarcoglycan are components of the dystrophin-glycoprotein complex, which is a membrane-associated muscle protein that spans the muscle sarcolemma. The dystrophin-glycoprotein complex is thought to have a key role in maintaining the stability of the muscle membrane. It is generally believed that the disruption of the dystrophin-glycoprotein complex would lead to a cascade of events that results in muscle cell damage. Replacement of a normal variant of mutant protein, either directly or by molecular manipulation, is one of the strategies underlying gene therapy.

For structural protein deficiencies, direct replacement is unlikely to be successful. Trials of dystrophin replacement in DMD by cell transfer using donor myoblasts have so far been disappointing.⁶⁴⁻⁶⁶ Molecular manipulation includes somatic-cell gene replacement therapy (SGRT) and 'gene fixing'. Somatic-cell gene replacement therapy works by inserting a functional allele of the mutant gene into the cells of vulnerable tissues. Experiments on SGRT have been undertaken in animal models of DMD, and α , γ , and δ sarcoglycanopathies. The general approach is to transfer the dystrophin gene with a high capacity, non-pathogenic, non-immunogenic vector via an acceptable route of administration. Numerous technical obstacles, however, remain to be overcome. Manipulation of DNA to correct defects—so called 'gene fixing'—constitutes a much greater challenge than SGRT. In DMD, gene fixing could consist of transforming an out-of-frame into an in-frame mutation by introducing an exon-skipping RNA oligonucleotide so that a more stable protein can be created. This would transform the DMD phenotype into a more benign Becker-like phenotype.

Motor neuron disease

Five to ten percent of cases of motor neuron disease (MND) are familial. The mode of inheritance is

autosomal dominant. In 15% to 20% of familial cases, the disease is associated with mutations in the *SOD-1* gene on chromosome 21.⁶⁷ This gene encodes the copper- and zinc-dependent superoxide dismutase (SOD-1). Superoxide dismutase-1 converts the superoxide anion into hydrogen peroxide, which is then metabolised into water and oxygen. Thus, *SOD-1* plays an essential role in free-radical scavenging. Most of the mutations seen result in a variable loss of *SOD-1* activity, but such a loss of activity is unlikely to be the main mechanism underlying MND. The current thinking is that the mutated *SOD-1* acquires a new cytotoxic activity. Free radicals, including hydroxyl radicals and nitronium ions, are paradoxically generated. The reason for motor neuron selectivity remains unknown. It is postulated that oxidative stress associated with *SOD-1* mutations renders motor neurons more vulnerable to glutamate-induced excitotoxicity.

It is reasonable to assume that MND has multiple aetiologies, which include the interaction of one or more susceptible genes, undetermined environmental factors, and physiological cell ageing. The underlying genetic defect is unknown in at least 98% of cases.

Huntington's disease

The genetic defect in Huntington's disease (HD) was found to be an unstable CAG repeat in the open reading frame of the *HD* gene at 4p16.3,⁶⁸ encoding huntingtin, which is of unknown function. The result of this defect is the expression of an elongated protein that has polyglutamine residues. The exact pathogenic mechanism of HD is not fully understood. Various hypotheses have been proposed, but none has yet been proven in vivo. It is thought that the polyglutamine expansion would contribute to a 'gain of function'. Intranuclear inclusions of huntingtin and ubiquitin are found in affected neurons. One possible explanation is that the mutant huntingtin conjugates with ubiquitin and is transported into the nucleus through an aberrant proteasome complex. The resulting protein-protein aggregates may accelerate apoptosis. A similar mechanism might also account for the neuronal damage seen in other CAG repeat disorders, such as some of the SCAs and DRPLA.

Presymptomatic HD can be diagnosed by genetic analysis, but application of genetic testing remains controversial. The test identifies a life-threatening genetic defect that has no available remedy. As the implications for individuals testing positive are immense owing to the high penetrance of the genetic

defect, guidelines on presymptomatic screening should be strictly observed.⁶⁹

The adult-onset form of DRPLA, commonly with choreoathetosis and psychiatric manifestations, is often misdiagnosed as HD. Hence, all patients with an HD-like illness but without the appropriate mutation should be tested for DRPLA.

Alzheimer's disease

Although approximately 30% of patients with Alzheimer's disease (AD) have a positive family history, most of them probably represent sporadic cases, because it is a very common condition with a prevalence of almost 50% by the age of 85 years.⁷⁰ The genetic basis of early-onset AD differs from late-onset AD. Approximately 50% of cases of early-onset AD involve mutations of the presenilin 1 (*PS1*) gene at 14q24.3.⁷¹ Less common loci include that coding for the amyloid precursor protein (*APP*) gene at 21q21.2⁷² and the presenilin 2 (*PS2*) gene at 1q31-q42,⁷³ both of which occur in less than 1% of cases. In late-onset AD, approximately 30% of cases involve α_2 -macroglobulin gene mutations at chromosome 12,⁷⁴ and 40% are associated with the apolipoprotein E (*APOE*) $\epsilon 4$ allele at 19q13.2.⁷⁵ Only a small proportion of late-onset disease is associated with *PS1*, *PS2*, or *APP* mutations. It is believed that early-onset AD is a heterogeneous set of disorders caused by genetic mutations that are unique to different families. In contrast, late-onset AD is a sporadic disease influenced by multiple environmental and genetic factors.

APOE- $\epsilon 4$ homozygous individuals have an eight-fold increase in risk for developing AD, the onset being on average 10 to 20 years earlier. Heterozygous carriers have a three-fold increase in risk, and disease onset is 5 to 10 years earlier. Conversely, the *APOE- $\epsilon 2$* allele may be protective against AD.⁷⁶ β -Amyloid ($A\beta$) is an important component of senile plaques and is thought to play a central role in the pathogenesis of AD. The affinity for $A\beta$ is different for the three variants of apolipoprotein E, with $\epsilon 4$ having the highest affinity. The *APOE- $\epsilon 4$* allele is associated with increased deposition of fibrillar $A\beta$, which is shown to be neurotoxic in vitro and in vivo.⁷⁷ The precise physiological function of APPs and presenilins is not fully understood. The mutated proteins result in altered amyloidogenesis through an abnormal processing of APP. This results in increased synthesis and accumulation of $A\beta$ peptides. The proteases β - and γ -secretases may be important mediators of this process. α_2 -Macroglobulin is a serum protease inhibitor. Its role in AD is also unclear.

The neurofibrillary tangles in AD are composed predominantly of hyperphosphorylated tau. Mutations of the *tau* gene on chromosome 17q21-22 are found in frontotemporal dementia and parkinsonism.⁷⁸ No *tau* gene mutations, however, have been identified in AD.

The diagnosis of AD is based on clinical criteria after the exclusion of other conditions that can cause dementia.⁷⁹ *APOE- $\epsilon 4$* mutation is only a risk factor, rather than mandatory, for the development of AD. Genotypic determination has no role in the diagnosis of AD, and no specific treatment that targets the underlying disease process is yet available. Use of anticholinesterases, such as tacrine and donepezil, produces benefits in cognitive function but must be viewed as palliative therapy. Genetic findings currently seem to have little impact on patient management. Recent experiments, however, might open up new therapeutic options. Schenk et al⁸⁰ showed that immunisation with $A\beta_{42}$ in transgenic mice carrying the mutant *APP* gene could prevent AD-associated pathological changes, although a functional correlate was not demonstrated. Whether non-*APP*-related models can also benefit from this potential therapy is uncertain. Other potential therapies include the use of specific inhibitors of β - and γ -secretases, and $A\beta$ fibrillogenesis.

Parkinson's disease

Results from twin studies initially did not appear to support a genetic component to the aetiology of idiopathic Parkinson's disease (PD).^{81,82} Familial parkinsonian syndromes broadly resemble idiopathic PD, but unusual features are seen, such as early age of onset, rapid progression, prominent dementia, or atypical pathology in some pedigrees. Molecular studies of large families have been crucial in understanding the pathogenesis of parkinsonism. Golbe et al⁸³ have linked the Contursi kindred to 4q21-23. Subsequent studies have also linked these families to a rare mutation in the α -synuclein gene,⁸⁴ but most familial parkinsonian syndromes and sporadic PD do not have this mutation.⁸⁵ Homozygous deletion of exons in the *Parkin* gene have recently been detected in Japanese families with autosomal recessive juvenile parkinsonism,⁸⁶ an atypical parkinsonian syndrome characterised by early onset, marked response to levodopa, and susceptibility to levodopa-induced dyskinesia.

Paroxysmal disorders and channelopathies

Ion channels are membrane proteins that typically consist of four to six subunits that are specifically adapted for transmembrane ion flux regulation. The subunits

are assembled to form channels that have different degrees of permeability to various ions and different opening and closing kinetics. Disorders of ion channels commonly affect excitable tissues such as muscle and nerve, thereby resulting in their paroxysmal dysfunctions. The first channelopathy to be characterised genetically was hyperkalaemia periodic paralysis. Electrophysiological studies in this disorder show defective activation of the muscle membrane voltage-gated sodium channels. The other two overlapping conditions are paramyotonia congenita and potassium-aggravated myotonia. These are of autosomal dominant inheritance.

Hyperkalaemia periodic paralysis is characterised by episodes of muscle weakness, which are triggered by rest after a heavy workload, with myotonia between attacks. Paramyotonia congenita manifests as a stiffening of muscles during exercise or exposure to cold that can develop into flaccid weakness that last several hours. Potassium-aggravated myotonia is characterised by exercise-induced myotonia with delayed onset (approximately 20 minutes). It can be precipitated by ingestion of potassium-rich food. The paralysis can be relieved by administering thiazide diuretics or acetazolamide, and the myotonia by class I antiarrhythmic drugs. Genetic studies have shown that these diseases are linked to the *SCN4A* gene at 17q23,^{87,88} which codes for the α subunit of skeletal muscle sodium channels. In all these disorders, the underlying pathogenic mechanism is essentially the same—failure to maintain muscle membrane in the refractory state due to a leaky channel. Other muscle voltage-gated channelopathies include hypokalaemic periodic paralysis, malignant hyperthermia, central core disease, and myotonia congenita. The first three involve the calcium channels and the last affects the chloride channel.

Neuronal voltage-gated channelopathies are potential candidates for common paroxysmal disorders such as migraine and epilepsy. Some rare forms of these conditions are now established to be genetic channelopathies. Familial hemiplegic migraine (FHM) is associated with mutations of the *CACNA1A* gene at 19p13 which encodes for the α_{1A} -subunit of P/Q-type voltage-gated calcium channel. Familial hemiplegic migraine is characterised by migrainous headache, which is preceded by an aura of hemiplegia that can last for hours to days. *CACNA1A* gene mutations are also found in SCA6 and episodic ataxia type 2 (EA2). Episodic ataxia type 2 and FHM are overlapping syndromes, with basilar migraine occurring in half of the EA2 families and ataxia in 20% of FHM families.⁸⁹ A second locus for FHM has recently been mapped to

chromosome 1q at or near the R type voltage-gated channel α_{1E} subunit gene.^{90,91} Generalised epilepsy with febrile seizure plus presents with febrile seizures beyond the age of 6 years, and non-febrile generalised seizures until puberty. The *SCN1B* gene at 19q13.1, which encodes the voltage-gated sodium channel accessory β_1 -subunit, is involved. Its disruption may result in neuronal hyperexcitability—a mechanism similar to that of myotonia. Benign familial neonatal convulsions are associated with mutations in the *KCNQ2* gene at 20q13⁹² or the *KCNQ3* gene at 8q,⁹³ which encodes for voltage-sensitive neuronal potassium channels. Autosomal dominant nocturnal frontal lobe epilepsy is linked to 20q13.2 and the mutations affect the α_4 -subunit of the neuronal nicotinic acetylcholine receptor.^{94, 95} Although the above conditions account for no more than 1% of cases of migraine and epilepsy, identification of their underlying disease mechanisms suggests that genetic susceptibility might exist for the more common paroxysmal disorders and that ion channel dysfunction may have an important pathogenic role.

Conclusion

The development of molecular biology has undoubtedly altered our understanding of the nervous system and its disorders. Our knowledge of the pathophysiology of neurological diseases is still relatively scant, but increased insights in molecular biology will eventually translate into potential rational therapies for neurological disorders, many of which are currently incurable. The identification of molecular defects is the first step in this direction. To date, advances in this area have certainly helped in the reclassification of some neurological disorders. Previous classifications, especially of inherited neurological disorders, have been based on clinical features, conventional laboratory methods and radiology, and they have been confounded by existing phenotypic heterogeneity. In certain neurological disorders—for example, HD—advances have translated into clinically viable diagnostic tests, which are useful in genetic counselling. Until viable treatment options are developed for these disorders, however, difficult ethical issues with such techniques remain. Whether these developments will lead to meaningful alterations in clinical management currently remains unclear.

Apart from hereditary disorders, molecular biology has undoubtedly assisted in exploring the pathogenic mechanisms of many sporadic neurological disorders including AD, PD, and MND. Although the development of molecular techniques is rapidly increasing, it

is by no means a panacea to the understanding and eventual rational therapy of neurological disorders. Development in other technologies such as cellular biology and bioinformatics will certainly play an integral role towards reaching this goal.

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