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The aetiology of idiopathic Parkinson's disease

D B Ramsden, R B Parsons, S L Ho, R H Waring

Abstract

Agents potentially involved in the aetiology of idiopathic Parkinson's disease are discussed. These include factors regulating dopaminergic neurogenesis (Nurr 1, Ptx-3, and Lmx1b) and related proteins, together with genes involved in familial Parkinson's disease (α synuclein, parkin, and ubiquitin carboxy terminal hydroxylase L1), and endogenous and environmental agents.

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Idiopathic Parkinson's disease appears not to be a single entity but rather a spectrum of conditions resulting from the death of the pigmented dopaminergic neurones of the substantia nigra, pars compacta, which ultimately leads to the one single, fatal endpoint. As such, this spectrum is unlikely to have a single cause. Despite an intense research effort over many years, these causes still await elucidation. Various factors contribute to the difficulties in the search. Some obvious ones are the long period between the initiation of the disease process and the manifestation of clinical symptoms, the lack of any distinctive blood biochemistry with which to trace the disease process, and the inadequacy of current animal models. Quite when idiopathic Parkinson's disease begins is uncertain. Based on the premise that clinical symptoms appear when approximately 50% of pigmented dopaminergic neurones are dead and the surviving ones can supply the striatum with only about 20–30% of its dopamine demand, mathematical models of neuronal death rates suggest that there may be as small a gap as three years or as large a one as 20. However, beyond the strictly mathematical, clinical case histories of twins suggest the presence of a "parkinsonian" personality that, in hindsight, was present in very early life. Whether such a personality exists and, if so, whether it implies the presence of the disease process so early in life remain obscure. Nevertheless, the concept that early life events might be of crucial importance has been moved from the realms of fancy with the recognition of the role of specific transcription factors in brain development, particularly the proteins Nurr 1 (and its related subfamily members), Lmx1b, and Ptx-3. These are transcription factors that are involved in determining neurogenesis in the basal ganglia. It has been hypothesised that immature neurones die by apoptosis because of neurotrophic factor deprivation, as a result of failing to make adequate contacts in their target sites, whereas mature neurones die because of

toxic insult. However, the quality of contact and/or degree of neurotrophic support in early life may be of importance in determining the length of survival. In addition, these proteins are expressed throughout life in the basal ganglia, which suggests that they have roles in maintaining the continuing health of specific neurones and makes them worthy of consideration in terms of the aetiology of idiopathic Parkinson's disease. These factors often have multiple names, which sometimes relate to the different species that research groups were investigating, or simply to different phenomena that were being considered at the time. For the sake of simplicity only one name will be used here in the main body of the text, but alternative names are listed.

Factors involved in dopaminergic neurogenesis

NUR RELATED FACTOR 1 (NURR 1) (ALSO KNOWN AS NOT, RNR-1, AND HZF-3)

Nurr 1 is a member of the superfamily of "zinc finger" transcription factors.[1] Over 90 members of the superfamily have been described. Included among these are the hormone receptors for triiodothyronine, oestradiol, cortisol, aldosterone, vitamin D, all trans retinoic acid, and 9-cis retinoic acid. Increasingly, molecules that at one time were thought of as unlikely candidate ligands for these transcription factors are now being recognised as such. In the past few years, it has been established that bile acids modulate gene transcription via farnesyl receptors.[2] Nevertheless, many members of the superfamily have no known ligands—so called "orphan" receptors (reviewed by Enmark and Gustafsson[3])—but despite this, the functions of these receptors are becoming recognised. Most recently, cholesterol entry and egress from cells has been shown to be regulated via such orphans.[4 5] Also among this class of receptors is Nurr 1.

Nurr 1 was first recognised as a transcription factor that was primarily expressed in the brain[6] and the regenerating liver[7] (some details of the gene, cDNA, and protein are given in table 1, together with those for Ptx-3 and Lmx1b). Its close structural relation to Nur 77 led to its identification in stimulated T cells, where it functions as an intermediate early response gene, with stimulation causing a rapid increase in mRNA without the need for de novo protein synthesis.[1] Inhibition of Nurr 1 binding to its response element led to inefficient clonal deletion of self reactive T cells.[34] It also functions as an intermediate early gene in the central nervous system (CNS), being rapidly induced in response to focal injury,[35] but an appreciation of its crucial importance in brain development came later, after it was

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Table 1 Transcription factors involved in dopaminergic neurogenesis

Nurr 1
- Member of "zinc finger" superfamily of receptors; its subfamily includes nerve growth factor inducible B (NGFI-B) and neurone derived orphan receptor 1 (NOR-1)[8]
- Human gene on 2q22–23; eight exons, spanning 8 kb. Murine, rat, and human genes have similar structures[9–12]
- Full length mRNA has 3427 bases, 1794 are translated; the protein has 598 amino acids (66 kDa)[1 6]; splice variants exist—for example, Nurr 2—a novel cryptic exon located upstream in the Nurr 1 promoter region—and alternative splicing at exons 1, 2, and 6[13]
- Potential regulatory region contains consensus binding sites for nuclear factor κB (NF-κB), cAMP response element binding protein (CREB), and Sp1[9 11 14]
- In the central nervous system (CNS) Nurr 1 expression occurs in the postmitotic late differential phase of dopaminergic precursor neuronal development,[15 16] detectable from rat embryonic day 10.5[17]
- Nurr 1 response elements: a single half site (AAAGGTCA) binds Nurr 1 as monomer; this is the same sequence as the NGFI-B response element[7 8]; a direct repeat (DR5) (AGGTCANNNAAAGGTCA) binds Nurr 1/RXR as a heterodimer, Nurr 1 homodimers, and the Nurr 1/NGFI-B heterodimer[18–20]
- Nurr 1 response elements are found in tyrosine hydroxylase (AAAGGTCA),[21] the dopamine transporter,[22] and other molecules such as proopiomelanocortin[23]

Ptx-3
- Structurally related to pentaxins—for example, C reactive protein and serum amyloid P; subfamily includes Ptx-1 and Ptx-2, and homeobox proteins such as Otx-1 and Otx-2[24 25]
- Human gene on chromosome 10.q25. has three exons[26]
- cDNA has 1861 bp; protein has 381 amino acids[25]; Ptx-3 is also a secreted, acute phase protein
- mRNA induced in endothelial, hepatic, and fibroblastic cells by interleukin 1β (IL-1β) and tumour necrosis factor α, but not by IL-6 and interferon γ; raised serum concentrations after bacterial lipopolysaccharide injection[25 27 28]
- Expression is restricted in the CNS[29]
- The Ptx-3 response element is GGCTTT
- There is a Ptx-3 response element in the tyrosine hydroxylase gene[30]
- Mutations are associated with autosomal dominant cataracts[26]

Lmx1b
- This transcription factor is related to members of the LIM family of homeobox proteins[31]
- The human gene is on 9q34 and has eight exons[32]
- Genetic mutations are associated with nail-patella syndrome[33]

shown to be expressed strongly in the midbrain region.[16 36] In mice in which the expression of Nurr 1 is prevented (−/− knockout), there is a failure of development of midbrain dopaminergic neurones, with a 98% reduction in striatal dopamine and a 30% reduction in noradrenaline.[37 38] In the olfactory bulb, another area that is important in idiopathic Parkinson's disease (70% of patients with idiopathic Parkinson's disease have olfactory bulb dysfunction, and anosia may precede other signs of the disease), Nurr 1 (−/−) mice have a 60% reduction in dopamine,[37] although claims concerning the details of Nurr 1 expression in the olfactory bulb are conflicting.[39 40] The importance of Nurr 1 for dopaminergic neurones can be seen in relation to tyrosine hydroxylase expression, as shown by the degree of coexpression of the two proteins in the adult mouse brain, namely: substantia nigra (96%), ventral tegmental area (95%), retrorubral field (91%), olfactory bulb (85%), linear nucleus raphe (91%), central grey (61%), paraventricular and periventricular hypothalamic nucleus (few), and arcuate nucleus and zona incerta (0%).[41] In the absence of the growth of the nigral dopaminergic neurones, the animals fail to thrive and die shortly after birth. In contrast, heterozygous animals (+/− Nurr 1 mice) are apparently healthy but have reduced midbrain dopamine values.[37] In humans, a similar role for Nurr 1 in dopaminergic neurogenesis is assumed.

Nurr 1 is not only expressed throughout life in midbrain neurones, but also in other regions unconnected with idiopathic Parkinson's disease,[42] suggesting that it plays an important role in maintaining the continuing health of all these cells. Exactly what the role of Nurr 1 is in

the development of idiopathic Parkinson's disease remains uncertain. Treatment of Nurr 1 deficient (−/+) animals with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) showed that they were more susceptible to the effects of the neurotoxin than normal (+/+) mice.[43] The identification of such a factor, which acts from early fetal life onwards, raises the possibility that differences in the amount of expression and/or the timing of the onset of expression in the fetal period may be important in predisposing these cells to an earlier that normal death and, thence, the development of idiopathic Parkinson's disease. Other intriguing possibilities that surround Nurr 1 centre on its potential ligand binding ability and its immune responsiveness. As mentioned earlier, Nurr 1 is classed as an orphan receptor. It may act as a transcription factor without an endogenous natural ligand,[44] either binding as a monomer to its response element, or as a dimer.[45] However, this does not of necessity rule out the possibility that it can bind ligands, and binding of any such ligand may alter its function beneficially or deleteriously for the health of the cell. Until developments in molecular biology led to a re-evaluation of the evidence in the past decade, idiopathic Parkinson's disease was thought to result exclusively from non-genetic causes. However, it is now generally accepted that genetics play a part. Nevertheless, as will be reviewed later, environmental causes are still thought to be of great importance. Any ability of Nurr 1 either to bind endogenous or exogenous ligands or to change in concentration in response to exposure to viruses or other noxious stimuli offers a portal to see how the health of these cells might be uniquely adversely affected by environmental factors. Furthermore, not only does Nurr 1 offer the possibility of theoretical insights, but also that of practical exploration of dopaminergic neuronal cell biology and replacement tissue for implantation. In the past it was possible to culture neuronal cells, but these cultures were highly heterogeneous mixtures of cells, making culture to culture comparisons extremely difficult. However, a culture system has been described that overcomes this problem. This involves three steps, namely: (1) the transfection of neuronal stem cells from mouse cerebellum with a Nurr 1 expression plasmid so that they overexpress Nurr 1; (2) the propagation of the cells in the presence of basic fibroblast growth factor; and (3) co-culture of the cells in the presence of type 1 astrocytes to supply other growth factors. The protocol leads to the development of a dopaminergic phenotype in approximately 80% of the resultant neurones.[46] Such cultures should provide a model in vitro system to enable a detailed exploration of the physiology of these unique cells. Further advances in cell culture techniques, such as that described by Kawasaki *et al*, which allow a slightly simpler method of inducing a dopaminergic phenotype from a modified embryonic stem cell line, will also greatly aid this exploration.[47] However, the close structural relation of Nurr 1 to other

members of its subfamily suggests that it should not be considered in isolation.

NERVE GROWTH FACTOR INDUCIBLE B (NGFI–B) (ALSO KNOWN AS NUR 77/TR3/NAK1/N10/ST59/TIS) AND NEURONE DERIVED ORPHAN RECEPTOR 1 (NOR–1) (ALSO KNOWN AS MINOR/TEC/CHN/NOR2)

Nurr 1, NGFI-B, and NOR-1 form a subfamily within the "zinc finger" nuclear receptor superfamily. Of the three, NGFI-B was the first to be characterised. Like Nurr-1, NGFI-B and NOR-1 play important roles in brain development, and both are expressed in tissues outside the CNS. The regional expression of NGBI-B and NOR-1 within the CNS is different from that of Nurr 1, with that of NGFI-B being wider, but the three do overlap, suggesting selective roles for these transcription factors in the regulation of motor function.[48 49] Table 2 summarises the expression of the three subfamily members in the CNS. Of possible relevance to idiopathic Parkinson's disease are the facts that: (1) both NGFI-B and NOR-1 are expressed in the caudate/putamen, the target site for dopaminergic neurones from the substantia nigra, but are not expressed in the substantia nigra itself; and (2) NGFI-B, NOR-1, and Nurr 1 are involved in the regulation of dopamineric neurone formation in the olfactory bulb, where NGFI-B is strongly expressed in the glomerular and granule cell layers.[51]

Human NGFI-B cDNA is 2498 bp in length, with an open reading frame of 1794 base pairs, which encodes a protein of 598 amino acids and a predicted molecular mass of 64 kDa.[52 53] The NGFI-B structural gene is encoded on human chromosome 12q13.1,[54] and the gene for NOR-1 is located on human chromosome 9q22, spans some 35 kb, and has eight exons.[55] The NOR-1 gene gives rise to two transcripts, which when translated result in a protein containing 626 amino acids, with an approximate molecular weight of 68 kDa. All three receptors bind to a common response element—AAAAGGTCA—as monomers.[56] Nurr 1 and NGFI-B bind to AGGTCA repeats as heterodimers with RXR isoforms,[17] as homodimers,[57] and as a heterodimer with each other.[58] NOR-1 is unusual in that it does not heterodimerise with RXR. *x* Ray crystallography has shown that, when NGFI-B binds as a monomer, the DNA binding domain of the receptor not only interacts with the core section of the response element (AGGTCA) in the major groove, but also with the AAA extension via the minor groove.[59]

Like Nurr 1, NGFI-B and NOR-1 are intermediate early genes. NGFI-B mRNA in brain was induced rapidly and transiently by growth stimulating agents,[53 60] and in hippocampal neurones in response to NMDA and muscarinic receptor stimulation.[61] Both NGFI-B and NOR-1 are expressed in the fetal brain and are important signals in mitogenesis[62] and apoptosis pathways in tissues outside the CNS.[63 64]

The role of these receptors in apoptotic signalling is complex. Both NGFI-B and NOR-1 generally are regarded as proapoptotic factors in tissue outside the CNS.[65 66] This appears to be true for NGFI-B in the adult human CNS too, with high amounts of the receptor being detectable in the brains of patients with Alzheimer's disease.[67]. However, as indicated above, both NGFI-B and NOR-1 are constitutively expressed in some regions of the brain in adult life where high rates of apoptotic neural death are not occurring, so obviously they have other functions, and in a model system where overexpression of NGFI-B was induced, it inhibited ceramide induced apoptosis but not the Fas–Fas ligand pathway.[68] Despite these somewhat contradictory strands of evidence, an understanding of the role of NGFI-B in apoptosis and gene transcription is beginning to emerge. An essential step in the initiation of apoptosis is the release of cytochrome c from mitochondria, which then activates the caspase cascade.[69] After exposure of the prostate cancer cell line LNCaP to 6-[3-(1-adamantyl)-4-hydroxyphenyl]-3-chloro-2-naphthalene carboxylic acid and other proapoptotic agents, human NGFI-B was induced and the protein was shown to move from the nucleus to the mitochondrion to trigger cytochrome c release. Therefore, in its role in apoptosis, human NGFI-B is not required to initiate gene transcription. Signals directing movement out of the nucleus were contained in both the N-terminus and C-terminus of the molecule. Apoptosis was inhibited by antisense human NGFI-B mRNA. In contrast, epidermal growth factor (EGF)—a non-apoptotic stimulus—also induced human NGFI-B mRNA, but the protein produced stayed within the nucleus and was capable of initiating gene transcription.[70] Therefore, it seems that the contrasting actions of human NGFI-B are modulated by its intracellular localisation, which in turn is dependent upon the nature of the signal to which the cell is exposed.

Export of human NGFI-B from the nucleus is also a means of modulating gene transcription via interaction with the retinoid signalling system. After nerve growth factor induced phosphorylation of NGFI-B serine residue 105, the NGFI-B–RXR complex leaves the nucleus, reducing the availability of RXR for heterodimerisation with RAR, which thus reduces the transcriptional activity of the RAR–RAR complex.[71] Export of the RXR–

Table 2   Regional expression of Nurr 1, NGFI–B, and NOR–1 mRNA in rat and mouse brain[12 18 48 50]

Area of brain	Nurr 1		NGFI–B		NOR–1	
	A	B	A	B	A	B
Neocortex		++		++		+
Caudate putamen		–		++		++
Hippocampus		++		++		++
Olfactory bulb	+		++		++	
Ventral tegmental area		++		–		–
Substantia nigra		++		–		–
Subiculum		++		++		++
Locus coeruleus		–		–		–
Cerebellum	++		++		++	
Spinal cord		–		–		–

A, early, neonatal life; B, later life.
++, strong expression; +, moderate expression; –, weak or no expression.

NGFI-B complex from the nucleus has also been suggested as the mechanism whereby retinoids inhibit the activation induced apoptosis of immature thymocytes. It was suggested that this occurred because of the reduced ability of NGFI-B to initiate gene transcription,[72] but it may be the result of the inability of the RXR–NGFI-B heterodimer to interact with the mitochondrion, in contrast to NGFI-B alone.

The recent work on the role of NGFI-B in apoptosis has been carried out largely in non-CNS cells, and whether the same actions and mechanisms apply in the CNS remains to be seen. However, NGFI-B is inducible in the dopaminergic target areas in the striatum in response to burst stimulation of the medial forebrain bundle,[73] and both NGFI-B and NOR-1 are inducible in response to therapeutic drugs (halopyridol)[74] and substances of abuse (cocaine and morphine).[75] The induction of NGFI-B and NOR-1 was suggested as a possible mechanism whereby retinoid signalling was disturbed, so that it might be of relevance in schizophrenia.[74] In contrast, compulsive running was shown to downregulate striatal NGFI-B and NOR-1 expression.[76] Thus, these two transcription factors appear to be involved in dopaminergic related personality characteristics. However it is still unclear whether Nurr 1 has similar properties to NGFI-B, and whether and how the expression of NGFI-B and NOR-1 changes and, if it does, how such changes coincide with any alterations in the expression of Nurr 1 in idiopathic Parkinson's disease. Because of the complex way that all three principle members of the NGFI-B family act as individuals and interact with each other and other liganded nuclear receptors, such as the retinoid and glucocorticoid receptors,[77] [78] these proteins have the capacity to modulate the transcription of many genes, only a few of which have been recognised, and to act as both survival and proapoptotic factors, not necessarily by modulating events in the cell nucleus. This complexity is increased by the fact that further proteins are formed from the same genes by either exon splicing or the use of different transcription start sites and promoters.[79] These also have transactivator properties; the Nurr 1 related protein (TINUR) derived by differential splicing is induced in T cells undergoing apoptosis.[80]

The elucidation of the actions of all three major proteins should give insights into dopaminergic neuronal cell survival and death in the substantia nigra, either because the function of Nurr 1 in these cells has changed or because the functions of NGFI-B or NOR-1 in neurones in target sites have changed, causing a lack of support for the incoming dopaminergic neurones. In turn, such knowledge may give insights into the biochemical basis of some of the behavioural and personality features of the disease, in addition to the cause of the neuronal death.

However, zinc finger nuclear receptors are not the only transcription factors that are of interest in dopaminergic neurone development and survival. Two others which are members of different families are being increasing recognised as important. These are Lmx1b and Ptx-3. Both are homeobox proteins.

LMX1B AND PENTAXIN 3 (PTX-3)

Although Nurr 1 expression is essential for the final differentiation of stem cells into dopaminergic neurones, it cannot initiate and complete this process by itself.[81] Neuronal development and differentiation occur as a consequence of the actions of successive waves of transcription factors. In the early phases of the transformation of stem cells sonic hedgehog and fibroblast growth factor 8 (FGF-8) appear to be principal players.[82] [83] Another transcription factor that acts at this early stage is Lmx1b. The expression of this factor is stimulated by FGF-8 and, as part of this cascade, Lmx1b in turn stimulates another transcription factor, wnt1.[84]

Lmx1b is a member of the LIM homeodomain protein family and was first recognised because of its role in dorsal–ventral limb patterning. It is expressed in numerous tissues where it affects skeletal, cranial, renal, and eye structures,[85–87] and the trajectory of motor neurones in limbs.[88] Subsequent studies have shown that loss of function mutations in the human gene are responsible for the nail-patella syndrome.[89–94] Its role in dopaminergic neuronal development is only just beginning to be elucidated. It is expressed in the neural tube at an early stage in the genesis of mesencephalic dopaminergic neurones in response to FGF-8 stimulation, where it maintains the expression of another transcription factor Wnt1,[84] and in the normal adult midbrain.[95] Lmx1b knockout mice fail to develop the full repertoire of dopaminergic neurones and their gene products. Although Nurr 1 and tyrosine hydroxylase are still expressed, there is a failure of expression of Ptx-3.

Later and at almost the same time as Nurr 1 is expressed, another transcription factor—Ptx-3—appears (embryonic day 11.5 in the rat).[16] In the CNS, this factor is found only in mesencephalic dopaminergic neurones, unlike Nurr 1 and the other factors mentioned above, but its expression does not by itself lead to the formation of the final dopaminergic phenotype. In Nurr 1 deficient (−/−) mice, Ptx-3 is expressed normally in the progenitor cells but these do not survive.[37] However, the importance of Ptx-3 for these neurones can be seen by the fact that a Ptx-3 response element (GGCTTT) is present in the 5' flanking regions of the human, rat, and mouse tyrosine hydroxylase genes, and binding of the transcription factor to this element results in pronounced upregulation of transcription.[30] Ptx-3 expressing neurones are reduced in number in parkinsonian substantia nigra and in that of the 6-hydroxydopamine lesioned rat. However, whether the neuronal loss in these situations is related to Ptx-3 function, or the reduction in Ptx-3 is simply a consequence of the death of these neurones, is uncertain. Nevertheless, it is principally the Ptx-3 expressing neurones that die.[94]

Genetic and environmental aetiological factors

The above discussion has concentrated on transcription factors that are involved in dopaminergic neurogenesis and have functions, as yet ill defined, in the adult CNS. Whether they have a role in the aetiology of idiopathic Parkinson's disease is very uncertain, but they clearly have the potential to be involved in this. By definition, the causes of idiopathic Parkinson's disease are as yet unknown. Over the past two decades, scientific opinion has varied between two extreme poles: from there being only environmental causes, to the position where genetic factors are considered to be the dominant aetiological feature. One major stimulus to this refocusing of emphasis on to genetics has stemmed from the ability to visualise dopaminergic neurones in vivo by positron emission tomography (PET) scanning. Early twin studies, before PET scanning was available, showed no significant difference between the incidence of idiopathic Parkinson's disease in the second twin when monogyzotic and dizygotic pairs were compared. On PET scanning of small groups, second monozygotic twins were found to have a greater dopaminergic deficit than dizygotic partners, suggesting than genetic parameters are important.[96] A second stimulus is the early finding that continues to be re-emphasised with studies of increasing sophistication, in terms of the elimination of potential confounding factors, is that a considerable proportion of patients with apparently sporadic idiopathic Parkinson's disease have a family history of the disease.[97–99] In addition, advances in molecular biological techniques, such as whole genome scanning, reviewed and illustrated in an earlier edition of this journal, have made the exploration of genetic factors possible.[100] This has been particularly important in elucidating the genes involved in familial Parkinson's disease.

In contrast to the twin study quoted above, other recent major studies not based on PET scanning have failed to detect a significant difference between the incidence of idiopathic Parkinson's disease in monozygotic compared with dizygotic twins, leading the authors to the conclusion that genetic factors are of minor importance in the aetiology of the disease.[101 102] The emphasis on causal environmental agents received a boost with the recognition of the selective toxicity of rotenone in mice.[103] This is quite widely used as a "safe" insecticide. The compound is also a potent inhibitor of mitochondrial complex 1 and thus shares a common mode of action with 1-methyl-4-phenylpyridinium ion (MPP^+) formed from in vivo oxidation of the protoxin, MPTP. The recognition that a new class of rotenone-like compounds might exist in the environment helps to counter one objection to the concept that environmental toxins might be involved in the aetiology of idiopathic Parkinson's disease, simply that MPTP is not encountered in nature. Although this is the case for MPTP, MPP^+-like compounds may be, and this possibility is discussed in a later section.

Whether either of these opposing opinions or a combination of the two is correct remains to be seen, but because most effort has been directed to elucidating the genetic basis of familial Parkinson's disease. Consequently, these developments will be reviewed first.

FAMILIAL GENETIC LOCI

To date, the genetic loci that have been found to be associated with Parkinson's disease with fairly typical symptoms are:

- A locus on chromosome 4q, which encodes the protein α synuclein. The mutations involved are A30P and A53T.[104 105]
- A locus on chromosome 6q, which encodes the protein parkin. Multiple mutations are involved.[106–108]
- A locus on chromosome 4p, which encodes the protein ubiquitin C-terminal hydrolase (UCTH). The mutation involved is I93M.[109]
- A locus on chromosome 4p, which encodes an unknown protein.[110]
- A locus on chromosome 2p13, which encodes two unknown proteins,[111 112] and cytochrome b.[113]

In addition, there are other loci associated with atypical symptoms. For example, a locus on chromosome 17q21–22, which encodes the protein tau and which is associated with frontotemporal dementia,[114–116] and a locus on chromosome 19q13, which is associated with rapid onset dystonia Parkinson's disease.[117] There are other loci that are associated with other modes of inheritance than the autosomal dominant transmission described in the original family with the α synuclein mutation. These include:

- Autosomal recessive—several mutations in the parkin gene.[106–108 118]
- Autosomal dominant inheritance because of parkin gene mutation.[119]
- Maternal transmission—mutations in mitochondrial DNA.[120]

Other loci are associated with both unusual features and mode of transmission—for example, a 12S ribosomal RNA gene point mutation causing disrupted protein synthesis resulting in parkinsonism, deafness, and neuropathy.[121]

Despite the increasing number of kindred being described, familial parkinsonism accounts for a relatively small proportion of the total patient population; therefore, the reason for the interest in these cases, apart from that of knowing the details of the causal mutation in each, is that they may shed light on the aetiologies of sporadic cases. This is proving to be the case to a surprising extent, although the mutations in the familial cases are not found in sporadic idiopathic Parkinson's disease.[122–125] (In fact, it has been suggested that another relatively common mutation in the UCTH gene, S18Y, is protective.[122]) What has been highlighted from this work is the importance of two inter-related pathways: α synuclein fibril formation and ubiquitin targeted protein catabolism.

α Synuclein is a member of a small family of proteins (other members being β synuclein and persyn). Under normal circumstances, it is strongly expressed in neurones in a limited

number of areas of the brain, including the dopaminergic neurones of the substantia nigra (a feature in common with parkin and in contrast to ubiquitin carboxy terminal hydrolase[126]), and comprises about 2% of total brain protein.[127] Its physiological role is ill defined, but it is present in nerve terminals. Its importance was recognised because of the fact that, together with proteasome and synphilin-1, and in whole and partially digested forms, it is a major component of Lewy bodies, which are a cardinal feature of the pathology of the parkinsonian brain. Of added importance are the facts that the proteins composing Lewy bodies are heavily ubiquitinylated and they are resistant to proteolysis.[128–132] The mutations discovered in the initial parkinsonian families were thought either to render α synuclein more resistant to proteolysis or to have a greater tendency to form fibrils, and in these ways accelerate Lewy body formation, thereby effectively clogging up the cytosol of the neurone and killing it.[133]

In support of this relatively simple scenario was the discovery of the mutation in the ubiquitin C-terminal hydroxylase gene. The ubiquitin targeted pathway of intracellular protein catabolism utilises initially three classes of proteins, corresponding to the three steps in the first phase of the pathway—ubiquitin activation, ubiquitin conjugation, and target protein ligation—to attach the polyubiquitin tail to the protein to be destroyed. Ubiquitin C-terminal hydroxylases in the final step release ubiquitin from this polyubiquitin tail for re-use after digestion of the target protein by the 26S proteasome complex.[134] An inability to release ubiquitin from the polyubiquitin tail, as a result of deficient ubiquitin C-terminal hydrolase activity, with the resultant absent or incomplete digestion of the target protein, could allow the accumulation of the building blocks for neurotoxic fibrils.

The first difficulty with this simple scenario in which toxicity occurs because of cytosolic blockage is the Japanese families with parkin gene defects. These defects give rise to an early onset form of Parkinson's disease. In the few individuals who underwent necropsy no Lewy bodies were found. While the function of the parkin gene product was unknown, this difficulty could be explained away, but it is now clear that the gene gives rise to a ubiquitin ligase.[135] These enzymes are of crucial importance for attaching the initial ubiquitin to the target protein and then extending the ubiquitin chain. Lack of Lewy bodies in individuals with gross genetic mutation that inactivate the enzyme presumably arises from the fact that α synuclein and other proteins are not ubiquitinylated, so they do not progress to the point where partially digested material is available for fibril formation. Hence, simple cytosol blockage by insoluble proteins is not the only cause of cell death, which, therefore, may be caused by the inability to clear soluble proteins in these cases.

Second, in most sporadic cases investigated no mutation in the α synuclein gene has been found and it is not clear from in vitro experiments that the variant α synucleins have a significantly greater rate of fibril formation when compared with that of the wild-type, which nevertheless does form fibrils.[136] These facts pose questions of how Lewy body formation occurs in sporadic Parkinson's disease and how this is related to cell death. Factors that may be more relevant than the rate of fibril formation are interactions with other proteins, such as synphilin-1[137]; the rates of formation of soluble oligomers of α synuclein, a step that precedes fibril formation,[138 139] which affects mitochondrial function[140]; and the nucleation of fibril formation.[141] In the last instance, the role of iron as the instigator of fibril nucleation has been suggested to be important.[142] In health, the pigmented dopaminergic neurones normally have relatively high concentrations of both iron and copper, and in the clinical phase of the disease increased amounts of low molecular weight iron and copper compounds are released in the cerebrospinal fluid.[143] Thus, the iron released from one dead or dying cell may be available to propagate fibril nucleation in other cells.

Third, it is worth repeating that none of the genetic abnormalities seen in the familial cases is seen in most of the patients. Therefore, if the inability to catabolise α synuclein and other proteins is not only the basis of Lewy body formation but the ultimate reason why the neurone dies, a defect must exist that precedes and precipitates this. Such a conclusion is supported by the existence of the well characterised familial cases that result from mitochondrial DNA mutations where genomic DNA mutations can be discounted.[120] This leads back to themes that are familiar to those in the immediate field: oxidative stress, mitochondrial complex 1 underactivity and energy production, excitotoxicity, and dopamine metabolism. In addition, as pointed out by Goldberg and Lansbury, one consequence of events before fibril formation occurring being of prime importance may be that a therapeutic approach directed solely at inhibiting the polymerisation of protein into fibrils might not be beneficial.[138]

ENDOGENOUS GENERATION OF SELECTIVE NEUROTOXINS

The evidence that the substantia nigra in idiopathic Parkinson's disease experiences free radical mediated oxidative stress has been reviewed exhaustively on numerous occasions,[144] and the abnormally low mitochondrial complex 1 activity well documented previously,[145] so neither will be discussed in detail here. That overexcitation of receptors such as the α1B-adrenergic receptor might lead to neurodegenerative disease also continues to receive support, as in the recent findings in multiple system atrophy.[146] However, in idiopathic Parkinson's disease the things that are not clear are: (1) whether oxidative stress precedes complex 1 inhibition or vice versa, and (2) how excitotoxicity relates to the two. The MPTP model of idiopathic Parkinson's disease would suggest that underactivity of complex 1 leads to oxidative stress. This

reduced activity could occur because of the presence of some inhibitory neurotoxin, or as a result of the accumulation of random replication errors in mitochondrial DNA. In terms of neurotoxins, two classes of compounds have been investigated extensively: cysteine-dopamine reaction products and MPP[+]-like molecules.

The salient feature of MPP[+] is the aromatic quaternary *N*-methyl, which allows it to bind to complex 1 but, because such a charged molecule would not pass the blood–brain barrier, intra CNS routes of synthesis are required, as in the case of the conversion of MPTP to MPP[+] by monoamine oxidase B. One such route is *N*-methylation, with simple pyridines, β carbolines, and tetrahydroisoquinolines as substrates. Members of these classes of compounds are present in the food chain, the general environment, and in the human brain and cerebrospinal fluid.[147] Enzymes that can convert them into their *N*-methylpyridinium analogues are also present in the mammalian brain.[148] In an apparently analogous fashion to MPP[+], the *N*-methyl derivatives are selectively neurotoxic, interact with the dopamine uptake system, and inhibit mitochondrial respiration. In the case of β carbolines, the molecule can be *N*-methylated at two sites, and the kinetics of the two reactions are different. However, the enzyme(s) responsible has not been isolated and cloned, so it is not clear whether the two activities observed represent different actions of the same enzyme or two entirely different ones. Following on from earlier work by Matsubara *et al*,[147] who showed that β carboline concentrations in the cerebrospinal fluid of patients with Parkinson's disease were higher than those of control subjects, Gearhart *et al*

have shown increased β carboline 9*N*-methyltransferase activity in the frontal cortex of parkinsonian brain compared with that of non-parkinsonian brain.[149] Thus, there appears to be both higher amounts of substrate and one form of enzyme activity in the disease population.

Our own work in this area has concentrated on nicotinamide-*N*-methyltransferase (NNMT), which, apart from catabolising the amide form of vitamin B3, has a wide substrate specificity that enables it to generate a variety of potentially toxic *N*-methylpyridinium ions. Its expression is dominated by an as yet undetermined, non-coding, genetic polymorphism, which results in a quarter of the general population having high hepatic enzymic protein and activity values.[150] Because nicotinamide is an integral part of NADH, which is the electron and hydrogen donor for complex 1, catabolism of nicotinamide could conceivably have an effect on intracellular NADH values. Thus, NNMT links, albeit tenuously, the ability to produce *N*-methylpyridinium ions, the metabolism of NADH, complex 1 activity, and the genetics of 25% of the general population. In exploring the hypothesis that NNMT may be an aetiological factor in idiopathic Parkinson's disease, some of our initial findings are that the enzyme is expressed in neurones and in higher amounts in two regions of the brain (caudate nucleus and cerebellum) in Parkinson's disease compared with control brain (cerebellum, fig 1). The enzyme is expressed in the substantia nigra,[151] but the destruction of so many neurones that has occurred in this area by the time subjects reach necropsy makes comparisons between parkinsonian and non-parkinsonian material difficult to interpret.
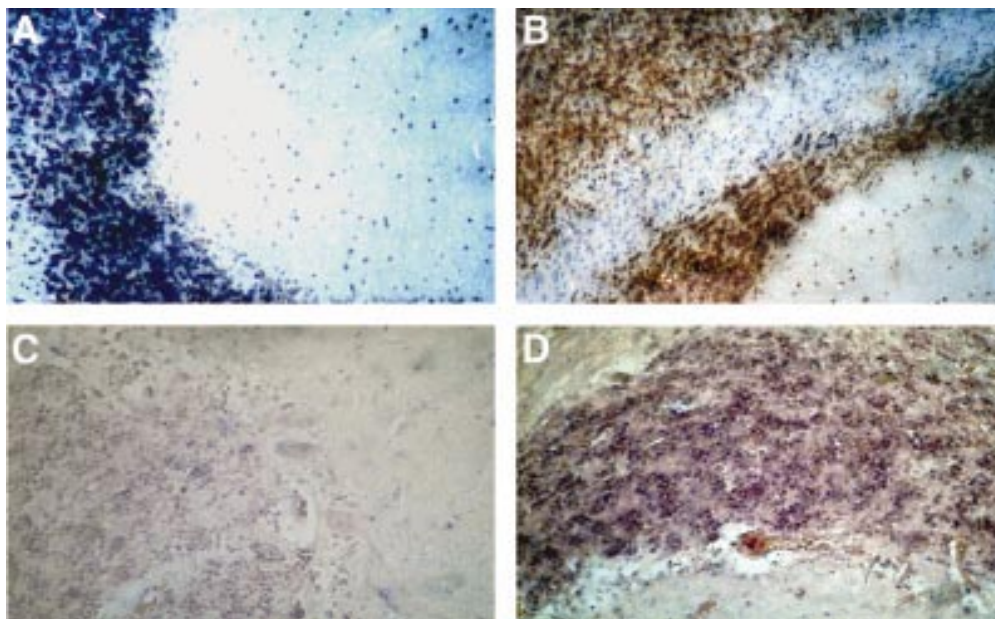

Figure 1   Expression of nicotinamide-N-methyltransferase (NNMT) protein and mRNA in control (non-Parkinsonian) and Parkinsonian cerebellum. (A) Protein expression in non-Parkinsonian cerebellum. Counterstain only (blue) is present. (B) Protein expression in the Parkinsonian cerebellum. Brown staining indicating the presence of NNMT protein is present solely in the neurones of the granular layer, with little or no staining in the white matter and the molecular layer. (C) mRNA expression in the non-Parkinsonian cerebellum. Little expression indicated by blue/purple staining was present in the granular layer. (D) mRNA expression in the Parkinsonian cerebellum. Pronounced blue/purple staining indicating the presence of NNMT mRNA is present solely in the granular layer, with no staining in either the white matter or the molecular layer.

How NNMT relates to β carboline *N*-methyltransferases and tetrathydroisoquinoline *N*-methyltrnasferase is unclear, but as Gearhart *et al* pointed out, these may all be the same entity.[149] If so, the collective evidence from the three areas would present a strong case for *N*-methylpyridinium ion formation being an important aetiological factor.

A second area of biochemistry where the generation of neurotoxins has been explored is that of dopamine metabolism. The arguments as to whether dopamine itself is deleterious to the neurone when considered in relation to L-DOPA treatment have been well rehearsed previously and will not be repeated here. One aspect of endogenous dopamine metabolism that has not received wide attention is that of its interaction with cysteine. Work in this area has been carried out in the main by the group led by Dryhurst. This group has shown that under mild oxidising conditions cysteine and dopamine or other catecholamines react together to form a variety of compounds, including dihydrobenzothiazines, which are potent inhibitors of complex 1, and free radical species.[152–158] If the products of these reactions are involved in the aetiology of the disease, because the neurone has evolved to generate dopamine, factors that control the intracellular concentration of the other reactant (cysteine) would be important. Within the hepatocyte, the enzyme cysteine dioxygenase is one such factor. Therefore, we explored the possibility that this enzyme, which has been shown to be expressed in brain previously,[159] is expressed in dopaminergic neurones. To date, we have shown that, in the rat, the enzyme is present in numerous brain regions and the highest unstimulated activity can be seen in the basal ganglia and olfactory bulb.[160] Inspection of chromosome 5 sequence data in GenBank shows a potential Ptx-3 response element within 2 kb of the transcription start site of the gene, suggesting that this exclusively dopaminergic transcription factor may be involved in the regulation of expression. Initial immunohistochemistry shows that the enzyme is present in neurones,[161] and further work is under way to characterise expression in the human substantia nigra. Relatively little work has been done on the genetics of cysteine dioxygenase regulation, but some phenotyping studies suggest that most of the population has a low to medium degree of constitutive activity, with a small proportion being in a high activity group.[162] A low activity would favour a high intracellular cysteine concentration and hence the formation of toxic reaction products.

THE GENETICS OF "NON-FAMILIAL" PARKINSON'S DISEASE: CANDIDATE GENES

The work outlined in the previous section may be categorised as a "candidate toxin" approach to elucidate the aetiology of idiopathic Parkinson's disease. However, the general emphasis on genetics has led to other themes such as xenobiotic metabolism and links with Alzheimer's disease. Polymorphic alleles are sought that are more or less common in the idiopathic Parkinson's disease population compared with the control population, controls being either age and sex matched to varying extents, or sibling pairs. Many genes have been investigated and a few phenotypic studies carried out. Examples of genes investigated in some recent studies are CYP1A1,[163] the dopamine D2 receptor gene,[164] tau,[165] and apolipoprotein E.[166] In addition, associations between Parkinson's disease and combinations of genetic loci, such as α synuclein and apo lipoprotein E alleles[167] have been investigated. On the whole, the results of such studies have been unconvincing. One group will present evidence of an allele being positively associated with the development of idiopathic Parkinson's disease, only for another group either to fail to find the correlation with the disease or to give another interpretation. An example of this is the case of the NAT2 alleles, where Bandmann and colleagues[168 169] claim a positive association between the "slow acetylator" genotype and the development of idiopathic Parkinson's disease in what they call a "familial" Parkinson's disease group—a group composed of individuals with at least one affected relative—as opposed to the classic, "large family" studies, which identified the mutant α synuclein and parkin genes referred to earlier. This was suggested in a smaller scale phenotypic study too.[170] In contrast, Harhangi *et al* had failed to find this connection in randomly selected patients with idiopathic Parkinson's disease and claim that the genotype is a marker of mortality in the general population.[171] We also failed to find this connection,[163] although patient selection was not identical to that of Bandmann *et al*.

The question arises, therefore, of how to interpret the results of such studies. Where two or more groups have put forward opposing findings, has one side simply got it wrong? If so, how does one tell which? In the case of CYP2D6 alleles, which were suggested to be important by both separate, early phenotyping[172] and genotyping[173] results, the weight of evidence from repeat studies by other groups has tipped the scales against these conclusions.[163 174–176] Nevertheless, it would be depressing to think that such a process would have to be gone through for every candidate gene that is dreamt up. It may be argued that whole genome scanning will obviate this problem. However, in the case where it has been most successful in identifying susceptibility loci in a common multigene disease—diabetes mellitus, type 1—the genetic link in families is stronger than in idiopathic Parkinson's disease, much larger population groups and family clusters were studied, and controls were carefully matched. Thus, if one accepts the multigene hypothesis, it presents huge logistical problems in terms of sample acquisition and classification to achieve adequate statistical power to recognise small differences in allelic frequencies. Moreover, the case for a genetically based aetiology is still strongly challenged,[100] and although the multigene hypothesis may form a convenient way of explaining a baffling problem, it is not necessarily correct.

In an essential monogenic disease such as familial amyloidotic polyneuropathy a single mutation in a single gene can give rise to greatly different phenotypic forms of expression. In the case of the Met30 variant transthyretin, the Portugese expression is one of lower limb involvement and renal failure,[177] whereas in a Danish family the heart is the organ primarily affected.[178] Presumably, this arises in part from the interaction of different levels of gene products in the two populations. A similar scenario (in this case a combination of apolipoprotein E and α synuclein alleles[179]), compounded by the effects of exposure to varieties of natural (such as homocysteine[180]) and man made toxicants (such as lead[181]), might also explain the multifarious features of the entity we call idiopathic Parkinson's disease.

Conclusions

We are some way from unravelling the conundrum of the aetiology of idiopathic Parkinson's disease, although two big advances have been made. The first is the elucidation of the genetic defects in large affected kindreds, which has pointed to the importance of defects in ubiquitin targeted α synuclein catabolism for Lewy body formation, and as a likely end reason for death of the dopaminergic neurone in idiopathic Parkinson's disease. However, the initial precipitating problem is uncertain. Whether it is falling ATP concentrations, the accumulative effects of the inadequate clearance of oxidative free radicals, overexcitation of the neurone, or low amounts of neurotrophic support remains to be resolved.

The second major advance is the increasing recognition of the factors involved in dopaminergic neurogenesis, which, in addition to the intrinsic importance of the knowledge, will allow the application of these factors to produce good model in vitro culture systems. Hopefully, such in vitro cultures, coupled with hybrid systems, which were first described a decade ago and that are beginning to elucidate mitochondrial defects,[182 183] will provide the tools that are needed to enable the conundrum to be explained.

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