Population genetic approaches to neurological disease: Parkinson's disease as an example

S. Gandhi, P. M. Abou-Sleiman, D. G. Healy, M. Weale, W. Gilks,
K. Ahmadi, D. B. Goldstein and N. W. Wood*

*Department of Molecular Neuroscience, Institute of Neurology, UCL, Queen Square,
London WC1N 3BG, UK*

Parkinson's disease (PD) is a common, progressive, incurable disabling condition. The cause is unknown but over the past few years tremendous progress in our understanding of the genetic bases of this condition has been made. To date, this has almost exclusively come from the study of relatively rare Mendelian forms of the disease and there are no currently, widely accepted common variants known to increase susceptibility.

The role that the 'Mendelian' genes play in common sporadic forms of PD is unknown. Moreover, most studies in PD can really be described as candidate polymorphism studies rather than true and complete assessments of the genes themselves. We provide a model of how one might tackle some of these issues using Parkinson's disease as an illustration. One of the emerging hypotheses of gene environment interaction in Parkinson's disease is based on drug metabolizing (or xenobiotic) enzymes and their interaction with putative environmental toxins. This motivated us to describe a tagging approach for an extensive but not exhaustive list of 55 drug metabolizing enzyme genes. We use these data to illustrate the power, and some of the limitations of a haplotype tagging approach. We show that haplotype tagging is extremely efficient and works well with only a modest increase in effort through different populations. The tagging approach works much less well if the minor allele frequency is below 5%. However, it will now be possible using these tags to evaluate these genes comprehensively in PD and other neurodegenerative conditions.

Keywords: Parkinson's; tagging; genetics

1. INTRODUCTION

Parkinson's disease (PD) is a common, progressive, incurable disabling condition. Clinically, it is characterized by the triad of bradykinesia, rigidity and tremor. Coupled with an initial responsiveness to levodopa, the clinical phenotype correlates very highly with the pathological phenotype of the disease, that is, nigral cell degeneration and the presence of inclusions termed 'Lewy bodies' (Hughes *et al.* 1992). The establishment of a clear phenotype for PD is a prerequisite for accurate studies on causation. Epidemiological studies have clearly shown that the disease becomes increasingly prevalent with age (de Rijk *et al.* 1995). In addition, there is an excess of a positive family history suggesting a hereditary component (Marder *et al.* 2003). For reasons as yet unclear, smoking appears to be protective against the development of PD (Allam *et al.* 2004). There is a well-developed literature base demonstrating that a variety of environmental factors, namely rural living, pesticide exposure and well water drinking contribute to the development of PD (Lai *et al.* 2002). The search for toxins in the environment that contribute to the development of the disease was fuelled by the observation in the 1980s that accidental exposure to the toxin methyl phenyl-tetrahydropyridine (MPTP) in

a small group of illicit drug users in the US produced an acute Parkinsonian syndrome (Ballard *et al.* 1985). This observation together with the epidemiological data provided evidence for the xenobiotic theory of Parkinson's disease pathogenesis (Ramsden *et al.* 2001). This theory postulates that Parkinson's disease is caused by an interplay of two factors. Firstly, the presence of unidentified environmental agents or toxins which increase the risk of developing the disease; secondly, the exposure to such agents is modulated by the particular activity profile of xenobiotic metabolizing enzymes of an individual, which is determined by the genetic variation in genes encoding those metabolizing enzymes. Ultimately, it is the combination of an environmental toxin handled by a certain functional set of metabolizing enzymes that would determine whether a person would develop PD.

At present there are no available therapies to modify the disease process. However, there is a wide range of symptomatic treatments. Principally, these treatments are aimed at manipulating the dopaminergic system, either by using L-dopa directly or by enhancing dopamine activity. There are many sources of variability in this disease: the natural history, the age of onset, the disease course, the response to drugs and the presence of additional features. Moreover, the natural history is potentially confounded by drug treatments as there is evidence from animal studies that some of these treatments may in themselves contribute to

* Author for correspondence (n.wood@ion.ucl.ac.uk).

One contribution of 12 to a Discussion Meeting Issue 'Genetic variation and human health'.

Table 1. This provides a list of all the genes currently implicated in Mendelian forms of PD. (Others—Nurr1 and tau have either been implicated in familial disease with Parkinsonian symptoms (tau) or the role is uncertain (Nurr1).)

symbol	name	locus
PARK1	<i>SNCA</i>	4q21–q23
PARK2	<i>parkin</i>	6q25–q27
PARK4	<i>SNCA</i> -triplication	
PARK5	<i>UCHL1</i>	4p
PARK6	<i>PINK1</i>	1p35–36
PARK7	<i>Dβ1</i>	1p36
PARK8	<i>dardarin</i>	12p11–q13

complications or even disease progression. As the disease progresses, it is extremely common for a patient to develop involuntary movements (dyskinesias). These are one of the most troublesome features of the disease and may be extremely difficult to manage. If the determinants of the development of dyskinesias could be identified and influenced, this would produce a major improvement in the lives of many PD sufferers (Jenner 2000).

The major focus of research in PD has been the attempt to identify the molecular pathways that lead to nigral cell degeneration. Until 1997, the pathway was almost exclusively studied using MPTP- or toxin-based animal and cell models of PD. As MPTP is an inhibitor of complex 1 in the respiratory chain of the mitochondria, it appeared that mitochondrial dysfunction was central to the pathogenesis of PD. Since 1997, however, this field of research has been dominated by the discovery of genetic factors causing PD. A number of rare families with a Mendelian pattern of inheritance of PD have been described. Using classical positional cloning strategies several genes have been now identified (see table 1) to be the cause of PD in these families. The discovery of these rare genetic factors has demonstrated that nigral cell loss can also be caused by primary dysfunction in the ubiquitin–proteasome pathway, and has, therefore, implicated protein handling and protein aggregation as central themes in PD pathogenesis, as with many other neurodegenerative diseases (Dawson & Dawson 2003).

Recently, two further pieces have been added to the puzzle. In collaboration we identified a gene called *PINK1* which causes autosomal recessive Parkinsonism. *PINK1* is a putative serine/threonine kinase which appears to be located in the mitochondria. This was the first time that a nuclear encoded mitochondrial protein involved in kinase signalling has been implicated in PD. Therefore, the discovery of this gene may provide some of the links between the varying hypotheses of neurodegeneration. Initial observations suggest that *PINK1* is a neuroprotective molecule which helps to prevent cell death in the face of cellular stress. This protective function is lost in patients with mutations in the *PINK1* gene.

To date, four studies have been carried out to evaluate the burden of *PINK1* mutations in PD patients. These studies have shown that 12 of the 22 families reported were found to carry either homozygous or

compound heterozygous mutations in *PINK1* (Hatano *et al.* 2004; Rohe *et al.* 2004; Valente *et al.* 2004a,b; Rogaeva *et al.* 2004). This indicates that *PINK1* mutations, although rare, account for a significant minority of autosomal recessive forms of PD.

In October 2004, we and others reported mutations in a gene called *LRRK2* which causes autosomal dominant PD (Paisan-Ruiz *et al.* 2004, Zimprich *et al.* 2004). *LRRK2* encodes a protein called dardarin whose role and function is currently poorly understood. Dardarin has a number of putative domains including a ras domain, a kinase domain, WD40 domains and a leucine-rich repeat (LRR) domain. Following the discovery of this gene, a number of early mutations have been also been reported and it appears that mutations in *LRRK2* account for the most common autosomal dominant forms of PD to date.

Although there has been tremendous progress in our understanding of single gene forms of PD, our knowledge of the genetic variation underlying the common sporadic form of the disease is extremely poor. There are two basic approaches to identifying genetic factors underlying susceptibility: linkage and association based. The former has proven incredibly useful in the identification of high penetrant major genetic factors as illustrated above for the monogenic forms of PD. However linkage techniques, principally affected sibling pair design, have been adopted for teasing out genetic risk factors for common forms of neurological disease including PD (Elston *et al.* 2005). This approach has several advantages, including the fact that it is model-free and robust, but has relatively low-power and poor ability to localize a precise region. A recent report by the European study in genetics of Parkinson's disease in 227 sib pairs identified six regions with an maximum likelihood score of greater than one (Martinez *et al.* 2004). Of these, two have been reported in other studies (5q23 and 2q11–q22). These regions are large and contain many genes and it is highly unlikely that fine refinement will be possible using the currently available sibling pair studies. Therefore, one can consider sib pair studies as providing a rough route map to relatively large regions containing many candidate genes which are worthy of further study. The association study design on these potential candidate genes will then prove itself most useful in trying to further identify common genetic factors.

There are a number of potential designs, some of which are addressed elsewhere in this issue, but perhaps the simplest design is the case control study. Basically one asks the question 'are there frequency differences for variance between cases and controls?' This is a potentially powerful approach but is susceptible to potential biases and the creation of false positive reports. One of the problems, hitherto, has been a relatively poor understanding of the linkage disequilibrium structure and the informativeness, or not, of the markers selected. With the completion of the HapMap project much of these difficulties can be overcome. This will greatly aid study design. The strategy is based around the so-called block structure of linkage disequilibrium, and that by identifying the minimum number of markers to capture or 'tag' the 'blocks', one can minimize the genotyping work while

Figure 1. An example of tag performance of a PD gene. Filled triangle represents haplotype r^2 and filled square represents minimum r^2 . h , number of haplotype tags.

maximizing (or at least quantifying) the information content (Wall & Pritchard 2003). One can then simply assess allele, genotype and haplotype frequency differences in cases and controls.

Although it is highly likely that genome-wide association studies will be possible in the near future, there are a number of hurdles to be overcome including exact strategy to be used, statistical analysis and cost. There is a strong motivation for adopting a candidate gene approach, i.e. choosing candidates on biological plausibility.

We have sought to apply a haplotype tagging approach in two ways. First, to take the genes which cause Mendelian forms of PD and investigate whether common variation in these genes affects susceptibility to sporadic PD. It is noteworthy that several studies have already been published, which report both positive and negative associations for several of these Mendelian genes, and it is therefore unclear whether common variation in these genes has an impact on PD susceptibility. Second, to elucidate more fully the xenobiotic theory of PD by studying whether variation in the genes encoding the drug metabolizing enzymes affects susceptibility to PD.

2. HAPLOTYPE TAGGING SNP SELECTION

There are broadly two criteria for selecting tags for capturing information: those based on capturing as much as possible of the original haplotype diversity present in the set of known single nucleotide polymorphisms (SNPs); and those based on establishing as high an association as possible between the reduced tagging SNP. For the purpose of assessing risk for a given variant, we are more interested in association and are less concerned with capturing diversity precisely (see Weale *et al.* 2003 for details and for *TagIT* software: <http://popgen.biol.ucl.ac.uk>).

Table 2. A demonstration of the efficiency of the tagging strategy.

gene	no. of tagging SNPs	genome size (approximately)
<i>parkin</i>	10	1200 kb
<i>Dβ1</i>	3	<15 kb
<i>alpha syn</i>	4	~120 kb
<i>Nurr1</i>	3	<15 kb
<i>UCHL1</i>	4	8–9 kb
<i>tau</i>	3	100 kb
<i>PINK1</i>	4	25 kb

Table 3. Putative associations and drug metabolizing enzymes in PD.

(In all cases the significance of these associations is unknown and either they have been refuted by other studies or replication has not been done.)

CYP2D6
DRD2, DRD4
DAT
MAOA/B
COMT
NAT2
GST T1
GS (M1, P1, Z1)

The association-based criteria concentrate on r^2 , the coefficient of determination (explained sum of squares/total sum of squares) from fitting a linear model defined in different ways depending on the criterion in question (based on haplotype information provided by the tags) to the allelic state information at a given locus.

We have previously shown that a minimum number of polymorphisms (tags) can be used to capture an 'acceptable' amount of the polymorphisms, both known and unknown. We calculate these tags using two methods. The first 'average-locus' method takes a weighted average of all assayed polymorphisms. The second 'worst-locus' method takes the minimum r^2 from the set of assayed polymorphisms. See example in figure 1.

We applied these methods to all the genes listed in table 2. In addition, we assessed the performance of the tags in relation to the previously reported associations. In the event of non-capture with the tags, the previously associated polymorphism was also included in the tagging set.

3. DRUG METABOLIZING ENZYMES AND PD

As part of a planned investigation into the xenobiotic theory of PD, as well as their generic usefulness for pharmacogenetic studies, we have also defined the tag behaviour for 55 principal drug metabolizing enzymes (Ahmadi *et al.* 2005). This dataset also allowed a number of observations to be made about performance of tags, including cross population utility and predictive value across a range of allele frequencies.

There are a large number of association studies evaluating the drug metabolizing genes in the literature (see table 3). Many of these studies are contradictory and/or inconclusive and as a result the true biological

Table 4. Drug metabolizing enzymes or transporters.

	enzyme	mRNA (bp)	Genbank	SNP dbSNP (cds//genomic)	total gene GDB (bp)	exons
phase I	CYP1A1	1653	X04300	4	70551	7
	CYP1A2	3066	M55053	1	6886	6
	CYP1B1	5102	U03688	22	8524	3
	CYP2A6	1748	U22027	4	6779	9
	CYP2B6	1415	X13494	1	25566	9
	CYP2C8	1866	Y00498	19	32898	9
	CYP2C9	1854	S46963	18	50650	9
	CYP2C18	2258	M61853	1	5201	9
	CYP2C19	1746	M61854	4	159924	8
	CYP2D6	1567	M20403	64	6420	9
	CYP2D7P– CYP2D8P	1493–1489	M33387	28	170416507	9
	CYP2E1	1649	J02625	12	6507	6
	CYP3A4	1704	AF182273	4	37887	13
	CYP3A5	1667	J04813	4	31790	13
	CYP3A5P1					3
	CYP3A5P2					2
	CYP3A7	1608	D00408	14	35987	14
	ALDH1	2348	XM_030473	3//80	51792	13
	ALDH2	1992	XM_007012	4//10	43030	13
	ADH3	1486	M12272	90	16253	9
	NQO1	2447	J03934	14	16280	6
	DPYD	465	XM_017469	8	2010	5
phase II	NAT1	872	NM_000662	3	987	2
	NAT2	1276	D90042	14	1175	1
	GSTA2	2268	8218076	4//20??	8969	7
	GSTM1	1117	J03817	29	5887	8
	GSTM3	2351	J05459	10	3806	8
	GSTT1	2368	X79389	5	8092	5
	GSTP1	741	X06547	19	2830	7
	UGT1A1	2351	M57899	5	26121	5
	UGT2B	2368	M57951	29	40021	5
	TPMT	741	XM_031946	2	1757	1
	ARSA	2039	NM_000487	2//6	3120	8
	G6PD	2631	XM_049337	3//12	15860	13
	HMG-CoA reductase	2667	AF273765	4	19519	20
transporters	MDR1	4549	XM_029059	18//129	97214	28
	MRP2	3645	XM_083829	1//1	53469	25

significance of these associations is unclear. Therefore, to aid further evaluation in this field, we have performed a haplotype tagging approach in 55 genes encoding most of the important metabolizing enzymes. In summary, we genotyped approximately 1000 polymorphisms in 64 Centre Étude Polymorphisme Humaine (CEPH) Japanese individuals with a target density of about 1 SNP per 2 kb in the genes shown in table 4. The details of the analyses are reported in Ahmadi *et al.* 2005.

First we were able to show that the tags selected in the initial set (in this instance CEPH trios) performed extremely well when subsequently applied to a second 'application' set. This paradigm mirrors that which applies in real life, i.e. one usually defines the tags in an initial set and then uses them in their case-control set. This observation confirms that the data in HapMap will be generally applicable to population studies. We were also able to assess the impact of minor allele frequency, and in essence the tags perform very well if their frequency is in excess of 5%. Below this level their ability to capture information is much more variable

and this caveat must be considered when planning studies of this type. It of course also influences the conclusions one can draw from negative studies. Finally, we were able to show that the polymorphisms performed very well for capturing functional variants where these were known.

4. MENDELIAN GENES AND PD

As detailed in §1, we have used the genes that have been implicated in Mendelian forms of PD (see table 1) as our starting point. We have assessed each gene in over 750 patients with PD. We ascertained two independent control series each again with over 700 samples. All patients and controls are of European Caucasian ancestry. The preliminary results of this ongoing work do not show any frequency difference between alleles and genotype for the genes tested (unpublished observations) between cases and controls.

There is however, a cautionary note to the study of the genetic basis of common forms of PD. In the recently found gene *LRRK2/Dardarin*, a commonly occurring mutation gly2019ser has been found in

approximately 5% of familial cases (Nichols *et al.* 2005). In our population of 'sporadic' disease, we found 1.6% of cases carried this single mis-sense mutation. To our knowledge this is the first instance of a rare variant contributing to a common disease such as Parkinson's disease (Gilks *et al.* 2005). This variant would not have been identified using a haplotype tagging approach.

5. DISCUSSION

It is now widely appreciated and even expected that association studies will provide the necessary tools for identifying genetic variants underlying risk for common diseases. However, there are very few examples of success to date and, moreover, there are still a number of issues to be combated. Some of the problems that bedevilled association studies of the past can now be directly addressed. Of major importance is the completion of the international haplotyping effort—HapMap. This provides the raw genetic polymorphism data to select, within certain and to some degree identifiable boundaries, the polymorphisms needed to capture the information held by other variants in high linkage disequilibrium with the tags. It is also now known that haplotype diversity is generally limited and therefore an efficient strategy of tagging SNPs suggests itself. This mapping approach is complemented by the more direct sequencing approach which will also help identify risk variants.

The data discussed here highlights some of the benefits and some of the limitations of such an approach. Of benefit, the tagging strategy is robust, informative and extremely efficient. The candidate gene strategy further simplifies the statistical analysis and is being driven by our improving knowledge of the molecular pathogenesis of this disease. The limitations include the fact that if a disease is caused by a rare variant, it is unlikely to be detected by this method. There is a limit to the power of any study design and the ability to absolutely exclude a given gene must always carry the caveat that if the variant plays a small role (ie low odds ratio) then this cannot be excluded. Despite this drawback this approach also allows one to quantify to what level a gene has been excluded.

We are, therefore, suggesting a systematic approach of robust genetic statistical and technical analysis based on the biological plausibility of a candidate gene list. The studies on the drug metabolizing enzymes can be viewed as a xenobiotic register which can now be applied to a range of phenotypes. Principal amongst them at this time is the investigation of the xenobiotic theory of neurodegenerative disease, in particular PD. Ideally for this to be fully comprehensive a detailed knowledge of exposure to putative xenobiotics by the patients and controls will be required. This almost certainly will need a prospective study design and until such an endeavour, we will have to suffice with as robust a retrospective approach as possible. This register can also be used for a range of pharmacogenetic studies, including assessment of efficacy, responsiveness and side effect profile. For PD one can hypothesize that genetic variants in these genes might have an impact on response to L-dopa as

well as influencing the production of additional abnormal movements—dyskinesias, which cause major disability for many PD patients. Success in determining the variants which lead to dyskinesias is more likely in our view to impact on clinical care earliest. If it were possible to delay dyskinesias by the informed selection of drugs earlier in the course of the illness, one could make a significant improvement to the lives of a large number of patients.

It is generally agreed that replication of positive association studies is necessary. What form of replication is required is still debated. Having established a truly positive association, the next step is then to identify the actual causal variant. Genetic approaches can be employed to help this identification, such as definition of an associated interval, thereafter finding all the associated polymorphisms and then prioritizing the polymorphisms (Soranzo *et al.* 2004). This prioritization might need a range of approaches including *in silico* techniques e.g. phylogenetic footprinting, the use of other populations and/or experimental functional evaluation.

Finally, it is important to consider the potentially large impact of this field on society. We need to address a number of issues such as how to interpret risk in a clear and meaningful manner for patients and their relatives, and how to communicate with society in such a way that is understood by the interested parties. Interested parties would include government, insurance companies and law and policy makers. The promise of understanding genetic variation leading to disease risk is tremendous but a dialogue with society is essential if the full potential is to be successfully and ethically realized.

REFERENCES

- Ahmadi, K. R. *et al.* 2005 A single-nucleotide polymorphism tagging set for human drug metabolism and transport. *Nature Genet.* **37**, 84–89. (epub. 19 December 2004).
- Allam, M. F., Campbell, M. J., Hofman, A., Del Castillo, A. S. & Fernandez-Crehuet Navajas, R. 2004 Smoking and Parkinson's disease: systematic review of prospective studies. *Mov. Disord.* **19**, 614–621.
- Ballard, P. A., Tetrud, J. W. & Langston, J. W. 1985 Permanent human Parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): seven cases. *Neurology* **35**(7), 949–956.
- Dawson, T. M. & Dawson, V. L. 2003 Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **5646**, 819–822.
- de Rijk, M. C., Breteler, M. M., Graveland, G. A., Ott, A., Grobbee, D. E., van der Meche, F. G. & Hofman, A. 1995 Prevalence of Parkinson's disease in the elderly: the Rotterdam study. *Neurology* **45**, 2143–2146.
- Elston, R. C., Song, D. & Iyengar, S. K. 2005 Mathematical assumptions versus biological reality: myths in affected sib pair linkage analysis. *Am. J. Hum. Genet.* **76**, 152–156. (epub. 11 November 2004).
- Gilks, W. P. *et al.* 2005 A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet* **365**, 415–416.
- Hatano, Y. *et al.* 2004 Novel PINK1 mutations in early-onset Parkinsonism. *Ann. Neurol.* **56**, 424–427.
- Hughes, A. J., Daniel, S. E., Kilford, L. & Lees, A. J. 1992 Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J. Neurol. Neurosurg. Psychiatry* **55**, 181–184.

- Jenner, P. 2000 Pathophysiology and biochemistry of dyskinesia: clues for the development of non-dopaminergic treatments. *J. Neurol.* **2**(Suppl. 2), II43–II50.
- Lai, B. C., Marion, S. A., Teschke, K. & Tsui, J. K. 2002 Occupational and environmental risk factors for Parkinson's disease. *Parkinsonism Relat. Disord.* **8**, 297–309.
- Marder, K. *et al.* 2003 Familial aggregation of early- and late-onset Parkinson's disease. *Ann. Neurol.* **54**, 507–513.
- Martinez, M. *et al.* 2004 French Parkinson's disease genetics study group; european consortium on genetic susceptibility in Parkinson's disease. Genome-wide scan linkage analysis for Parkinson's disease: the European genetic study of Parkinson's disease. *J. Med. Genet.* **41**, 900–907.
- Nichols, W. C. *et al.* 2005 Parkinson study group—PROGENI investigators. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. *Lancet* **365**, 410–412.
- Paisan-Ruiz, C. *et al.* 2004 Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* **44**, 595–600.
- Ramsden, D. B., Parsons, R. B., Ho, S. L., Xie, T., Waring, R. H. & Williams, A. C. 2001 Further studies in xenobiotic metabolism and Parkinson's disease. *Adv. Neurol.* **86**, 105–113.
- Rohe, C. F., Montagna, P., Breedveld, G., Cortelli, P., Oostra, B. A. & Bonifati, V. 2004 Homozygous PINK1 C-terminus mutation causing early-onset Parkinsonism. *Ann. Neurol.* **56**, 427–431.
- Rogaeva, E. *et al.* 2004 Analysis of the PINK1 gene in a large cohort of cases with Parkinson disease. *Arch. Neurol.* **61**, 1898–1904.
- Soranzo, N., Cavalleri, G. L., Weale, M. E., Wood, N. W., Depondt, C., Marguerie, R., Sisodiya, S. M. & Goldstein, D. B. 2004 Identifying candidate causal variants responsible for altered activity of the ABCB1 multidrug resistance gene. *Genome Res.* **14**, 1333–1344.
- Valente, E. M. *et al.* 2004a Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**, 1158–1160.
- Valente, E. M. *et al.* 2004b PINK1 mutations are associated with sporadic early-onset parkinsonism. *Ann. Neurol.* **56**, 336–341.
- Wall, J. D. & Pritchard, J. K. 2003 Haplotype blocks and linkage disequilibrium in the human genome. *Nature Rev. Genet.* **4**, 587–597.
- Weale, M. E., Depondt, C., Macdonald, S. J., Smith, A., Lai, P. S., Shorvon, S. D., Wood, N. W. & Goldstein, D. B. 2003 Selection and evaluation of tagging SNPs in the neuronal-sodium-channel gene SCN1A: implications for linkage-disequilibrium gene mapping. *Am. J. Hum. Genet.* **73**, 551–565. (epub. 29 July 2003).
- Zimprich, A. *et al.* 2004 Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44**, 601–607.