

Role of Chronic Infection and Inflammation in the Gastrointestinal Tract in the Etiology and Pathogenesis of Idiopathic Parkinsonism

Part 3: Predicted Probability and Gradients of Severity of Idiopathic Parkinsonism Based on *H. pylori* Antibody Profile

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ABSTRACT

Background. Eradicating *Helicobacter* may convert rapidly progressive idiopathic parkinsonism to quieter disease, however only a minority of probands have evidence of current infection.

Aim. To explore the cross-sectional fit of parkinsonism as an extra-alimentary consequence of *Helicobacter pylori*, using the serum antibody profile.

Methods. A discriminant index for parkinsonism was based on the Western Blot pattern of IgG antibodies against electrophoretically separated *H. pylori* antigens in 124 subjects with idiopathic parkinsonism, 196 without. In parkinsonism, association was assessed between index and 1, anthropometric measures; 2, current and 3, increase over 4 years in hypokinetic and psychomotor/psychometric disability; and 4, a global score of current severity.

Results. Predicted probability of being labeled parkinsonian was greatest with cytotoxin-associated-gene-product (CagA) positivity and vacuolating-toxin negativity ($p = .03$ and $.004$, respectively, for antibody-age interactions), and urease-B negativity ($p = .03$, irrespective of age). In this circumstance,

the odds for parkinsonism increased fivefold by age 80 years ($p = .001$). *Helicobacter* status, according to anti-urease enzyme-linked immunosorbent assay (ELISA), did not complement the model. Gradients, of clinically relevant size, were found between index and disease burden, despite the potentially confounding effect of antiparkinsonian medication. The higher the index 1, the worse was posture, as gauged by forward displacement of occiput ($p = .04$), 2, the shorter mean stride-length ($p = .003$), longer reaction time ($= .002$) and lesser cognitive efficiency ($= .03$), 3, the greater their deterioration ($p = .006$, $.002$, and $.03$ respectively), and 4, the greater the overall severity of parkinsonism ($< .001$).

Conclusion. The apparent importance of *H. pylori* in the etiology/pathogenesis of idiopathic parkinsonism is not confined to those with evidence of current infection.

Keywords. Biomarker, idiopathic parkinsonism, *Helicobacter pylori*, serum antibody immunoblot profile, objective measures facets, biological gradients.

In piecing together the relationship of *Helicobacter* infection to idiopathic parkinsonism, some key findings appear contradictory. On the one hand, eradicating *Helicobacter* is beneficial and failed eradication accompanied by rapid deterioration [1,2]. Cross-sectional data link facets of idiopathic parkinsonism and their progression to

circulating cortisol and tumor-necrosis-factor- α [3,4]. On the other hand, any peptic ulceration is usually prodromal to parkinsonism by decades [5], implying a time shift between zenith of gastric response and crossing the diagnostic threshold. Moreover, cross-sectionally, the lower the serum anti-urease antibody titer (measured by enzyme-linked immunosorbent assay), the greater the global severity of parkinsonism [6]. Indeed, the odds for having developed the characteristic flexed posture are more than twofold greater with seronegativity [7].

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A unifying explanatory hypothesis would be that an immune/inflammatory pathogenic process continues, albeit more quietly, after *Helicobacter* infection is no longer detectable by routine methods. Beyond the diagnostic threshold for idiopathic parkinsonism, overt *Helicobacter* infection is already the exception [2]. It might continue to drive the process by persisting in low density, in 'resting' nonulcerogenic forms (e.g. coccoid bodies), or as isolated antigen. An analogy might be found in gastric atrophy, where microbial load lightens as pH rises [8]. Anti-urease seropositivity is no longer so predictive of seeing *Helicobacter* in gastric biopsies [9], but eradication therapy is said [10] still to improve atrophic changes. However, vitamin B₁₂ deficiency has not been associated with parkinsonism [11], where any *Helicobacter*-associated atrophy tends to be mild [1,2].

We explore the discriminant ability for idiopathic parkinsonism inherent in the serum immunoblot *H. pylori* antibody profile. Biological gradients strengthen a causal hypothesis [12]. The relationship of the 'best' discriminant index to anthropometric measures, neurological disease load (physical, psychomotor, and psychometric), and progression should thus be investigated. In modelling for cause and effect, even the most sensitive elements of a subjective global rating scale for parkinsonism do not match an appropriate continuous measure [13]. Stride-length at free-walking speed, when corrected for relevant demographic/anthropometric characteristics, discriminates between those with and without diagnosed parkinsonism with good sensitivity (true positive rate) and specificity (true negative rate) (0.81 and 0.90, respectively, using a probability of having parkinsonism of 0.5 as cut-point) [14]. Reaction time testing [15] discriminates with good specificity for psychomotor and psychometric disabilities (0.87 and 0.85 using the same cut-point), but lacks sensitivity (0.53 and 0.49).

A prognostic index sets the discriminant in the context of the general population.

Methods

Subjects

The study was approved by local ethics committees and written informed consent obtained from all participants. Consecutive independently living patients, with "clinically definite" idiopathic

Table 1 Inclusion and exclusion criteria for probands

Inclusion
Independently living subjects with clinically definite ^a idiopathic parkinsonism.
Any antiparkinsonian medication had to be at steady-state, and evenly spaced to minimize iatrogenic fluctuations in performance.
Caucasian with English as first language, and living in south-east of England ^b
Exclusion
1. Secondary parkinsonism, 'parkinsonism-plus' syndromes and other wider clinical entities [16,17]
2. Clinical depression [18], dementia [19] or other mental illness
3. Other specific neurological condition
4. Exposure to specific antimicrobial/antisecretory therapy against <i>Helicobacter</i>
5. Inflammatory bowel disease, or history of major gastrointestinal surgery
6. Other progressive or resolving disorders affecting physical ability or performance, or sufficient underlying incapacity to prevent assessments (e.g. use of a walking aid)
7. Cardiovascular/respiratory symptoms during normal activities
8. UK MRC Muscl Strength Score < 4/5
9. Arthropathy, musculo skeletal disorder, overt abnormalities of, or history of orthopaedic surgery to, joints of spine or lower limbs
10. Concurrent therapy with drugs which might be antidopaminergic, or with hypnotics or sedatives
11. Recent change in life situation (e.g. bereavement or change in marital status/domicile)

^aAny combination of three of the features: resting tremor; rigidity; bradykinesia, or impairment of postural reflexes. Alternatively sufficient were two of these features, with one of the first three displaying asymmetry [16].

^bTo constrain ethnic and/or geographical confounding influences with respect to infection, host response, anthropometry, and culture.

parkinsonism [16], attending clinic were eligible for inclusion (Table 1). Exclusions included secondary parkinsonism, "parkinsonism-plus" syndromes, and other wider clinical entities [16,17]. Any antiparkinsonian medication had to be at steady state. Similar inclusion and exclusion criteria (except that parkinsonism was an exclusion) were applied to consecutive responders to a call for healthy adult volunteers from the same locality. No attempt was made to exclude those with covert (< threshold for routine diagnosis) signs from the control group.

At least 12 men and 12 women in each decade were required, aged from 30 years (40 years in parkinsonism) to 89 years. Data from 124 patients with idiopathic parkinsonism and 196 controls recruited over the same time period [1] were used to construct the discriminant index and explore biological gradients. Immunoblot data from a further 18 probands and 17 controls were available for the prognostic index.

Clinical Measurements

Demographic characteristics and anthropometric measures. Age, gender, social status, and any

medication were recorded. Anatomical posture, gauged by forward displacement of occiput (≤ 10 cm scored 0; > 10 cm, 1), and height were measured: buttocks and heels against a wall, but otherwise standing relaxed [20]. Leg length, superior edge of symphysis pubis to medial malleolus while supine, was taken as a measure of stature relatively independent of the postural abnormality. Body weight was recorded.

Objective performance measures. In all subjects, objective measures relating to hypokinetic and psychomotor/psychometric facets of parkinsonism were made. Forty-two percent had been assessed, using the same methods, conditions, and venue, approximately 4 years previously. Treated probands with fluctuating performance were assessed during a "therapeutic window," to err in favor of underestimating the deficit due to the condition.

For gait analysis [21], following a practice, rested subjects walked unaided for 40 m, or for 1 minute, in a 2.5 m wide empty corridor, "at your own speed." Mean stride length for "steady state" gait was analyzed [22].

Psychomotor performance was measured as time taken to lift left or right index finger from its touch-sensitive support [15]. At a random (1 and 3 seconds) interval before the imperative, an alerting signal did or did not warn whether left or right was to be lifted. A practice of four replicates of the four conditions (randomized order) preceded the test proper of 10 replicates. If other components of reaction time are constant, "unwarned" minus "warned" reaction time should reflect the efficiency of response to the warning with respect to central processing time. This difference was taken as "cognitive efficiency."

Global rating. Severity of parkinsonism was rated on a 30-point scale [23] in probands.

Serology

Serum from venous blood was stored at -20°C . Test samples were stratified for age, gender, and subject group, the same proportion from each stratum being included in every run of an assay. Within run, they were ordered randomly. All immunoblot kits were from the same batch, as were all enzyme-linked immunosorbent assay (ELISA) kits.

Western Blotting (RIDA *Helicobacter* Blot IgG, Quadrantech Diagnostics Ltd, Epsom, UK) was used to detect antibodies against *H. pylori*

strain ATCC 60 190, antigens of which had been separated electrophoretically, by molecular weight, on nitrocellulose membrane. Specific antibodies bind to corresponding protein bands. Each assay contains one negative and one positive quality control strip and 18 test membrane strips. Strips, which have been exposed to test or quality control serum, are stained and compared with the developed control (k) strip for that kit. The k strip is labelled to identify bands relevant to the prediction of current infection status. All strips were scanned and magnified to maximize precision. A smooth curve was fitted through all distance/molecular weight (kiloDalton) coordinates of the k strip. From this a calibrator (marked in 1 kDa steps) was made for reading the sample strips.

Immunoglobulin G-antibody, directed against high molecular weight, cell-bound *H. pylori* urease, was measured by ELISA (SIA *H. pylori* [HM-CAP], Sigma-Aldrich Ltd, Poole, UK). Duplicate aliquots of each sample were positioned in mirror image on the ELISA plate. A calibration curve converts light absorbance to an "ELISA value" (EV). The between-assay coefficient of variation was 13.0, 8.0, and 6.0% at an EV of 0.8, 2.4, and 5.9, respectively. An EV > 2.2 is the recommended cut-point of seropositivity from equivocal seronegative.

Statistical Methods

Logistic regression was used to examine whether the dependent variable, having an established diagnosis of parkinsonism, or not, was associated with any immunoblot bands, between molecular weights 12 and 132 kDa. A number of regions on the strip were identified as predictive of neurological disease status, either directly or by interaction with age. These were candidate covariates, along with age (interaction with which was re-examined) and gender, in constructing a multivariable logistic regression model, the "discriminant index." Unlike the prevalence of *H. pylori* infection, that of parkinsonism is not related to social class [6]. That inclusion of social class in the final model would not influence predictive ability was tested. The relevance of current infection status (according to anti-urease ELISA) was explored.

In order to provide a "prognostic index" with "absolute" values for the general population, the inverse of the sampling probability was used to weight the multivariable logistic regression

Table 2 Characteristics of subjects

Characteristic	Mean (sd)		Difference (95% CI)
	With parkinsonism n = 124	Without n = 196	
Age (y)	71.8 (11.4)	63.7 (15.7)	8.1 (5.0, 11.1)
Height (m)	1.64 (0.11)	1.66 (0.09)	-0.02 (-0.04, 0)
Leg length (m)	0.72 (0.18)	0.75 (0.09)	-0.03 (-0.06, 0)
Weight (kg)	67.5 (13.7)	71.6 (12.2)	-4.1 (-7.0, -1.2)
Cognitive function score ^a	13.9 (2.2)	15.1 (1.0)	-1.2 (-1.6, -0.8)
Depression score ^b	6.1 (3.9)	3.7 (2.8)	2.4 (1.7, 3.2)

^a16-point scale [19].^b20-point scale [18].

analysis. The population estimate (Office of National Statistics, London) for each year of age (between 30 and 89 inclusive), in England at mid-2000, was taken. The total number of people of a given age labeled as having "Parkinson's disease" was estimated as follows: the number of incident cases for each year of age was calculated from published estimates, and the corresponding number of prevalent cases by summing the incidence for all younger years of age, after allowing for attrition by mortality. This assumed that determinants of the age structure of the general population also applied in the disease state (e.g. no excess mortality in Parkinson's disease) and that no cases were labeled below age 30. The predicted probability of having parkinsonism was obtained by $\exp(xb)/[1 + \exp(xb)]$, where xb , the linear predictor, is the prognostic index. Performance of the index, in classifying subjects by presence/absence of parkinsonism, was estimated by calculating the predicted probabilities for all 320, and applying a 0.5 cut-point.

Analysis of covariance was used to examine whether posture and performance, adjusted for predetermined covariates [14,15], were dependent on the discriminant index. As a preliminary, interaction between disease status and index was assessed. To allow for any shift in the grand mean (e.g. due to a learning effect), change in a performance measure, in an individual, was expressed as the difference between standardized measurements (i.e. $[(x_c - x_{mc})/sd_c] - [(x_i - x_{mi})/sd_i]$, where x_i and x_c are the initial and subsequent values in that individual, and sd_i and sd_c are the standard deviations about the corresponding grand means, x_{mi} and x_{mc} , for all subjects). Variables exhibiting a positively skewed distribution were transformed (natural logarithmic) to approximate normality.

All analysis was performed using STATA 8.2 [24].

Results

Discriminant Index

Definition of subject groups. Table 2 shows the characteristics of the 320 subjects, from whose immunoblot data the index was derived. Approximately half were male (i.e. 52% of 124 with idiopathic parkinsonism and 51% of 196 controls). In each group, the median social status was IIIN, with an interquartile range of II-IIIIN (UK Registrar General's Social Classification: I, II, IIIN, and IIIM, where N is nonmanual and M is manual; IV and V). Eight percent of probands were not receiving antiparkinsonian medication. Individual drug categories exhibited were levodopa/decarboxylase inhibitor combination (81%), dopamine agonist (19%), selegiline (58%), and antimuscarinics (19%). Functional impairment on a 5-point scale [25] was Stage II in 29%, III in 42%, and IV in 29%. Anti-urease ELISA was seropositive in 47% of probands and 39% of controls.

Predictors of neurological disease status. Eight regions on the immunoblot were identified as important, individually. These corresponded to molecular weights of 115-121, 93-95, 86-87, 61-62, 53-55, 46-48, 35-37, and 12-14 kDa. The presence/absence of a band in the 93-95 kDa region (labeled VacA on k strip) was predictive as an interaction with age ($p = .04$). In controls, the odds increased (4.7% [95% CI 2.1, 7.4] per year, $p < .001$), but the proportion seropositive was less than that in parkinsonism until the 8th decade. In parkinsonism, the odds for having antibodies against VacA did not change with age. (This is analogous to the birth-cohort effect for anti-urease ELISA antibody in controls being obliterated in parkinsonism [6].) The other seven blot regions were directly predictive for parkinsonism (e.g. odds ratio [probands/controls] for

Table 3 Discriminant index for idiopathic parkinsonism based on immunoblot serum antibody profile

Predictor	Estimated odds ratio (presence/absence band)	95% CI	<i>p</i> value ^a
CagA	0.03	0.00, 3.80	0.2
Age (year)	1.04	1.02, 1.06	< 0.001
CagA age interaction ^b	1.06	0.99, 1.14	0.07
VacA	765	4, 132,000	0.01
VacA age interaction	0.9	0.85, 0.98	0.01
Urease B ^b	0.59	0.34, 1.02	0.06
Flagellin ^b	1.82	0.96, 3.44	0.07
46–48 kDa band	0.41	0.20, 0.83	0.01

At the stage of formulating the discriminant index, a strict $p < .05$ criterion was not applied. Thus, three predictive terms^b within the model do not reach conventional levels of "statistical significance", but do provide evidence that an association should not be ruled out.

presence of a band at 115–121 kDa [CagA] was 1.83 [1.11, 3.02], $p = .02$).

There was evidence, in the context of the multi-variable logistic regression model, that an association should not be ruled out for five of the above bands (Table 3): CagA and VacA band status as interactions with age; flagellin (53–55 kDa) as a positive predictor; and urease B (61–62 kDa) and a band at 46–48 kDa as negative. Here, the rate of increase with age in the proportion seropositive for CagA antibodies was numerically greater in probands than in controls, as if the birth cohort effect was accelerated. All five bands turned out to be among the 11 recommended (RIDA Blot package insert) for assessment of *H. pylori* infection status. The model was able to classify subjects as having not passed the routine diagnostic threshold for parkinsonism with reasonable certainty, but not *vice versa*. It had good specificity (0.82), but lacked sensitivity (0.42).

Inclusion of social class had no significant effect (likelihood ratio test, $\chi^2 = 6.74$, DF = 4, $p = .2$).

Is anti-urease ELISA complementary? Current infection status, as judged by the cut-point of seropositivity from equivocal negative in anti-urease ELISA, did not complement the discriminant index, either directly or by interaction with age. Neither did the actual EV. Moreover, performance of the index was similar in seropositive and equivocal negative subjects (specificity 0.81 and 0.85, respectively, sensitivity 0.41 and 0.38).

There was some congruency between immunoblot urease-B antibody status and EV. The odds for detecting a urease-B band increased by

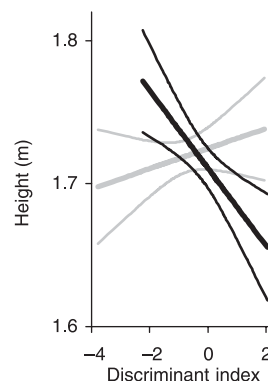


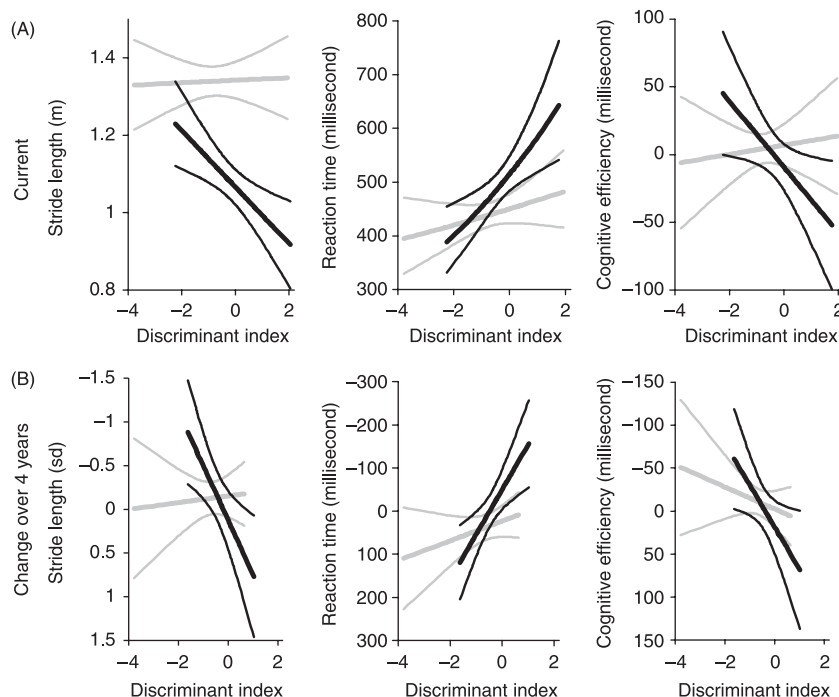
Figure 1 Relationship of discriminant index to height in those with (black) and without (grey) parkinsonism. Regression lines (95% C.I.) are shown. Standing height is adjusted as if all subjects were males of 65 years.

1.40 (95% CI: 1.28, 1.54) per unit higher the EV ($p < .001$), but goodness of fit (pseudo r^2) was only 0.14 (where 1 represents perfect prediction, 0 no predictive ability).

Relationship of discriminant index to clinical criteria. Clinical measurements were made in 312 of the 320 subjects. Gait and reaction time had been measured in 135 of these (39 probands and 96 controls) at a geometric mean of 4.1 (90% data interval: 3.2, 4.5) years previously. Figs 1 and 2 plot the continuous clinical measures against the discriminant index. Here, indices of -4, -3, -2, -1, 0, 1, and 2 correspond to predicted probabilities of having parkinsonism of 0.02, 0.05, 0.12, 0.27, 0.50, 0.73, and 0.88, respectively. Eighty-two percent of controls, but also 58% of probands, had an index ≤ 0 (see specificity and sensitivity).

Anthropometric measures of postural abnormality. Figure 1 shows the relationship of the discriminant index to standing height to be dependent on neurological disease status (status-index interaction: $p < .001$, adjusted for age and gender). Whereas, height was 2.7 cm (95% CI: 1.1, 4.3) shorter per unit higher the index in parkinsonism ($p = .001$), they were not associated in controls. Overall, leg length was shorter in parkinsonism by 2.1 cm (0.8, 3.5) ($p = .002$, adjusted for age and gender), a finding not explained by the index. The flexed posture of parkinsonism would have more effect on standing height than recumbent leg length, and indeed, the odds for the presence of stoop (63% of probands) increased by 2.29 (1.06, 4.93) per unit higher the index ($p < .04$, adjusted for age). Any effect of *Helicobacter* on

Figure 2 Relationship of discriminant index to (A) current clinical assessments and (B) their change over 4 years in those with (black) and without (grey) parkinsonism. Regression lines (95% CI) are shown. Change in performance is expressed as previous values minus current, so +ve represents deterioration and -ve improvement for stride length and cognitive efficiency, and vice versa for reaction time. Stride is adjusted as if all subjects were male of 65 years, with height of 1.65 m and with cognitive function score of 15/16; reaction time as if all were of above age and cognitive score, with depression score of 5/20; and cognitive efficiency to above age and cognitive score. Change in mean stride length is expressed as a proportion of the between subject standard deviation (sd).



growth [26] was not described by the discriminant index.

Hypokinesia of gait. Figure 2 shows the relationship of discriminant index to current, and change in, mean stride length (neurological disease status-index interactions: $p = .005$ and $.003$, respectively, adjusted for age, gender, height, and cognitive function [14]). In parkinsonism, the higher the index, the shorter the stride and the greater its deterioration ($p = .003$ and $.006$). Stride was shorter by 7.3 cm (2.6, 12.0) per unit higher the index. Over 4 years, there had been a reduction of two-thirds (0.62 [0.18, 1.06]) of the between-subject standard deviation in stride per unit higher the index (see Statistical methods). In controls, stride was not influenced by index.

Psychomotor and psychometric performance. Their relationship to the discriminant index is also shown in Fig. 2.

The relationship of index to current, and change in, mean reaction time was dependent on neurological disease status (status-index interactions: $p = .04$ and $.01$, respectively, adjusted for age, age², cognitive function and depression scores [15]). In parkinsonism, reaction time was longer by 13% (5, 23) per unit higher the index ($p = .002$). Over 4 years, there had been an increase of 104 milliseconds (38, 169) per unit

higher the index ($p = .002$). In controls, reaction time was not influenced by index.

Neurological disease status also affected the relationship of index to current cognitive efficiency, tending to affect the relationship to change only under the more discriminant [15] condition of using the nondominant hand (status-index interactions: $p = .02$ and $.1$, respectively, adjusted for age and cognitive function). In parkinsonism, cognitive efficiency was less by 24 milliseconds (2, 46) per unit higher the index ($p = .03$). Over 4 years, it decreased by 49 milliseconds (4, 93) per unit higher the index ($p = .03$). In controls, efficiency was not influenced by index.

Global rating of severity of diagnosed parkinsonism. Mean (sd) severity on the 30-point scale [23] was 14.4 (5.1). It was greater by 2.07 (95% CI 0.53, 3.62) per unit higher the index ($p = .009$, adjusted for age).

Prognostic Index for Being Labeled as Having Idiopathic Parkinsonism

Table 4 shows that only three immunoblot antibody bands contributed to the discrimination after applying weightings to make our patient sample representative of the country. In this context, the worst case was defined as aging with cagA seropositivity and vacA negativity, and

Table 4 Prognostic index for being labeled as having idiopathic parkinsonism, based on immunoblot serum antibody profile

Predictor	Coefficient	95% CI	<i>p</i> value
CagA	-6.40	-12.86, 0.06	0.05
Age (year)	0.09	0.07, 0.11	< 0.001
CagA age interaction	0.10	0.01, 0.19	0.03
VacA	9.05	2.70, 15.41	0.005
VacA age interaction	-0.13	-0.22, -0.04	0.004
Urease-B	-0.58	-1.11, -0.05	0.03
Constant	-11.42 ^a	-12.78, -10.07	< 0.001

^aThe only coefficient in the model to be affected by the sampling probability.

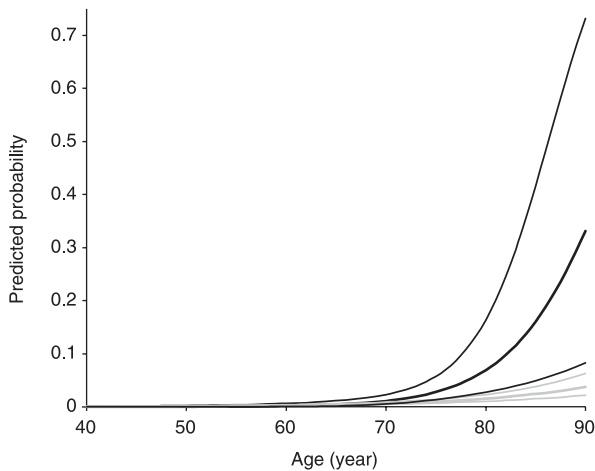


Figure 3 Comparison of mean (95% CI) age-specific predicted probability of being labeled as having idiopathic parkinsonism in presence of CagA, but absence of VacA and urease-B immunoblot bands (back), with that in absence of all three bands (grey). Sampling probabilities for a different population or time would not affect relative difference.

being negative for urease-B, irrespective of age (Fig. 3). By 80 years old, the odds ratio for having idiopathic parkinsonism would increase by 4.75-fold (95% CI 1.82, 12.43) in the presence of a cagA band, but absence of VacA and urease-B bands, compared with absence of all three bands ($p = .001$).

Discussion

In Rothman's words, "The isolated null hypothesis between two variables serves as a useful statistical contrivance for postulating probability models" [27]. Adjusting for multiple comparisons implicitly extends this concept to "a universal null hypothesis" that "only purely random processes govern the variability of all the observations in hand" [27]. In contrast, our empirical approach presumes that, underlying

the observed variability, there is a network of factors connecting the *Helicobacter* antibody profile causally to idiopathic parkinsonism. Our strategy is hypothesis generation. Associations are ranked using *p* values, erring in favor of accepting false positive associations, in order not to reject false negative. The balance is set for exploration, not pragmatic testing.

Irrespective of current infection status, according to ELISA anti-urease serology, a CagA antibody-band, unaccompanied by VacA and urease-B bands, appears to bestow an increasingly bad prognosis for acquiring the label of parkinsonism as age advances. The validity of this finding is supported by well documented links of parkinsonism to increasing age, and of severe gastro-duodenal pathology and extra-alimentary manifestations of *H. pylori* to cagA positive strains. The additional [1,2] insight afforded is of a disease whose relationship to *Helicobacter* may go beyond an aggravating effect of overt infection.

Biological gradients, of clinically relevant size, between the antibody profile-based discriminant index and concurrent measures of diagnosed parkinsonism strengthen the causal hypothesis. The association of index with neurological disease burden was robust. Any reversal of neurological deficits by antiparkinsonian medication did not mask it. The association encompassed objective assessments of anatomical postural abnormality, hypokinesia of gait, psychomotor and psychometric disabilities, and a global rating of severity. Moreover, the index reflected temporal change. There had been progression of parkinsonism with a high index, improvement with a low. The comparative absence of biological gradients in controls implies a bimodal population, with respect to susceptibility to developing parkinsonism or to uncharacterized properties of the infection (such as a dual infection of *Helicobacter* plus *Mycoplasma* [28] or a lymphopenia [2]-inducing virus). It is possible to reach old age with gait, reaction time, and cognitive efficiency virtually intact [15,29]. Whether this feat corresponds to avoiding/loosing *Helicobacter* infection and the CagA antibody band remains to be seen. Unexpected outcomes, where more than one infection is involved, may depend on which is antecedent. (E.g. Beneficial effects on CD4-cell count and viral load with cotrimoxazole prophylaxis against opportunistic infection in HIV [30] or, speculatively, a viral trigger of acute inflammatory demyelinating polyradiculoneuropathy

in a chronically *H. pylori* infected individual [31].)

Thus in parkinsonism, the discriminant index may represent a graded insult, determined by dose (load and neuropathogenicity of organism) and exposure (duration of insult or time since its termination/suppression), modulated by host response. Low sensitivity relative to specificity would fit with dilution of the control group by covert parkinsonism, and of probands by parkinsonism associated with *cagA* negative species/strains [1] and by those who lack/have lost CagA antibody response. A degree of neuronal rescue/compensation, following deliberate [1,2], accidental or spontaneous eradication of infection, may drive the syndrome underground temporarily in those with a persistent CagA band. Absence of a CagA band in some probands may be a consequence of lymphopenia [2].

Difficulty in unravelling the chain of events leading to idiopathic parkinsonism may have grown out of all proportion, because of the boundaries imposed by medical and scientific specialities. A history of peptic ulcer or any dyspepsia concurrent with parkinsonism may carry little weight at the point of care delivery. That the antiparkinsonian medication is gastro-protective [32,33] goes unrecognized. Use of statistical modeling to describe the disease from insult to outcome (with the possibility of pragmatic intervention studies at different stages in the natural history) is akin to a classical functional pharmacological approach to drug design. This contrasts with “compartmental” description (e.g. by genetics or proteomics) in search of a target.

Our starting point for homing in on a link between this common syndrome and a ubiquitous environmental insult is a usable, purely clinical definition of idiopathic parkinsonism [16]. The approach is centered on objective quantification of somatic motor facets, but we acknowledge that other manifestations (e.g. olfactory and autonomic) may precede them [34]. Consensus and mathematical criteria defining the syndrome according to predicted pathological “hallmarks” are not optimal for fitting it to an environmental insult. The same insult might result in neuronal deposition of different aberrant proteins, each having its spectrum of phenotype associations [17]. Aged brains are, indeed, frequently affected by more than one “neurodegenerative” disease, a large proportion of parkinsonian probands having coexistent Alzheimer pathology [35]. Even when familial parkinsonism is associated with mutations

in a single gene, presence of mutation is not always reliable in predicting hallmark (e.g. neuronal loss and gliosis in substantia nigra ± Lewy bodies, tau deposition or ubiquitin with eosinophilic granules, loss of anterior horn cells, senile plaques, and neurofibrillary tangles) or phenotype (parkinsonism, alone or in combination with dementia, dystonia and amyotrophy) [36]. The interaction of genetic constitution and environmental insult must be all important, but the extent to which the genetics of parkinsonism is that of susceptibility/response to infection is unknown. Other environmental factors may be classified as modulators. Smoking is protective for parkinsonism [37], whilst immune-compromise [2], perhaps as a consequence of *Helicobacter* or viral infection or toxic chemicals, is potentially detrimental.

Further evaluation of being CagA immunoblot positive, VacA and urease-B negative, as a biomarker of risk of idiopathic parkinsonism and its rate of progression is needed. Progression needs to be compared prospectively between CagA seropositive and seronegative probands, who are biopsy negative for *Helicobacter* on histopathology and culture. Both physiological and cognitive facets of parkinsonism [38–41] are commonly labeled only as aging. The ubiquity of these facets might well fit the prevalence of *Helicobacter*, but objective follow up over a longer period than in the present study might be needed to show whether, in the absence of overt infection, the CagA seropositive fare worse. A biomarker lying directly in the causal pathway is, of course, much sought after [42].

The work was supported by the Psychiatry Research Trust, which received grants from the Cyril Corden Trust, the Hayward Foundation, the Cecil Pilkington Charitable Trust; and by the North-West London Hospitals General Charitable Fund. Donations were received from Abbott Laboratories (Dr Steve Coles, currently Director of European Clinical Operations). Barclays Corporate Social Responsibility Ambassador, Nicholas Smith, coordinated a fundraising program with the help of patients and carers. Malcolm and Jean Plant coordinated the network of support.

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