

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
13 August 2009 (13.08.2009)(10) International Publication Number
WO 2009/100243 A2(51) International Patent Classification:
C12Q 1/68 (2006.01) *G01N 33/50* (2006.01)(21) International Application Number:
PCT/US2009/033253(22) International Filing Date:
5 February 2009 (05.02.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
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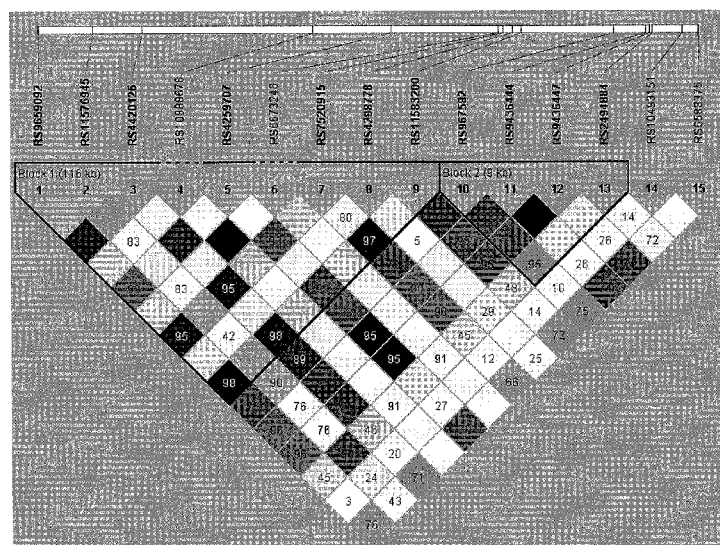
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG,

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(54) Title: ELAVL4 AS A PREDICTOR OF DEPRESSION IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE PATIENTS

Fig. 1



(57) Abstract: Methods of screening patients with Alzheimer's Disease or Parkinson's Disease for risk of late-onset depression, by assaying a biological sample for the presence of ELAVL4.



SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, ZA, ZM, ZW.

MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR),
OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,
MR, NE, SN, TD, TG).

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

ELAVL4 as a predictor of Depression in Alzheimer's Disease and Parkinson's Disease Patients

5 This invention was developed in part with a grant from the U.S. National Institutes of Health. The Government of the United States of America has certain rights in the invention.

FIELD OF THE INVENTION

10 The invention relates to methods of screening patients with Alzheimer's Disease or Parkinson's Disease for risk of late-onset depression, by assaying a biological sample for the presence of ELAVL4.

BACKGROUND

15 Alzheimer's disease (AD) is the most common neurodegenerative syndrome, affecting five million Americans (Alz. Assoc. 2008). Early efforts at gene discovery in AD met with notable success. Traditional positional cloning techniques identified APP, PSEN1, and PSEN2 in rare forms of autosomal dominant AD (1-4). These studies benefitted from using age-at-onset (AAO) as a stratifying variable, to select rare families with an unusual early-onset form of AD.

20 In recent years, discovery of genetic variants governing risk for AD has been challenging. While there are individual reports implicating literally hundreds of genes, few have been replicated or widely accepted (5). Thus far only the APOE-4 variant has a well accepted role in late-onset AD. APOE-4 was initially identified through linkage analysis (6) and has been consistently confirmed using association
25 analyses (7-9). The APOE gene could be identified through this approach because the E-4 variant is common, has a large effect size, and appears to increase risk across many populations (10). Since discovery of the role of APOE-4, progress in characterizing the remaining genetic risk for AD has been slow.

Genetic heterogeneity complicates the search for additional AD risk genes.
30 With many different common variant risk loci, each possessing a low overall effect size, it can be difficult to reliably detect risk conferring genetic variants. Reducing genetic heterogeneity within a dataset promises to improve the signal to noise ratio and power to detect relevant risk loci. Published reports have thus far defined AD consistently yet broadly, and as yet, there are no validated attempts to subphenotype

AD into more genetically homogenous subsets. The critical step then, is to identify methods capable of reducing genetic heterogeneity.

Phenotypic features of a disease might effectively inform the subphenotyping process. Phenotypic characteristics may act as useful surrogates for complex biological processes and by extension the genetic architecture underlying those processes. For instance, the presence of depression in AD is linked to more severe cognitive impairment and may involve distinct neuropathogenic mechanisms in brainstem nuclei (11,12). There is evidence to support susceptibility loci that both influence neuronal changes and confer risk for AD (13-15). Additionally, depressive illness is a common feature of AD (16) and may result in a more aggressive AD phenotype (17). Interestingly, depression appears to be specific to a distinct subset of individuals with AD and does not appear to be a general inevitable consequence of AD pathology (18). This depression that often accompanies AD is phenotypically distinct from the classic major depressive episodes that are more conventionally recognized in younger individuals. Specifically, depression with an onset that is associated with AD shows less sexual dimorphism, is less cyclic in clinical course, and the symptoms are more neurovegetative in nature (19). As a biologically relevant and clinically identifiable variant of AD, depressed AD represents a less heterogeneous subphenotype of AD than a mix of depressed and euthymic individuals with AD. As such, depressed AD may act as an effective subphenotype of AD to reduce genetic heterogeneity within an AD sample.

Therefore it would be useful to have biological markers and/or methods of identifying individuals who may suffer from AD related depression. As depression is often the herald symptom of AD, early recognition provides an opportunity to intervene in the disease process before further damage to brain tissue occurs. Additionally, AD with depression is known to have a more aggressive clinical and pathological course, so timely intervention is likely to be more critical.

Parkinson's disease (PD) is the second most common neurodegenerative disorder of adults and shares a number of phenotypic and histopathological features with AD. Like AD, the majority of PD cases have a complex etiology. Where AD begins with cognitive dysfunction and progresses to motor symptoms, PD presents with motor symptoms before affecting to cognitive function. The primary lesion in AD, amyloid plaques, contain pathological aggregates of alpha-synuclein, and the primary lesion in PD, Lewy bodies, also contain pathological aggregates of alpha-

synuclein. Furthermore, both AD and PD are commonly accompanied by sleep and mood disturbances (37). Interestingly, like AD, the presence of depression has also been suggested to be correlated with more severe cognitive loss in PD (38,39).

Furthermore, just as in AD, depression has been suggested to be marker for more phenotypically severe PD (40).

Previously, our group reported an association between ELAVL4 and PD AAO (but not risk) in the UPDRCE (rs967582; $p=0.006$; (21)). A total of 643 families (1155 PD affected individuals) were used in the original analysis (21) demonstrating the association of ELAVL4 with AAO in PD. Subsequently, two other groups reported a risk association for the same ELAVL4 allele in independent case-control PD datasets (22,23). However, once we stratified the UPDRCE dataset based on depression, we discovered that the AAO signal was driven by the depressed subset (as in AD) and not the euthymic subset.

As with Alzheimer's disease, it would be useful to identify individuals who may suffer from PD related depression for the purposes of early intervention and treatment.

SUMMARY

We hypothesized that the distinct nature of late-onset depression in AD defines a more genetically homogeneous subphenotype of AD patients. Our analyses used data from a published AD genome-wide association study (GWAS) (20) followed by dense SNP mapping to refine associations. We found the strongest association with the AD depression subset is in a gene, ELAVL4, which has previously been reported to be associated with PD in multiple studies. (21-23). Accordingly, we extended our investigation to an independent dataset of affectively characterized PD participants. The second dataset provided the opportunity to determine if the affective subphenotype association generalizes to a second neurodegenerative disorder and whether the same SNP alleles are involved in both depressed AD and depressed PD.

It is an object of the invention to provide a method of screening AD and PD patients for increased risk of late-onset depression. In one embodiment, the invention provides method of screening for elevated risk of depression in a patient afflicted with AD or PD comprising screening a biological sample from the patient for the

presence of ELAVL4, wherein the presence of the ELAVL4 variant in said sample indicates that the patient has an increased probability of late-onset depression.

By "ELAVL4" is meant the gene known as embryonic lethal, abnormal vision, Drosophila-like 4 or Hu antigen D, the sequence being deposited in GenBank under
5 accession no. AY033998.

The method also includes variants of the ELAVL4 gene. By "variants" is meant genetic polymorphisms within the coding and regulatory regions of ELAVL4

By "presence of" is meant that a detectable amount of, for example, a gene or gene product is present in, e.g. a biological sample. In general, a "detectable amount"
10 is an amount that is statistically significant above background. By "background" is meant the mean value of a group of controls who are "normal" and/or are not believed to carry the gene or gene product.

By "increased probability" is meant at least a 10%, preferably 20%, 30% or 40%, most preferably greater than 50% increase over a baseline probability. A
15 baseline probability is, for example, the probability of a control subject having the indicated disorder, disease or condition, e.g. in the present instance, depression. For example, if the baseline probability is 5%, an increase of 10% means that the subject has a 5.5% probability of having or developing the condition.

By "depression" or "late-onset depression" is meant depressed mood or
20 anhedonia of greater than two weeks duration with an onset after 55 years of age.

Screening for such risk will allow the patient to be monitored for depression and for early intervention and treatment.

Tissue samples to be measured include whole blood, packed cells, buffy coats, cultured cells, cell lysates, buccal cells, and other suitable tissues as will be
25 familiar to those of skill in the art.

The presence of ELAVL4 can be measured by any suitable means known to those of skill in the art, particularly polymerase chain reaction genotyping, DNA sequencing, allele specific oligonucleotide assays, by use of a DNA hybridization microarray, e.g. a genechip, including those currently available commercially, or
30 custom-made arrays made by similar means. Using such systems, other genes of interest in diagnosis/treatment of patients may also be simultaneously measured.

This application claims priority to U.S. Provisional Application No. 61/006,903, filed February 5, 2008, which is hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

- 5 Figure 1: Linkage disequilibrium map of peak ELAVL4 SNPs (D').
Figure 2: Association results for ELAVL4 region of chromosome 1.

DETAILED DESCRIPTION

MATERIALS METHODS

10 *Ascertainment and Samples*

Alzheimer Disease: Our AD analyses use a clinically based case-control design. The AD sample set is derived from the Collaborative Alzheimer Project (CAP: The Miami Institute for Human Genomics at the University of Miami Medical Center and The Center for Human Genetics Research at Vanderbilt University Miller
15 School of Medicine). After complete description of the study to the subjects, written informed consent was obtained from all participants in agreement with protocols approved by the institutional review board at their contributing center. For inclusion, each AD affected individual meets the NINCDS/ADRDA criteria for probable or definite AD had an age at onset >60 years of age and screening for the possible
20 presence of depression (24). Age-at-onset (AAO) for AD was determined from specific probe questions within the clinical history provided by a reliable family informant or documented significant impairment in the medical record. Controls were spouses, friends, and other biologically unrelated individuals who were frequency age and gender matched to the cases, and all were from within the same
25 clinical catchment areas. All controls were examined and showed no signs of depression or dementia or movement disorder by history, interview, physical exam, and depression screening. Additionally, each control has a documented Mini-Mental State Exam (MMSE) ≥ 27 or Modified Mini-Mental State Exam (3MS) ≥ 87 (25). Depression status was determined by score on the Geriatric Depression Scale (GDS)
30 greater than 10 (26) at ascertainment or history of depression and treatment documented in the medical record. Since the GDS is a reliable measure of depression in individuals with MMSE score greater than 15 (27), all those with MMSE ≤ 15 (i.e. severe dementia) at assessment were dropped from the dataset. Additionally, those with missing or incomplete depression data were dropped from the dataset.

Parkinson Disease: The PD sample set was collected through the University of Miami Morris K. Udall Parkinson Disease Research Center of Excellence (Miami Udall). Of 2173 patients now available in the UM UPDRCE, 597 had available depression screening data. Similar to the AD dataset, depression status was determined by GDS>10 screening or medical history of depression with treatment. PD affected individuals meet criteria for clinical PD diagnosis based on the United Parkinson's Disease Rating Scale (21,28). Age-at-onset was defined as the age at which an affected individual first noticed one of the cardinal signs of PD(29).

10 *Genotyping*

We extracted DNA from buffy coats derived from whole blood of each participant (see Table 1) with the Puregene system (Gentra Systems, Minneapolis, MN, USA). Genotyping for AD was performed using the Illumina Beadstation and Illumina 550K HumanHap Beadchip following recommended conditions, with the exception of requiring the more conservative gencall score of 0.25. The ABI 7900 Taqman® system was used to generate *APOE* genotypes corresponding to allele combinations at SNP +3937/rs429358 and SNP +4075/rs7412 (Applied Biosystems, Foster City, CA). To refine the strongest association findings, additional fine mapping SNPs identified through HapMap were genotyped also using the Taqman® system. All genotyping in the validation UPDRCE PD dataset used the ABI 7900 Taqman® system.

Sample Quality Assurance

After genotyping and before the statistical analysis, samples must pass a variety of quality control tests (described below) to ensure the integrity of the genetic data. The discovery AD GWAS genotyping efficiency was greater than 99% and sample quality assurance was achieved by including 2 asymmetrically arranged CEPH controls per 96 well plate that were genotyped multiple times. The lab was blinded to affection status and quality control samples. After GWAS genotyping, samples were subject to a battery of quality assurance tests. An extensive description of quality assurance procedures for this report are available in Beecham et al. 2008, and briefly reviewed here. Individual samples with genotyping efficiency less than 0.98 were dropped from the analysis. Many samples were previously genotyped on the Illumina Goldengate and Taqman platforms for SNPs that are included in the GWAS (80% of samples were previously typed at one hundred or more SNPs). Duplicate genotypes

were used to validate correct sample acquisition. Gender concordance was examined using X-linked SNPs; inconsistent samples were dropped from the analysis. Each SNP was subject to several tests for quality before being analyzed. First, all genotype calls were made using standard HapMap cluster files. Second, all genotypes were recalled based on the empiric clusters from this experiment, to correct for any missed calls due to ill-defined HapMap clusters. Third, all SNPs with less than 95% efficiency were dropped from the analysis. Fourth, to reduce error, SNPs with minor allele frequencies of less than 0.10 were subjected to a more stringent efficiency cutoff of 99%.

Taqman® APOE, fine mapping, and all validation PD genotyping achieved greater than 99% genotyping efficiency. Quality assurance was achieved by including a total of 14 quality control samples (including 2 CEPH controls) per 384 well plate. The laboratory was blinded to affection status and quality control samples.

Statistical Analysis

In the AD discovery dataset, tests for deviations from Hardy-Weinberg equilibrium (HWE) were conducted in cases and controls using the exact test from the Genetic Data Analysis software(30). In the family-based PD dataset, tests for deviations from Hardy-Weinberg equilibrium (HWE) were conducted in unrelated cases and unrelated controls (selecting one affected individual and one unaffected individual per family) also using the exact test from the Genetic Data Analysis software (31). Measures of linkage disequilibrium (LD) were computed with GOLD(32). We report the normalized disequilibrium coefficient (D') between all pairs of SNPs.

AD risk association analyses were performed using Armitage's trend test for association (33). This method is equivalent to the score statistic from a logistic regression model with no covariates. It tests for a linear trend in the number of alleles at a single locus. In addition to the standard trend test, we performed logistic regression with APOE status, age, and gender as covariates. All analyses were performed using PLINK (34). False discovery rate genome-wide multiple testing correction was applied using the beta-uniform distribution (35). SNPs with q-values less than a 0.20 false discovery rate were declared genome-wide significant (36). APOE status was designated as the number of e4 alleles. For the PD age-at-onset analyses, we used the generalized estimating equation implemented in Statistical

Analysis System software version 9 (SAS Institute, Cary NC) to allow for correlations within families.

Example 1

The discovery AD dataset contained a total 1049 white/caucasian individuals who were genotyped on the Illumina 550K chip at the MIHG genotyping core facility. There are 518 LOAD cases aged 71.7 years +/- 7.2 years and 531 cognitive controls aged 74.4 years +/- 5.9 years. Each group is 63% female. The final data set analyzed contained a total of 988 Caucasian individuals. There are 492 LOAD cases aged 72.9 years +/- 6.6 years and 496 cognitive controls aged 74.3 years +/- 6.5 years. Cases are 63% female and controls are 61% female (Table 1). (Table 1). The strongest association with the depressed subphenotype of AD is with SNPs on chromosome 1 at 50.3Mb at the ELAVL4 gene (Table 3). The peak SNP rs11583200, has an association p-value= 2.8×10^{-7} and an odds ratio of 1.8 (95% confidence interval 1.5 – 2.3). The association meets FDR criteria for genome-wide significance ($q < 0.20$).

Table 1: Alzheimer Disease Case-Control Sample Information

	All	Depressed AD Cases	Euthymic AD Cases	AD Controls
Total	988	207	285	496
% Female	62.3	63.3	63.9	61.3
AAO/AEE ± (SD)	-----	71.9 (5.5)*	73.4 (5.5)	74.2 (5.6)
APOE4 freq.	0.27	0.41	0.42	0.13
GWAS Efficiency	99.8%	99.8%	99.8%	99.8%

AAO = age-at-onset of Alzheimer disease

AEE = age-at-exam of Control Individual

* Depressed cases are significantly younger than euthymic cases ($p=0.003$)

Table 3: Peak ELAVL4 SNPs: Nominal association trend p-values.

SNP	Overall AD risk n=492	Depressed AD risk n=207	Euthymic AD risk n=285	Overall PD AAO n=597	Depressed PD AAO n=219	Euthymic PD AAO n=378
RS9659092	0.0046	2.81E-05	0.1129	0.5438	0.7318	0.3408
RS11576945	0.7956	0.1418	0.3280	0.6512	0.9649	0.0688
RS4420126	0.0049	2.23E-06	0.1584	0.1686	<i>0.0007</i>	0.4714
RS10888678	0.0352	0.1006	0.0642	0.6512	<i>0.0134</i>	0.8467
RS4259707	0.0545	1.64E-05	0.5118	0.1453	<i>0.0007</i>	0.3708
RS6673246	0.0438	0.1006	0.0865	0.2652	<i>0.0111</i>	0.8079
RS7520915	0.0004	5.25E-07	0.0038	0.2566	<i>0.0143</i>	0.9085
RS4298778	0.1349	0.0753	0.4410	0.9057	<i>0.0414</i>	0.1557
RS11583200	0.0018	2.82E-07*	0.0211	0.8194	<i>0.0055</i>	0.1397
RS967582	0.0094	8.18E-05	0.4967	0.7101	<i>0.0034</i>	0.1169
RS9436444	0.0145	2.03E-05	0.8933	0.9308	0.0521	0.1267
RS9436447	0.0145	2.03E-05	0.8933	0.9552	<i>0.0436</i>	0.1228
RS2494884	0.4588	0.7046	0.5642	0.9223	0.5088	0.3024
RS10493151	0.0878	0.2078	0.7512	0.8391	0.5768	0.2714
RS6588376	0.0883	0.01066	0.6484	0.2965	<i>0.0282</i>	0.9360

- AAO= age-at-onset.
- * = odds ratio 1.8; 95% confidence interval 1.5 - 2.3.
- **Bold** = genome-wide significance in the AD risk analyses (q<0.20).
- *Italics* = nominal significance (p<0.05) in the PD AAO analyses.
- **Shaded** = genome-wide significance in the AD risk analyses (q<0.20) and nominal significance (p<0.05) in the PD AAO analyses.

10 Example 2

To test the hypothesis that the previously identified ELAVL4 effect on PD age-at-onset can be accounted for by PD cases with depression, we genotyped individuals in the UPDRCE dataset. The UPDRCE PD validation dataset is independent from the discovery AD dataset, and has been extended since the original Nouredine et al. 2005 report of an ELAVL4 association with PD age-at-onset at SNP rs967582 (n=2116, p=0.006). Significance (p=0.0056) was essentially

unchanged in a re-analysis of the overall extended PD dataset (n=2173) which includes all subsequently ascertained PD cases. For this study, each of the SNPs defined by the discovery AD GWAS and TaqMan fine mapping were genotyped in the depression screened PD dataset (n=597). Typical of PD epidemiology, the UPDRCE has a preponderance of PD affected males, and the age-at-onset is much younger than the AD dataset (Table 2). Validating our hypothesis, the significant age-at-onset effect at SNP rs967582 is restricted to the much smaller depressed subset of PD participants (n=219; p=0.003, in Table 3). No significant deviations from Hardy-Weinberg equilibrium expectations were identified among the discovery AD cases or controls or the PD cases or controls among the significant SNPs.

Table 2: Parkinson Disease Family-Based Sample Information

	All PD Cases	Depressed PD Cases	Euthymic PD Cases
Total	597	219	378
% Female	36.0	37.0	36.0
AAO $\pm$ (SD)	57.9 (11.7)	56.2 (11.5)*	58.6 (11.7)
APOE4 freq.	0.14	0.18	0.13

AAO = age-at-onset of Parkinson disease

* Depressed PD cases are significantly younger than euthymic PD cases (p=0.02)

CONCLUSIONS

It is clear that major genetic risk factors such as APOE-4 are common to both depressed AD and euthymic AD (Table 1). However, we found in addition a strong association (SNP rs11583200, $p=2.8 \times 10^{-7}$; odds ratio = 1.8; 95% confidence interval 1.5 – 2.3) between variation in ELAVL4 is associated with the depressed AD subphenotype in our dataset. The association exceeds FDR criteria for genome-wide significance ($q < 0.20$). Despite, significant risk association and a small AAO effect for depression itself, no significant AAO effects for ELAVL4 SNPs were identified. To our knowledge, ELAVL4 has not previously been implicated as a risk factor in AD or affective disorders.

In the overall GWAS, the most strongly associated chromosome 1 SNP is rs7520915 (p-value = 0.0004). Empirically, there are more than 230 SNPs that are more strongly associated with AD than rs7520915 (Table 3). Therefore, whether using conservative Bonferroni correction, false discovery rate or even taking the 100

“top hits” regardless of their significance, the ELAVL4 gene would not be identified from this screening GWAS as an important contributor to AD, unless the dataset is stratified based on the depressed subphenotype. This demonstrates that our phenotype (i.e. depression) stratification approach was necessary to identify the role of ELAVL4.

5 It is noteworthy that among the euthymic subphenotype of AD, two SNPs, rs7520915 and rs11583200, do reach statistical significance if uncorrected for multiple testing ($p=.0038$, and $p=0.0211$ respectively in Table 3). Several different phenomena could be responsible for the association. First, some AD affected participants may carry ELAVL4 risk variants, but not express depression at the time
10 of screening so they are subphenotyped into the euthymic AD group. Second, the depression screening is not completely sensitive (Sheikh and Yesavage 1986) so some depressed participants may inadvertently fall into the euthymic subset. Finally, the apparent association could be stochastic.

In addition to the positive association between depressed AD and ELAVL4,
15 the same association is seen with age-at-onset in the depressed PD subphenotype.

Using the same subphenotyping technique employed in the discovery AD dataset, we defined a depressed PD subphenotype. This method identified a stronger age-at-onset effect at SNP rs967582 ($p=0.003$) in the UPDRCE PD dataset specific to the depressed PD subphenotype (Summarized in Table 3). Additionally, we
20 uncovered even stronger age-at-onset effects at SNPs rs4259707 and rs4420126 ($p=0.0007$ for each) in the depressed PD subphenotype. Moreover, both of these SNPs achieved genome-wide significance in the depressed AD subphenotype as well (Table 3). Note that the original UPDRCE report did not require depression
25 screening, so that dataset was much larger ($n=1155$ PD affected participants in Nouredine et al. 2005 versus $n=597$ (of which only 219 have depression) that are included in this report). Consequently the original study had more power to detect genetic effects that are shared across subphenotypic strata. Similar to the original Nouredine et. Al. 2005 report, no risk effect for ELAVL4 was identified in the UPDRCE dataset.

30 It is interesting that ELAVL4 is associated with risk of depressed AD, and AAO of depressed PD. Risk and AAO are both important clinical phenomena, but the relationship between risk and AAO in late-onset disorders remains unclear. While there is no consensus on the clinical meaningfulness of the distinction between risk and AAO, we do know that some genes are consistently reported to affect risk alone

in some datasets, while affecting AAO alone in others. For instance among published PD reports, APOE, GSTO1, GSTO2, and ELAVL4 all follow a pattern of a risk association in case-control datasets, and an AAO association in family-based datasets (41). There are a number of potential reasons for this phenomenon.

5 Participants in family-based studies are presumptively loaded with risk alleles, so that it may not be possible to discriminate the risk conferred by an individual locus. In contrast AAO is a quantitative trait, which provides more information that may be particularly relevant within families who have members with similar genetic backgrounds. Furthermore, while it is well known that the AAO range of sporadic cases of AD and PD vary broadly, case-control datasets often restrict the AAO range under investigation and match them to controls so that AAO effects may be less apparent (5,41).

Despite association of the same ELAVL4 alleles with the depressed forms of AD and PD, it remains premature to conclude that ELAVL4 has a definitive role in AD and PD. Linkage disequilibrium is extensive in the gene rich ELAVL4 region of chromosome 1 (see Figure 1). While the association is not as strong in neighboring genes and does not reach genome-wide significance, the association extends to the AGBL4 gene (Figure 2). Little is known about AGBL4. The AGBL4 gene codes for ATP/GTP binding protein like-4. It is known to be expressed in brain, and from structural evidence is predicted to have carboxypeptidase function (42,43). Previously Oliveira et al demonstrated that it was associated with AAO in PD, using AGBL4's previous designation of FLJ14442 (44).

Extreme ELAVL4 dysregulation has been most widely study in paraneoplastic syndromes (45). The normal function of the ELAVL4 gene is to code for HuD protein in brain. HuD is an RNA binding protein that specifically binds acetylcholinesterase (ACHE) transcript in hippocampal neurons and has been demonstrated to regulate ACHE expression in situ ((46). As such, ELAVL4 represents an excellent functional candidate for the more subtle cholinergic dysfunction seen in cognitive and affective disorders.

30 The present data most strongly suggests a role for ELAVL4 in AD and PD when depression is present. It remains unclear whether ELAVL4 acts as a common risk factor (i.e. pleiotropy) for both AD and PD that includes depression as part of the core phenotypes of those two neurodegenerative disorders or whether ELAVL4 acts to increase susceptibility to late-onset depression in the context of either of these two

neurodegenerative disorders (and possibly others). Either of these two hypotheses could lead to the present findings. Regardless, the data suggest common etiologic mechanisms are shared between the depressed forms of AD and PD. Additionally ELAVL4 may be important for understanding the pathogenesis of AD and PD, and as
5 part of the cholinergic system, ELAVL4 represents a potential target for intervention. More generally, the data suggest that refining the phenotypes of complex diseases into subphenotypes may reduce genetic heterogeneity that obscures important genetic associations.

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WE CLAIM:

1. A method of screening for elevated risk of depression in a patient afflicted with Alzheimer's Disease (AD) or Parkinson's Disease (PD) comprising screening a biological sample from said subject for the presence of ELAVL4, wherein the
5 presence of ELAVL4 in said sample indicates that the patient has an increased probability of depression.
2. The method of claim 1 wherein ELAVL4 is measured using a DNA microarray.
- 10 3. The method of claim 2 wherein the DNA microarray is a genechip.
4. The method of claim 1 wherein the biological sample is selected from the group consisting of whole blood, packed cells, buffy coats, cultured cells, cell lysates and buccal cells.
- 15 5. The method of claim 1 wherein the sample is also assayed for the presence of at least one of the AGBL4 genes.
6. The method of one of claims 1-5 wherein the patient has AD.
- 20 7. The method of one of claims 1-5 wherein the patient has PD.

