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# Glutathione *S*-transferase omega-1 modifies age-at-onset of Alzheimer disease and Parkinson disease

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**We previously reported genetic linkage of loci controlling age-at-onset in Alzheimer disease (AD) and Parkinson's disease (PD) to a 15 cM region on chromosome 10q. Given the large number of genes in this initial starting region, we applied the process of 'genomic convergence' to prioritize and reduce the number of candidate genes for further analysis. As our second convergence factor we performed gene expression studies on hippocampus obtained from AD patients and controls. Analysis revealed that four of the genes [stearoyl-CoA desaturase; NADH-ubiquinone oxidoreductase 1 beta subcomplex 8; protease, serine 11; and glutathione *S*-transferase, omega-1 (GSTO1)] were significantly different in their expression between AD and controls and mapped to the 10q age-at-onset linkage region, the first convergence factor. Using 2814 samples from our AD dataset (1773 AD patients) and 1362 samples from our PD dataset (635 PD patients), allelic association studies for age-at-onset effects in AD and PD revealed no association for three of the**

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**candidates, but a significant association was found for GSTO1 ($P = 0.007$) and a second transcribed member of the GST omega class, GSTO2 ($P = 0.005$), located next to GSTO1. The functions of GSTO1 and GSTO2 are not well understood, but recent data suggest that GSTO1 maybe involved in the post-translational modification of the inflammatory cytokine interleukin-1β. This is provocative given reports of the possible role of inflammation in these two neurodegenerative disorders.**

## INTRODUCTION

Genetic studies of common complex diseases such as Alzheimer's disease (AD; MIM 104300) and Parkinson disease (PD; MIM 168600) have focused on identifying risk genes as targets for developing new treatments and improved diagnoses. This approach led to the identification of several genes contributing to risk in either AD or PD (1,2). However, risk is only one mode of genetic expression; age-at-onset of AD and PD is also genetically controlled (3,4). Identifying genes that control age-at-onset may allow therapeutic intervention to delay disease onset beyond the natural lifespan. The present study looks specifically at genes that affect age-at-onset in these two neurodegenerative disorders, not genes that affect the risk of developing the disease.

We previously reported the first genetic linkage screen for age-at-onset in AD and PD and identified several chromosomal regions that may harbor novel age-at-onset genes. The most interesting finding was a ~15 cM linkage region surrounding D10S1239 and D10S1237 on chromosome 10q (Fig. 1). This linkage region was found in both AD and PD families (4), suggesting the existence of a common gene affecting age-at-onset in both disorders. Here, we follow up the chromosome 10q region to search for novel age-at-onset genes for AD and PD.

This linkage peak is large, spanning over 15 Mb. Thus an effective method is needed to narrow the number of genes for further study. We have previously reported the concept of 'genomic convergence' (5) to prioritize the evaluation of candidate genes. This approach converges the results of two or more different genomic techniques, with the premise that genes shown to be significant in several types of tests have a higher likelihood of being important in the disease process. In this study, we hypothesized that genes demonstrating significant differences in expression level between AD and control hippocampus may play a potential role as disease modifiers or disease susceptibility genes. We used AD gene expression study as our second convergence factor, combined with our previously reported linkage data for age-at-onset (not risk), to select candidate genes that modify the age-at-onset of AD.

Convergence identified four candidate genes mapping to the chromosome 10q linkage region: stearoyl-CoA desaturase (SCD; MIM 604031); NADH-ubiquinone oxidoreductase 1 beta subcomplex 8 (NDUFB8; MIM 602140); glutathione *S*-transferase, omega-1 (GSTO1, also called GSTO1-1; MIM 605482); and protease, serine 11 (PRSS11; MIM 602194). Upon further evaluation we identified a second actively transcribed member of the glutathione transferase omega class, GSTO2, which lies 7.5 kb downstream of GSTO1 (6) and was not included in the arrays used in the study. GSTO2 has 64% amino acid identity with GSTO1. These five genes (SCD, NDUFB8, GSTO1, GSTO2 and PRSS11), therefore, become excellent candidate genes to evaluate for a role in the control of age-at-onset in AD and PD. Using single-nucleotide polymorphisms (SNPs), association analysis (an approach independent of the gene expression study) was performed for each gene to test the relationship between the markers and age-at-onset in AD and PD.

## RESULTS

### Candidate genes

Eight GeneChips (Affymetix), one array per sample, were used to test probes for about 22 000 predicted or known human genes. After applying initial filtering criteria to exclude the genes with low signal intensity, 1072 genes remained for further analysis. Fifty-two genes demonstrated significant differences in gene expression levels between AD cases and controls. Four of these genes (SCD, NDUFB8, GSTO1 and PRSS11) map to the chromosome 10q linkage region. The average raw signal intensity and its standard deviation for AD and control samples are listed in Table 1. Clearly, the transcripts of these four genes were significantly down-regulated in AD in comparison to controls. Figure 1 depicts the genomic locations of these genes relative to D10S1239, D10S1237 and our linkage peaks for PD and AD. Among them, GSTO1 is located under the linkage peak (LOD = 2.62) of the combined AD and PD dataset (4). Lying 7.5 kb downstream of GSTO1 is GSTO2, which was not on the Affymetrix GeneChip U133A array.

### Association study

We initially genotyped 18 SNPs spanning these five candidate genes in the 1773 AD patients and 1041 relatives. All SNPs were in HWE. Strong linkage disequilibrium (LD; $r^2 > 0.85$) was seen between two pairs of SNPs in the GSTO1 and GSTO2 region (SNP7 and SNP9, SNP8 and SNP11) as well as between SNP15 and SNP17 in PRSS11 (Table 2). Other SNPs were in strong linkage disequilibrium (LD) neither with each other nor with any SNPs located in GSTO1, GSTO2 or PRSS11, indicating that the glutathione *S*-transferases and PRSS11 form two independent LD groups.

Age-at-onset was analyzed as a quantitative trait. The mean age-at-onset ± SD was 72 ± 8 years (range 40–97 years) for the AD dataset and 59 ± 13 years (range 12–90 years) for the PD dataset. The *P*-values obtained from the orthogonal model (OM) and Monks–Kaplan (MK) methods for all 18 SNPs genotyped in the AD dataset are depicted in Figure 2. Both GSTO1 SNP7 and GSTO2 SNP9 showed the strongest association with age-at-onset in AD by the OM method

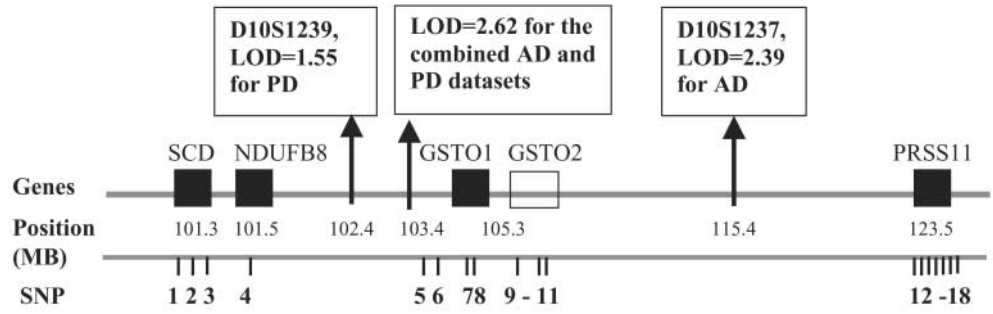


**Figure 1.** The locations of candidate genes used in the study in relationship to the linkage region reported for AD and PD in Li *et al.* (4). The solid black boxes indicate the genes significantly down regulated in the microarray study. The short vertical lines represent the SNPs genotyped in the AD dataset. Their exact positions are listed in Table 4. The physical position is in megabases (Mb, based on NCBI build 31).

**Table 1.** List of genes in chromosome 10q showing significant differential expression between AD and controls

| Affymetrix probe ID | AD,[a] mean ± SD | Control,[a] mean ± SD | Nominal $P$[b] (empirical $P$) | Description |
|---|---|---|---|---|
| 200832_s_at | 1134 ± 460 | 2167 ± 119 | 0.039 (0.024) | Stearoyl–CoA desaturase (SCD) |
| 201185_at | 1134 ± 121 | 2101 ± 119 | 0.0009 (0.005) | Protease, serine, 11 (PRSS11) |
| 201227_s_at | 925 ± 203 | 1475 ± 277 | 0.027 (0.038) | NADH–ubiquinone oxidoreductase 1 beta subcomplex, 8 (NDUFB8) |
| 201470_at | 521 ± 73 | 833 ± 342 | 0.044 (0.029) | Glutathione-*S*-transferase, omega-1 (GSTO1) |

[a]Average and standard deviation of the raw signal intensity in each group.
[b]The nominal $P$ was from a two-sample $t$-test and the empirical $P$ was from the permutation test.

($P = 0.007$ for SNP7 and 0.005 for SNP9). Both SNPs were also confirmed by the MK method ($P = 0.023$ for SNP7 and 0.024 for SNP9). SNP 18 in PRSS11 showed a significant $P$-value ($P = 0.028$) by the OM method, but this finding was not confirmed by the MK method ($P = 0.06$). Independent examination of SNP7, SNP9 and SNP18 in the smaller PD dataset demonstrated significant association for SNP7 ($P = 0.026$ for OM and 0.028 for MK) and SNP9 ($P = 0.042$ for OM and MK), but not SNP18 ($P = 0.07$ for OM and 0.38 for MK). The increased power generated by the combined AD and PD datasets resulted in similar findings for both SNP7 and SNP9 ($P < 0.008$ for OM and $P < 0.02$ for MK).

SNP7 (Ala140Asp) in GSTO1 creates a non-conservative amino acid change from a hydrophobic to a hydrophilic residue. The MK method detected that the less common asparagine (Asp) allele for GSTO1 SNP7 and the −183T allele for GSTO2 SNP9 are associated with later age-at-onset for both AD and PD. The inclusion of APOE as a covariate had little effect on the association results ($P$-values remained similar for each SNP), suggesting that the association between the SNPs and age-at-onset is independent from any APOE effect.

## DISCUSSION

The convergence of genetic linkage, gene expression, and association studies showed that SNPs in GSTO1 and GSTO2 are associated with age-at-onset in AD and PD. We chose SNPs with similar spacing (approximately 5 kb) across all genes in the candidate region. Although extensive SNP detection and genotyping are always the best choice, the availability of the SNPs and cost considerations are the barriers to such an approach. Here, we have used a reasonable density of SNPs for each gene. Although SNP18 in PRSS11 was significantly associated with age-at-onset in the AD dataset by the OM method, the overall evidence was not strong enough to conclude that PRSS11 is associated with age-at-onset of AD and PD. This is interesting given this gene has the lowest $P$-value in the gene expression study. The $P$-value could, however, reflect only secondary changes of cell loss. Overall, the genomic convergence approach proposed here allows us to obtain more meaningful candidates than a single method alone.

The similar association pattern seen with age-at-onset in SNP7 (GSTO1) and SNP9 (GSTO2) would appear to be due to the strong LD between these two SNPs. The pairwise LD estimations revealed three pairs of markers (SNP7 and SNP9, SNP8 and SNP11, and SNP15 and SNP17) in strong LD ($r^2 > 0.85$), which led to similar association results. The SNPs in GSTO1 and GSTO2 are not in LD with those in SCD, NDUFB8 and PRSS11. No LD structure was found in SCD or most of PRSS11. Furthermore, we did not detect any association between the SNPs genotyped in these three genes (SCD, NDUFB8 and PRSS11) and age-at-onset. Based on the pattern of LD structure and association results, we believe that the region in GSTO1 and GSTO2 flanked by SNP7 and SNP9 is the primary candidate region for a susceptibility allele for age-at-onset in AD and PD.

While our current study focused on genes relating to age-at-onset, we also examined whether these genes might contribute to risk of developing AD or PD. We tested GSTO1 and GSTO2 for risk in AD and PD by using the pedigree disequilibrium test (PDT) (7), in which the disease phenotype is the trait of

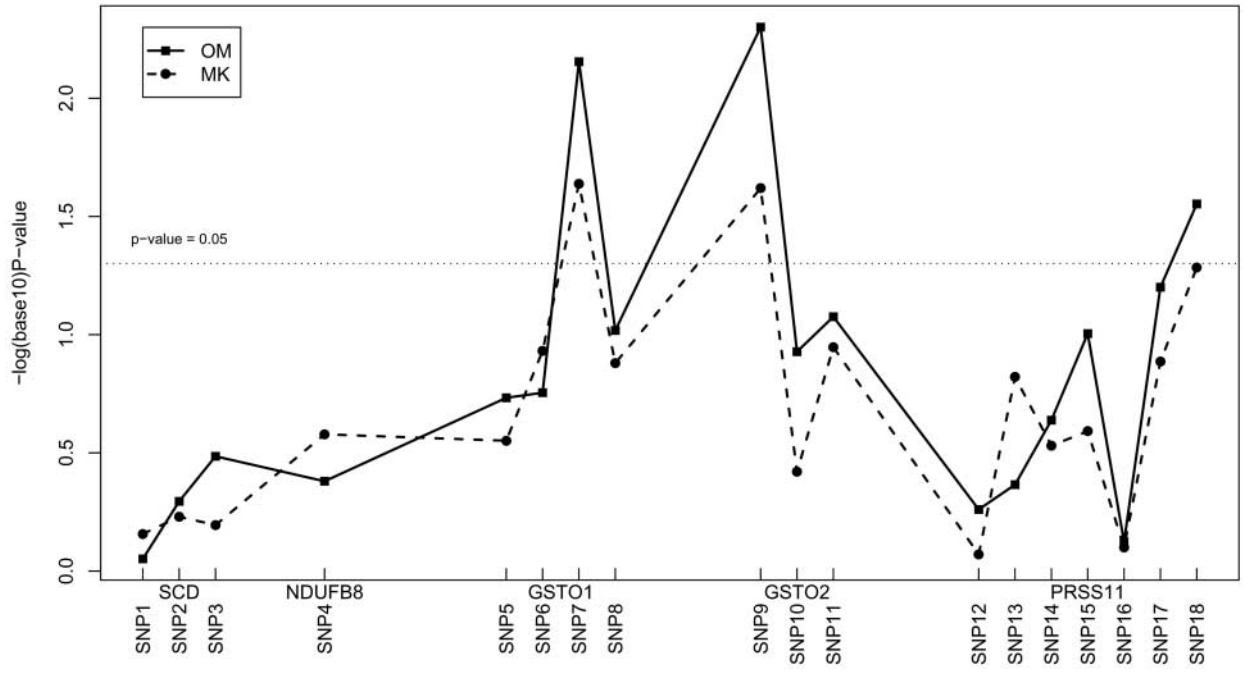


**Figure 2.** *P*-values derived from both OM and MK methods for all SNPs genotyped in the AD dataset. The nominal significant level of 0.05 is indicated by the horizontal line.

interest. There was no evidence for allelic association of any of the SNPs with risk of developing either disease. Since our datasets are relatively large (711 families in AD and 298 in PD), we believe that the PDT method has enough power to detect the risk effect if present, according to the power study in Martin *et al.* (7). The effect of GSTO1 thus appears to be specific to age-at-onset as is implied by our previously reported linkage results.

In the genomic convergence approach, whether a gene is over- or under-expressed in the disease tissue is not important; neither is the level of significance. Rather, importance is placed on whether a gene has any significant difference in expression levels, suggesting it has the potential to be involved in the disease process. Certainly, genes demonstrating expression changes between these two sample groups may be involved in primary or secondary pathways of disease. One of the great challenges with expression analyses is the sheer numbers of genes demonstrating differences and the complex interactions that lead to these differences. Placing importance on over- or under-expression can often be misleading, without a complete understanding of the biological processes involved. In our approach, those genes with significant expression differences between control and disease tissue that also lie within the independently determined age-at-onset linkage regions have an increased likelihood to control age-at-onset. These 'converged' genes can then be tested for genetic association with age-at-onset, an analysis independent of their expression changes and the genetic linkage results. When converged genes subsequently demonstrate significant genetic association with age-at-onset, the likelihood that they play an important role in the primary disease processes greatly increases.

GSTO1 is a member of the glutathione *S*-transferase (GST) family of genes that utilize glutathione in reactions and contribute to the biotransformation and disposition of many compounds, including drugs, carcinogens, and the products of oxidative stress. GSTO1 is expressed in a wide range of tissues, specifically neural glial cells (8). GSTO1 is unusual in that it has a different substrate profile than other members of this family, notably a glutathione-dependent thiotransferase activity similar to glutaredoxins. In addition, Dulhunty *et al.* (9) have reported that, in cells containing ryanodine receptors, it may have a role in protecting against calcium-induced apoptosis.

Recently, it was demonstrated that the activity of the Asp allele of SNP7 in GSTO1 (10) responds differently from the Ala allele depending on the substrate provided, further strengthening the hypothesis that this SNP has functional significance in AD and PD. SNP9 is 183 bp upstream of exon 1 of GSTO2, and could conceivably have an effect on this gene's promoter. However, the lack of association with SNP10 and SNP11, located in intron 3 and exon 4 of GSTO2, and the strong LD between SNP7 and SNP9 support the hypothesis that GSTO1, rather than GSTO2, is the primary candidate gene affecting age-at-onset of AD and PD. However, further investigation will be needed to solidify this point.

Recently reported work (11) provides the most exciting potential mechanism for the GSTO1 effect we have observed on age-at-onset. Laliberte *et al.* (11) report that current experimental drugs at Pfizer known as cytokine release inhibitory drugs, which inhibit interleukin-1β (IL-1β) post-translational processing, have GSTO1 as their direct target. This implies that GSTO1 may be involved in the activation of IL-1β, and thus variation in GSTO1 could alter the efficacy of IL-1β post-translational processing, modulating an inflammatory response. IL-1β is a fundamental component in inflammatory response, a mechanism that has been suggested as contributing to the pathogenesis of both AD and PD (12–15). Indeed, several studies have suggested that IL-1β is

over-expressed in AD and PD brains (12,13), and is induced by the amyloid Aβ peptide (16).

In summary, association of GSTO1 and possibly GSTO2 with age-at-onset for both AD and PD has been demonstrated. This continues to support the premise that AD and PD pathogenesis share many common mechanisms. The confluence of this GSTO1 association with previous findings demonstrates genomic convergence as a sensitive and viable approach for the genetic mapping of complex diseases.

## MATERIALS AND METHODS

### RNA isolation and preparation for hybridization

While either AD or PD (or both) could have been chosen for initial expression studies, we chose only one (AD) to limit the number and cost of microarrays needed for analysis. Brain tissues were collected by the Kathleen Price Bryan Brain Bank, in the Alzheimer's Disease Research Center, Duke University Medical Center, following the rapid autopsy protocol. RNA was isolated from the hippocampus of six AD patients in Braak and Braak (B&B) stage IV or V (17,18) and two normal controls (B&B stage I; Table 3). We chose AD patients of Apolipoprotein E (APOE) 4/4, 4/3 and 3/3 genotypes and controls with the APOE 3/3 genotype judged cognitively normal at the time of entry into the program. The range of controls' age-at-death was similar to affecteds. All patients died without hypoxia. The post-mortem delay ranged from 1 to 17 h for AD patients and 4–10 h for controls. The age-at-onset of AD cases was between 52 and 75 years old (Table 3).

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To label the RNA for hybridization to the microarray chip, 7 µg of total RNA were used for double-stranded cDNA synthesis using the SuperScript Choice System (Gibco BRL Life Technologies, Rockville, MD, USA) in conjunction with a T7-(dT)-24 primer (Geneset Oligos, La Jolla, CA, USA). The cDNA was purified using Phase Lock Gel (5 Prime →3 Prime, Inc., Boulder, CO, USA). *In vitro* transcription was performed to produce biotin-labeled cRNA using a BioArray HighYield RNA Transcript Labeling Kit (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. The biotinylated cRNA was cleaned using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) (19,20).

### Hybridization to high density oligonucleotide microarrays

Twenty micrograms of biotinylated cRNA were fragmented and hybridized at 45°C for 16–18 h using Affymetix Human Genome U133A arrays (Affymetrix Inc., Santa Clara, CA, USA), in which about 22 000 genes were spotted. After being washed, the array was stained and amplified as described (19) and scanned on an HP Gene Array Scanner (Affymetrix Inc., Santa Clara, CA, USA). The intensity for each feature was captured with Affymetrix Microarray Suite 5.0 software, according to standard Affymetrix procedures by performing global scaling with an average 'target intensity' of 100 for all probe sets.

**Table 2.** Pairwise Pearson correlation ($r^2$) for all SNPs genotyped in the AD dataset. The lower triangle is for the unaffected group and upper triangle is for the affected group

| Gene | SCD | | | NDUFB8 | GSTO1 | | | | GSTO2 | | | PRSS11 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | | |
| 1 | — — | 0.11 | 0.46 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | | |
| 2 | 0.12 | — — | 0 | 0.05 | 0 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 3 | 0.43 | 0 | — — | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 4 | 0.02 | 0.11 | 0.02 | — — | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.01 | | |
| 5 | 0 | 0 | 0.01 | 0 | — — | 0.59 | 0.56 | 0.41 | 0.50 | 0.21 | 0.36 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | | |
| 6 | 0 | 0 | 0.01 | 0.01 | 0.71 | — — | 0.79 | 0.72 | 0.72 | 0.40 | 0.66 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | | |
| 7 | 0 | 0 | 0.01 | 0 | 0.62 | 0.76 | — — | 0.78 | **0.89** | 0.41 | 0.69 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | | |
| 8 | 0 | 0.01 | 0.01 | 0 | 0.52 | 0.74 | 0.80 | — — | 0.70 | 0.54 | **0.89** | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | | |
| 9 | 0 | 0 | 0.01 | 0 | 0.53 | 0.68 | **0.89** | 0.71 | — — | 0.50 | 0.78 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | | |
| 10 | 0 | 0.01 | 0.01 | 0.01 | 0.23 | 0.37 | 0.40 | 0.50 | 0.49 | — — | 0.64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 11 | 0 | 0.01 | 0.01 | 0.01 | 0.46 | 0.68 | 0.70 | **0.87** | 0.81 | 0.61 | — — | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | | |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0 | — — | — — | 0 | 0.01 | 0.01 | 0.01 | 0.01 | | |
| 13 | 0 | 0 | 0.01 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — — | 0.13 | 0.13 | 0.15 | 0.13 | 0.22 | | |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0.14 | — — | 0.48 | 0.11 | 0.47 | 0.22 | | |
| 15 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0.10 | 0.48 | — — | 0.05 | **0.97** | 0.53 | | |
| 16 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.12 | 0.09 | 0.06 | — — | 0.05 | 0.08 | | |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0.11 | 0.46 | **0.93** | 0.06 | — — | 0.52 | | |
| 18 | 0.01 | 0 | 0.01 | 0 | 0 | 0 | 0.01 | 0 | 0.01 | 0 | 0 | 0 | 0.22 | 0.20 | 0.48 | 0.11 | 0.47 | — — | | |

Bold numbers represent strong LD ($r^2 > 0.85$) in both the affected and unaffected groups. $r^2 < 0.005$ is shown as $r^2 = 0$.

**Table 3.** Sample description for the microarray study

| Disease status | ID | Sex | PMD[a] | Pathology[b] | Age-at-onset | Age-at-death | APOE genotype |
|---|---|---|---|---|---|---|---|
| Control | 1 | M | 4:15 | B&B I | N/A | 80.8 | 3/3 |
| | 2 | F | 10:38 | B&B I | N/A | 93.1 | 3/3 |
| AD | 3 | F | 1:01 | B&B IV | 75.4 | 95 | 3/3 |
| | 4 | F | 3:36 | B&B V | 56 | 65 | 3/3 |
| | 5 | F | 17:28 | B&B V | 52.2 | 65.8 | 3/3 |
| | 6 | F | 2:15 | B&B V | 71 | 82 | 3/4 |
| | 7 | M | 2:57 | B&B IV | 69.4 | 83.2 | 4/4 |
| | 8 | M | 6:40 | B&B V | 71.9 | 80 | 4/4 |

[a]Post-mortem delay.
[b]Braak and Braak Stage for AD (18).

For quality control, RNA samples were checked by northern blotting (28S, 18S and 260/280 ratio) and judged to be of high quality prior to use. All arrays were visually inspected to identify arrays with production defects. To control for partial RNA degradation, 3′/5′ end ratios for the housekeeping genes actin and GAPDH were examined. Arrays with high 3′/5′end ratios (>5) suggestive of partial RNA degradation were excluded from further analysis.

### Microarray data analysis

Since genes with low signal intensity often cause high variability between arrays, and northern blots usually do not confirm positive results for genes with signal intensity less than 500, only genes with average expression intensities of ≥500 were considered for further analysis. $Log_2$ (logarithm base 2) was used for data normalization, so data within each chip are in agreement with a normal distribution. A two-sample *t*-test was used to calculate the nominal *P*-value to test whether the gene expression between case and control groups was significantly different. In addition, disease status was randomly assigned to each sample 1000 times to estimate an empirical *P*-value for each gene. A nominal significance level of 0.05 was compared with both nominal and empirical *P*-values to declare a result significant.

### Family data

The AD dataset includes 606 multiplex and 105 discordant sibpair families. Clinical diagnosis was based on consensus criteria (21). At least one individual in each of the 711 families has AD diagnosis of either 'definite' (autopsy confirmed) or 'probable' (clinically affected without autopsy). The source of families was described in our previous genomic screen study (4) and most families used in present study are the same as in the previous one. Even in early-onset families, carriers of PS1, PS2 and APP mutations are very rare, and such mutations have not been found in intensive screening of late-onset families (22–24). The inclusion of these rare early-onset mutation families should not have much, if any, effect on our results, because we are searching for genes that modulate age-at-onset independently of any underlying risk cause. Age-at-onset for AD patients was defined as the age at which the caregiver, family and/or individual first noted cognitive problems sufficient to interfere with independent daily activities. The PD dataset consists of 282 multiplex and 16 discordant sibpair families with a total of 635 PD patients. Diagnosis for PD patients followed consensus criteria (25). Age-at-onset for PD patients was defined as the age at which an affected individual first noticed one of the cardinal signs of PD (25). All participants or their legal representatives gave informed consent prior to joining the study, and data were collected according to protocols approved by each contributing group's institutional review board.

### SNP detection and genotyping

Public domain databases (Japanese JSNP, http://snp.ims.u-tokyo.ac.jp, NCBI dbSNP, www.ncbi.nlm.nih.gov/SNP/ and Applied Biosystems, ABI, Foster City, CA, www.appliedbiosystems.com) were used to identify SNPs located in or near the five candidate genes. In total, we selected 18 SNPs based on the principles of equal density across genes (approximately 1 SNP per 5 kb) and the SNP availability (Table 4). Owing to the different size of each gene, the number of SNPs included varies among these five candidate genes. We used a two-step genotyping strategy for the analysis. Given that both AD and PD were linked to this region, we chose the larger dataset (AD) to screen for association, then genotyped those SNPs with positive or suggested association in the PD dataset. Thus we genotyped 18 SNPs for the AD dataset. Taqman primers and probes were designed for genotyping SNP7 and SNP8 in GSTO1, SNP9-11 in GSTO2, and SNP18 in PRSS11 (Table 4). All other SNPs were obtained using assays-on-demand from ABI. Genotyping efficiency ranged between 95–98%. Following our genotyping strategy, SNP7, SNP9 and SNP18 were followed up in the PD dataset.

Genomic DNA was extracted from whole blood using the PureGene system (Gentra Systems, Minneapolis, MN, USA) and the SNPs were genotyped using the TaqMan allelic discrimination assay. APOE genotyping was performed as previously described (26,27).

### Association Analysis

All SNPs were tested for Hardy–Weinberg equilibrium (HWE) and LD in the affected group (one affected from each family) and the unaffected group (one unaffected from each family). An exact test implemented in the Genetic Data Analysis (GDA) program (28) was used to test HWE, in which 3200 replicate samples were simulated for estimating the

**Table 4.** Primers and probes used for SNP genotyping. The position and the base pair/amino acid change for each SNP are indicated

| Gene | SNP (dbSNP no.) | Position Chr. 10 (NCBI Build 31) (change) | Primers and probes sequence (5′–3′) or ABI assay-on-demand number |
|---|---|---|---|
| SCD | SNP1 (rs3870747) | 101 347 325 (IVS3-505 C > T) | C_1345738_10 ABI assay-on-demand |
| | SNP2 (rs3829160) | 101 348 653 (IVS4 + 618 A > G) | C_1345737_10 ABI assay-on-demand |
| | SNP3 (rs6544) | 101 355 833 (3′ UTR + 1497 C > G) | C_11743455_10 ABI assay-on-demand |
| NDUFB8 | SNP4 (rs1800662) | 101 522 724 (IVS3 + 109 A > C) | C_8866465_1 ABI assay-on-demand |
| GSTO1 | SNP5 | 105 241 160 (−7155 C > T) | C_2952474_10 ABI assay-on-demand |
| | SNP6 (rs2164624) | 105 247 082 (−1233 A > G) | C_2086928_10 ABI assay-on-demand |
| | SNP7[a] (rs4925) | 105 256 426 (Ala140Asp) | TGTCTAGGTGCCATCCTTGGT<br>TCCTCTAGCTTGGTAAATTCTTTACGA<br>FAM-AAGACTATGCTGGCCTA-MGBFNQ<br>VIC-AAGACTATGATGGCCTAA-MGBFNQ |
| | SNP8 (rs1147611) | 105 258 895 (IVS4-584 G > T) | GCTGCAGTGAACATTCACATAACAT<br>TGCATACTCATCACCCAGCAAT<br>VIC-ACTTGGCAATGTAAC-MGBNFQ<br>FAM-TGTACTTGGAAATGTAAC-MGBNFQ |
| GSTO2 | SNP9[a] (rs2297235) | 105 268 128 (−183 C > T) | ACTCTCGGGCTTCCAAATCTG<br>GCGATCTGGAGCAGGAGCTA<br>FAM-CCCAGGTTAAGTTAC-MGBNFQ<br>VIC-CCAGGTTAAATTTAC-MGBNFQ |
| | SNP10 (rs157077) | 105 271 531 (IVS3 + 20 G > A) | GCCAAAAGATGTTATTGGAGCTATTT<br>TTGGGAAAGACATGCAAAGTAAAAT<br>FAM-TGTGAGTGGCTTTT-MGBFNQ<br>VIC-CAGTGTGAGTGACTTT-MGBFNQ |
| | SNP11 (rs156697) | 105 272 822 (Asn142Asp) | GCCTGGTAGCGTTGAGATGTG<br>TTTTGTACCTCTTCCAGGTTGCT<br>FAM-AGAATGCACTAATCTGAAGGCAGCCC-BHQ1<br>TET-AGAATGCACTGATCTGAAGGCAGCC-BHQ1 |
| PRSS11 | SNP12 | 123 473 796 (IVS1 + 8972 A > C) | C_11197887_10 ABI assay-on-demand |
| | SNP13 | 123 480 796 (IVS1-10806 A > G) | C_2761707_10 ABI assay-on-demand |
| | SNP14 (rs2268345) | 123 488 667 (IVS1-2935 G > T) | C_2761716_1 ABI assay-on-demand |
| | SNP15 (rs2268349) | 123 493 568 (IVS3 + 1242 G > T) | C_2761720_1 ABI assay-on-demand |
| | SNP16 (rs714816) | 123 499 529 (IVS3 + 7203 G > A) | C_2347168_1 ABI assay-on-demand |
| | SNP17 (rs2250511) | 123 505 603 (IVS3-3788 A > G) | C_2761733_1 ABI assay-on-demand |
| | SNP18[a] (rs2293871) | 123 516 855 (IVS8-36 T > C) | CATGTAAAGTCAGACCAGGAGGAA<br>TGCAACACAAAGGGAAACACA<br>VIC-TGGAAACATGAAACAT-MGBFNQ<br>FAM-AAACACGAAACATTG-MGBFNQ |

[a]SNPs genotyped in both AD and PD datasets.

empirical *P*-value. We used the GOLD (Graphical Overview of Linkage Disequilibrium) program (29) to estimate the Pearson correlation ($r^2$) of alleles for each pair of SNPs as the measurement of LD. The $r^2$ ranges from 0 (no LD) to 1 (perfect LD). However, there is no clear definition how to interpret the intermediate $r^2$. In general, $r^2 > 0.3$ is considered to be a minimum useful value for detecting association with an unmeasured variant related to disease risk by genotyping a nearby marker in LD with that variant (30). Here, we consider two markers in strong LD if $r^2 > 0.85$.

The orthogonal model (OM) (31), which applies to general pedigrees, was used to test allelic association between each SNP and age-at-onset. To examine the direction of the effect on age-at-onset, the Monks–Kaplan method (MK) was applied, which uses parental and sib-pair data only (32). Owing to the computational intensity of the permutation test, we only computed the empirical *P*-values for the markers with significant nominal *P*-values (<0.05). For those markers, we performed 10 000 permutations to avoid false-positive results. We tested for allelic association between all

18 SNPs and age-at-onset in AD. The same approach was used for PD dataset. Although multiple test correction is often suggested, the Bonferroni correction will lead to a very stringent threshold of 0.001 based on a total of 36 tests. Here, we prioritized the markers based on the *P*-value and the testing methods. The smaller the *P*-value, the higher the priority of the marker. In addition, a significant marker needs to be confirmed by both MK and OM methods. Since APOE, particularly the APOE-4 allele, is associated with age-at-onset in AD (1), and more recently in PD (33), we also perform association analysis by incorporating the number of APOE-4 alleles as a covariate.

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## REFERENCES

1. Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L. and Pericak-Vance, M.A. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*, **261**, 921–923.
2. Martin, E.R., Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Lyons, K., Pahwa, R., Stern, M.B., Colcher, A. *et al.* (2001) Association of single-nucleotide polymorphisms of the tau gene with late-onset Parkinson disease. *JAMA*, **286**, 2245–2250.
3. Daw, E.W., Heath, S.C. and Wijsman, E.M. (1999) Multipoint oligogenic analysis of age-at-onset data with applications to Alzheimer disease pedigrees. *Am. J. Hum. Genet.*, **64**, 839–851.
4. Li, Y.J., Scott, W.K., Hedges, D.J., Zhang, F., Gaskell, P.C., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Pahwa, R. *et al.* (2002) Age at onset in two common neurodegenerative diseases is genetically controlled. *Am. J. Hum. Genet.*, **70**, 985–993.
5. Hauser, M.A., Li, Y.J., Takeuchi, S., Walters, R., Noureddine, M., Maready, M., Darden, T., Hulette, C., Martin, E., Hauser, E. *et al.* (2003) Genomic convergence: identifying candidate genes for Parkinson's disease by combining serial analysis of gene expression and genetic linkage. *Hum. Mol. Genet.*, **12**, 671–677.
6. Whitbread, A.K., Tetlow, N., Eyre, H.J., Sutherland, G.R. and Board, P.G. (2003) Characterization of the human Omega class glutathione transferase genes and associated polymorphisms. *Pharmacogenetics*, **13**, 131–144.
7. Martin, E.R., Monks, S.A., Warren, L.L. and Kaplan, N.L. (2000) A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am. J. Hum. Genet.*, **67**, 146–154.
8. Board, P.G., Coggan, M., Chelvanayagam, G., Easteal, S., Jermiin, L.S., Schulte, G.K., Danley, D.E., Hoth, L.R., Griffor, M.C., Kamath, A.V. *et al.* (2000) Identification, characterization, and crystal structure of the Omega class glutathione transferases. *J. Biol. Chem.*, **275**, 24798–24806.
9. Dulhunty, A., Gage, P., Curtis, S., Chelvanayagam, G. and Board, P. (2001) The glutathione transferase structural family includes a nuclear chloride channel and a ryanodine receptor calcium release channel modulator. *J. Biol. Chem.*, **276**, 3319–3323.
10. Tanaka-Kagawa, T., Jinno, H., Hasegawa, T., Makino, Y., Seko, Y., Hanioka, N. and Ando, M. (2003) Functional characterization of two variant human GSTO 1-1s (Ala140Asp and Thr217Asn). *Biochem. Biophys. Res. Commun.*, **301**, 516–520.
11. Laliberte, R.E., Perregaux, D.G., Hoth, L.R., Rosner, P.J., Jordan, C.K., Peese, K.M., Eggler, J.F., Dombroski, M.A., Geoghegan, K.F. and Gabel, C.A. (2003) Glutathione s-transferase omega 1-1 is a target of cytokine release inhibitory drugs and may be responsible for their effect on interleukin-1beta posttranslational processing. *J. Biol. Chem.*, **278**, 16567–16578.
12. Griffin, W.S. and Mrak, R.E. (2002) Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. *J. Leukoc. Biol.*, **72**, 233–238.
13. Czlonkowska, A., Kurkowska-Jastrzebska, I., Czlonkowski, A., Peter, D. and Stefano, G.B. (2002) Immune processes in the pathogenesis of Parkinson's disease—a potential role for microglia and nitric oxide. *Med. Sci. Monit.*, **8**, RA165–RA177.
14. Grimaldi, L.M., Casadei, V.M., Ferri, C., Veglia, F., Licastro, F., Annoni, G., Biunno, I., De Bellis, G., Sorbi, S., Mariani, C. *et al.* (2000) Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Ann. Neurol.*, **47**, 361–365.
15. Mattila, K.M., Rinne, J.O., Lehtimaki, T., Roytta, M., Ahonen, J.P. and Hurme, M. (2002) Association of an interleukin 1B gene polymorphism (-511) with Parkinson's disease in Finnish patients. *J. Med. Genet.*, **39**, 400–402.
16. Giri, R.K., Selvaraj, S.K. and Kalra, V.K. (2003) Amyloid peptide-induced cytokine and chemokine expression in THP-1 monocytes is blocked by small inhibitory RNA duplexes for early growth response-1 messenger RNA. *J. Immunol.*, **170**, 5281–5294.
17. Braak, H. and Braak, E. (1991) Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. *Brain Pathol.*, **1**, 213–216.
18. Braak, H. and Braak, E. (1997) Staging of Alzheimer-related cortical destruction. *Int. Psychogeriatr.*, **9** (Suppl. 1), 257–261.
19. Lockhart, D.J., Dong, H., Byrne, M.C., Follettie, M.T., Gallo, M.V., Chee, M.S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H. and Brown, E.L. (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.*, **14**, 1675–1680.
20. Warrington, J.A., Nair, A., Mahadevappa, M. and Tsyganskaya, M. (2000) Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. *Physiol. Genom.*, **2**, 143–147.
21. McKhann, G., Drachman, D. and Folstein, M. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*, **34**, 939–944.
22. Sorbi, S., Nacmias, B., Tedde, A., Forleo, P., Piacentini, S., Latorraca, S. and Amaducci, L. (1997) Presenilin-1 gene intronic polymorphism in sporadic and familial Alzheimer's disease. *Neurosci. Lett.*, **222**, 132–134.
23. Gomez-Isla, T., Wasco, W., Pettingell, W.P., Gurubhagavatula, S., Schmidt, S.D., Jondro, P.D., McNamara, M., Rodes, L.A., DiBlasi, T., Growdon, W.B. *et al.* (1997) A novel presenilin-1 mutation: increased beta-amyloid and neurofibrillary changes. *Ann. Neurol.*, **41**, 809–813.

24. Liddell, M.B., Bayer, A.J. and Owen, M.J. (1995) No evidence that common allelic variation in the amyloid precursor protein (APP) gene confers susceptibility to Alzheimer's disease. *Hum. Mol. Genet.*, **4**, 853–858.
25. Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Lyons, K., Pahwa, R., Stern, M.B., Colcher, A., Hiner, B.C. *et al.* (2001) Complete genomic screen in Parkinson disease: evidence for multiple genes. *JAMA*, **286**, 2239–2244.
26. Saunders, A.M., Strittmatter, W.J., Schmechel, D., George-Hyslop, P.H., Pericak-Vance, M.A., Joo, S.H., Rosi, B.L., Gusella, J.F., Crapper-MacLachlan, D.R. and Alberts, M.J. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*, **43**, 1467–1472.
27. Vance, J.M. and Ben Othmane, K. (1998) In Haines, J.L., Pericak-Vance, M.A. (eds), *Approaches to Gene Mapping in Complex Human Diseases*. Wiley-Liss, New York, pp. 213–228.
28. Zaykin, D., Zhivotovsky, L. and Weir, B.S. (1995) Exact tests for association between alleles at arbitrary numbers of loci. *Genetica*, **96**, 169–178.
29. Abecasis, G.R. and Cookson, W.O. (2000) GOLD—graphical overview of linkage disequilibrium. *Bioinformatics*, **16**, 182–183.
30. Ardlie, K.G., Kruglyak, L. and Seielstad, M. (2002) Patterns of linkage disequilibrium in the human genome. *Nat. Rev. Genet.*, **3**, 299–309.
31. Abecasis, G.R., Cardon, L.R. and Cookson, W.O. (2000) A general test of association for quantitative traits in nuclear families. *Am. J. Hum. Genet.*, **66**, 279–292.
32. Monks, S.A. and Kaplan, N.L. (2000) Removing the sampling restrictions from family-based tests of association for a quantitative-trait locus. *Am. J. Hum. Genet.*, **66**, 576–592.
33. Vance, J.M., Li, Y.J., Zhang, F., Booze, M.W., Martin, E.R., Scott, W.K., Haines, J.L., Nance, M.A., Hubble, J.P., Koller, W.C. *et al.* (2002) Apolipoprotein E controls the risk and age at onset of Parkinson disease. *Am. J. Hum. Genet.*, **71**, 180.

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# CORRIGENDUM

# Glutathione *S*-transferase omega-1 modifies age-at-onset of Alzheimer disease and Parkinson disease

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Two authors were inadvertently omitted from the title page of this recently published paper. They were Christine Hulette (affiliation 1) and Kathleen A. Welsh-Bohmer (affliation 16). The authors apologise for this error.