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A polymorphism in *SOD2* is associated with development of Alzheimer's disease

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Genes involved in cellular mechanisms to repair oxidative damage are strong candidates as etiologic factors for Alzheimer's disease (AD). One important enzyme involved in this mechanism is superoxide dismutase 2 (SOD2). The gene for this enzyme lies within a single haplotype block at 6q25.3, a region showing evidence for linkage to AD in a genome scan. We genotyped four single nucleotide polymorphisms (SNPs) in *SOD2* in families of the National Institute of Mental Health-AD Genetics Initiative (ADGI): rs2758346 in the 5′ untranslated region (UTR), rs4880 in exon 2, rs2855116 in intron 3 and rs5746136 in the 3′UTR. Under a dominant model, family-based association tests showed significant evidence for association of AD with the first three loci in a candidate gene set of families with individuals having age of onset of at least 50 years and two affected and one unaffected sibling, and in a late-onset subset of families (families with all affected individuals having age of onset of at least 65 years) from the full ADGI sample. The alleles transmitted more frequently to cases than expected under the null hypothesis were T, C, G, and G. Global tests of the transmission of haplotypes indicate that the first two loci have the most consistent association with risk of AD. Because of the high linkage disequilibrium in this small (14 kb) gene, and the presence of 100 SNPs in this gene, 26 of which may have functional significance, additional genotyping and sequencing are needed to identify the functionally relevant SNP. We discuss the importance of our findings and the relevance of SOD2 to AD risk.

Keywords: Alzheimer's disease, genetic association, oxidative damage, SOD2

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There is a significant body of evidence indicating that oxidative damage is involved in the aging process, and in particular neurodegenerative diseases such as Alzheimer's disease (AD) (Beal *et al.* 1997). The brain, with its high oxygen consumption, high content of easily oxidized lipids, and shortage of strong antioxidant defenses, is particularly susceptible to oxidative injury and stress (Smith *et al.* 1996, 1998). All levels of cell metabolism in the AD brain appear to be more affected by free-radical damage than in the normal aging brain (see Christen 2000; Markesbery 1999; Miranda *et al.* 2000; Varadarajan *et al.* 2000, for review). There is increased oxidative damage to nucleic acids and mitochondrial dysfunction in AD brains (Coskun *et al.* 2004; Davies 1995; Gabbita *et al.* 1998; Mecocci *et al.* 1994; Nunomura *et al.* 1999; Szczesny *et al.* 2003). Oxidation of proteins by free radicals can result in cross-linking, aggregation and fibril insolubility, like that seen in the aggregated form of beta amyloid (Aβ) (Dyrks *et al.* 1992), and the hyperphosphorylation of tau in neurofibrillary tangles (NFTs) (Smith 1997).

Oxygen-utilizing cells have evolved complex enzymatic and nonenzymatic defenses such as superoxide dismutases (SODs) and peroxiredoxins to offset oxidative challenges in different subcellular compartments (Fridovich 1978; Krapfenbauer *et al.* 2003). Superoxide dismutases are the key enzymes involved in the detoxification of the superoxide radical (Fridovich 1995). Levels of antioxidant enzymes, like SODs, increase with age in cortical tissues of Tg2576 mice, an AD transgenic mouse model (Apelt *et al.* 2004). One SOD, SOD2 or Mn SOD, is located in the mitochondria and is the only isoform that is induced and regulated by reactive oxygen species (Pinteaux *et al.* 1996; Warner *et al.* 1996; Weisiger & Fridovich 1973). The Sod2 null mice develop a severe neurological phenotype and neuronal cell death as a result of mitochondrial oxidative stress (Hinerfeld *et al.* 2004; Lynn *et al.* 2005), while reduction of Sod2 in transgenic mice carrying amyloid precursor protein (APP) mutations triggers exacerbation of neuronal and vascular AD pathology (Esposito *et al.* 2006). The SOD2 is expressed in most human tissues (Wan *et al.* 1994), including regions of the brain that are affected in AD (Zhang *et al.* 1994).

The *SOD2* gene is located at 6q22 in a candidate region of interest we identified in a recent genome scan of the National Institute of Mental Health (NIMH)-AD Genetics Initiative (NIMH-ADGI) set of families (Blacker *et al.* 2003) and has been identified in other genomic scans of AD (Ashley-Koch *et al.* 2005; Kehoe *et al.* 1999; Lee *et al.* 2006; Pericak-Vance *et al.* 2000). We now present results using family-based association testing to determine whether four nucleotide changes located in known and possible functional areas of *SOD2* are associated with increased risk of AD in the NIMH-ADGI families.

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Materials and methods

NIMH-ADGI families

We identified a subset of families recruited as part of the NIMH-ADGI following a standardized protocol using the NINCDS-ADRDA criteria for diagnosis of definite and probable AD (McKhann *et al.* 1984). This subset included sibships containing at least two affected and one unaffected sibling, fitting the criteria for family-based association testing (Laird *et al.* 2000; Lake *et al.* 2000). This group was denoted the candidate gene set (CGS) and consisted of 795 individuals in 203 families containing 211 sibships. The affected individuals in these families had a mean age of onset (MAO) of 70.9 ± 7.4 years, and the mean number of affected individuals per family was 2.18. The CGS was 67% female, and 60.6% of the affected individuals were females. When five non-Caucasian families were excluded from analyses, no qualitative differences were noted in the results, so these families were included in the analyses. A subset was formed from the CGS by including only those families (142 families containing 146 sibships) with affected individuals having age of onset of at least 65 years (MAO = 73.5 ± 5.7 years, range 65–97 years). This group was referred to as the late age of onset group (LOAD). There were 542 individuals in this set, of which 69.2% were female, and 61.9% of the females were affected, and the mean number of affected individuals per family was 2.21. All procedures involving human subjects followed Institutional Review Board (IRB) protocols approved by their respective institutions, and informed consent was obtained from all subjects or legal guardians.

Genotyping of SOD2 single nucleotide polymorphisms, rs2855116 (G → T), rs4880 (C → T), rs2758346 (C → T) and rs5746136 (A → G)

Isolation of genomic DNA and general procedures for polymerase chain reaction (PCR)–restriction fragment length polymorphism genotyping have been described (Perry *et al.* 2001), and all primers were ordered from IDT (Coralville, IA, USA). Primers and conditions to amplify the (G → T) single nucleotide polymorphism (SNP) located at position –59 of intron 3 of *SOD2* (rs2855116) have been described (Tomkins *et al.* 2001), except 100 ng genomic DNA, a standard 10× PCR buffer and 31 cycles were used. Eight microliters of product was digested with 2 U of *Nla*III in a 15 μl reaction overnight. Digestion products (137, 125, +12 bp) were run on a 3% agarose gel and photographed on a Fluor-S Imager (Bio-Rad, Hercules, CA, USA).

Primers to specifically amplify the Ala16Val (C → T) SNP of *SOD2* (rs4880) have been described (Shimoda-Matsubayashi *et al.* 1996) (primers 2 and 4), except a C was dropped from the 5′ end of forward primer 4. Reagents and conditions for amplification are the same as used for rs2855116, except 34 cycles of 94°C/45 seconds, 60°C/40 seconds and 72°C/1 min were used. Four microliters of product was digested with 2.5 U of *BsaW*I (N.E. Biolabs, Beverly, MA, USA) in a 12 μl reaction for 12 h. Forward (5′-TGAACCGTTTCCGTTGCTTC-3′) and reverse (5′-ACCCAACACGTAGCCCTAGTTAC-3′) primers to amplify the (C → T) SNP (rs2758346) in the 5′ untranslated region (UTR) of *SOD2* and the forward and reverse primers (5′-GATGCCTTTC-TAGTCCTATTC-3′ and 5′-TCAGTTCACCTGCTACATT-3′, respectively) to amplify the (A → G) SNP (rs5746136) in the 3′UTR of *SOD2* were designed using OLIGO 6.0 primer design software (Molecular Insights, Inc., Cascade, CO, USA). The PCR reagents for both SNPs were the same as above, and a touchdown protocol was used to amplify both SNPs. After an initial denaturation for 2.5 min, there were 21 cycles of 30 seconds at 94°C, 30 seconds at a variable annealing temperature and then 30 seconds at 72°C. The variable annealing temperature was set to 63°C on the first cycle for rs2758346 (64°C for rs5746136) and was decreased by 0.5°C on each succeeding cycle for 10 cycles. An additional 20 cycles was then run at the last annealing temperature. Digestions of products were performed as above with 3 U of *Bsr*GI for SNP rs2758346 at 37°C for 4 h and with 3 U of *Taq*I for SNP rs5746136 at 65°C for 2 h. Digestion products for the latter three SNPs were run on a 2.5% agarose gel and photographed as above.

Statistical analyses

Family-based association testing within the NIMH-ADGI data set was performed using the FBAT program (Horvath *et al.* 2004; Laird *et al.* 2000; Lake *et al.* 2000). This program tests for association in the presence of linkage by comparing the genotypes received by the children from their parents to the genotypes expected to be received under the hypothesis of no association. Either single-locus genotypes or multilocus haplotypes can be tested. For single-locus genotypes, a comparison of all possible genotypes (the general genotype model) is available. This model considers simultaneous transmissions from both parents, and separately tests for reception of each genotype by the children compared to what would be expected in the absence of association. More restrictive models, which can be applied for both single-locus and multilocus tests, include the additive model (in which the statistic used is the number of alleles of interest received by each affected child) and the dominant model (in which the statistic used is the presence or absence of the allele of interest in each affected child). The expected value in all situations is conditional on all available genotype data in the nuclear family. All results presented here use the empirical variance estimate, which assumes *a priori* evidence for linkage. Association between risk for AD and genotypes at the *APOE* locus is well known (Blacker *et al.* 1997; Corder *et al.* 1995; Farrer *et al.* 1997). We addressed the possible confounding between genotypes at the *SOD2* locus and the *APOE* locus in two ways. We first stratified the CGS data according to whether or not the ε4 homozygote genotype was present for any member of the pedigree (64 families) or not (139 families). These subgroups are referred to as the HM4 and NH4 subgroups, respectively. The other method was to adjust the outcome variable for *APOE* genotype and use the resulting adjusted value as a quantitative trait to be analyzed by FBAT, similar to the technique discussed by Lunetta *et al.* (2000). This latter technique is the method suggested by the authors of FBAT for quantitative traits.

We used the genotype IBD sharing test (GIST) (Li *et al.* 2004) to determine whether the genotypes of the four SNPs examined here could account for the linkage evidence previously noted in this region of chromosome 6 (Blacker *et al.* 2003).

Results

The minor allele frequencies of each of the four SNPs in *SOD2* in the CGS (203 families) are shown in Table 1 (the allele frequencies in the LOAD subsample were not appreciably different from the frequencies of the CGS). These values are calculated from all the typed individuals in the respective sample using the AFREQ function available in FBAT. Single-locus association results for both the CGS and the LOAD subset assuming a general genetic model are shown in Table 2. These results indicate that the alleles resulting in heterozygotes at each locus are consistently, although not always significantly, transmitted more frequently to cases than expected under the null hypothesis of no association between the locus and occurrence of AD (CGS: $P = 0.010$, 0.037, 0.064 and 0.343, respectively; LOAD: $P = 0.007$, 0.018, 0.044 and 0.104, respectively). At all but the 3′UTR locus, the major allele homozygous genotype (genotype C/C, T/T, and T/T respectively) is transmitted less frequently to cases than expected under the null hypothesis (CGS: $P = 0.009$, 0.026 and 0.029, respectively; LOAD: $P = 0.026$, 0.025 and 0.031, respectively). The transmission disequilibria of the minor allele homozygous genotypes at these three loci (genotypes T/T, C/C and G/G, respectively) are not significant, nor are any of the results at the fourth locus with this general genotypic model. Based on these results, all haplotype analyses were done assuming a dominant genetic model.

Table 1: Genotyped loci in the CGS

Locus	Name	Location	Major allele	Minor allele	Minor allele frequency
1	rs2758346	5′UTR (nt-1222)	C	T	0.487
2	rs4880	Exon 2 (nt1183)	T	C	0.493
3	rs2855116	Intron 3 (nt5777)	T	G	0.464
4	rs5746136	3′UTR (nt11115)	G	A	0.309

The frequency of the minor allele in the full CGS is shown.

The results of single-locus, family-based tests of association, assuming a dominant genetic model, are shown in Table 3. It is worth noting that restricting the analysis to the LOAD group resulted in similar results, except now the associations for all four SNPs are significant, even though the sample sizes are smaller. When the analyses are repeated for the *APOE*-adjusted traits, we noted little qualitative difference in the results (data not shown), so only the results based on the affection status are reported.

When these tests were performed in the NH4 subgroup, there were no qualitative differences in the results. None of the association results were significant in the HM4 subgroup, possibly because of the loss of power in this much smaller group, but the direction of the association was consistent with the above results. Several *ad hoc* adjustments using *APOE* genotype were attempted, and none resulted in any qualitative difference in results.

Haplotype frequencies, estimated within the FBAT program, were very similar between the LOAD subset and those of the CGS (see Table 4). The haplotype and single-locus allele frequencies were used to form an estimate of the measure of linkage disequilibrium (LD) between pairs of loci. Specifically, in the CGS, the value of D′ between loci 1 and 2 is 0.980 and $r^2 = 0.960$; between loci 2 and 3, D′ = 0.996 and $r^2 = 0.897$; and between loci 3 and 4, D′ = 0.972 and $r^2 = 0.372$. Within the LOAD subset, between loci 1 and 2, D′ = 0.984 and $r^2 = 0.960$; between loci 2 and 3, D′ = 1.0 and $r^2 = 0.908$; and between loci 3 and 4, D′ = 0.972 and $r^2 = 0.355$. We, thus, have very strong LD among the first three loci, and slightly less LD with the fourth locus. Data available from the HapMap project (HapMapConsortium 2003) indicate that this entire gene lies within a single haplotype block.

Results of association tests between haplotypes among the four loci and development of AD are summarized in Table 4. Because of the high LD in this region, we cannot view these tests as independent. For this reason, we do not feel that a conservative multiple test correction, such as the Bonferroni correction, would be appropriate. The global tests of haplotypes reported in Table 4 allow us to partially offset the multiple testing problem. Although every possible combination of loci has a haplotype that is preferentially transmitted to cases under a dominant model, the global tests indicate that the most promising results include only the first three loci.

When the data were analyzed with the GIST program, we found that only genotypes at the fourth locus explained a significant proportion ($P = 0.004$) of the linkage signal at

Table 2: Family-based, single-locus association test results with general genotype model

		Full CGS sample			LOAD subsample		
Locus	Genotype	Families*	$Z^\dagger$	$P^\ddagger$	Families*	$Z^\dagger$	$P^\ddagger$
1	C/C	82	−2.597	0.009	59	−2.22	0.026
1	C/T	129	2.584	0.010	90	2.698	0.007
1	T/T	74	−0.704	0.482	47	−1.245	0.213
2	C/C	78	−0.451	0.652	49	−0.787	0.431
2	C/T	132	2.082	0.037	93	2.358	0.018
2	T/T	81	−2.225	0.026	59	−2.237	0.025
3	G/G	75	−0.151	0.880	49	−0.372	0.710
3	G/T	131	1.85	0.064	96	2.01	0.044
3	T/T	84	−2.185	0.029	63	−2.16	0.031
4	A/A	46	−1.281	0.200	33	−1.855	0.064
4	A/G	114	0.948	0.343	75	1.624	0.104
4	G/G	86	−0.159	0.874	55	−0.45	0.652

A positive value of *Z* indicates transmission in excess of that expected under the null hypothesis of no association in the presence of linkage, while a negative value indicates a deficiency of transmission below that expected under the null hypothesis.

*Number of families informational for a test of transmission of each genotype to affected offspring.

†Normalized test statistic.

‡*P* value for the test of transmission.

Table 3: Family-based, single-locus association test results

		CGS sample		LOAD subsample	
Locus	Allele*	Families†	*P* value	Families†	*P* value
1	T	80	0.01	57	0.03
2	C	78	0.03	56	0.03
3	G	80	0.03	60	0.03
4	G	45	0.16	32	0.03

*The allele transmitted more frequently to affected offspring assuming a dominant genetic model.

†Number of families informational for the test of transmission.

6q. Genotypes at the first two loci explained a marginal proportion of the linkage signal ($P = 0.064$, and 0.065, respectively). The full four-locus haplotype (TCGG vs. all others) also explained only a marginal proportion of the linkage signal ($P = 0.058$).

Discussion

Oxidative damage is believed to be an important factor in the pathogenesis of AD, and SOD2 plays a key role in repairing oxidative damage. All four SNPs in SOD2 examined are located near known or potentially functional regions of the gene and thus could possibly contribute to the associated risk. For example, locus 1 (rs2758346) is located in the *SOD2* promoter, 65 bp upstream of a nuclear factor κB (NF-κB) transcription factor binding site (TFBS) (Xu *et al.* 2002). The TFBS for other transcription factors are located within 1 kb of this SNP (Wan *et al.* 1994; Xu *et al.* 2002) Locus 2 is the well-characterized C → T (Ala16Val) SNP, rs4880, in the mitochondrial targeting sequence (MTS) of SOD2. The C allele, which retains the alpha helical structure of the protein for normal activity of the enzyme, is over-represented in the patients. The presence of the Ala variant in the MTS of SOD2 for normal insertion and activity in the mitochondria, in addition to other genetic changes, could result in increased activity of SOD2 and induction of oxidative stress (Shimoda-Matsubayashi *et al.* 1997). The third SNP, rs2855116, is located in intron 3 and is ∼6.5 kb downstream from an additional regulatory region located in intron 2 of *SOD2*, the tumor necrosis factor responsive element (TNFRE), that is a potent transcription enhancer with binding sites for several transcription factors, including tumor necrosis factor, NF-κB (Guo *et al.* 2003; Jones *et al.* 1997; Mao *et al.* 2006). Another TFBS that plays a role in glial cells, PAX 6, is located within 100 bp of rs2855116 (Heins *et al.* 2002; Nacher *et al.* 2005), and nearby intronic sequences are specific to *Homo sapiens* only (Karolchik *et al.* 2003; Kent *et al.* 2002). The last SNP, rs5746136, is located ∼65 bp downstream of the poly A site. It is also <1 kb upstream from SP1 and NF-κB transcription element sequences(Wan *et al.* 1994).

The LD information in this region indicates that the tests of association among the first three SNPs are almost redundant because of the high values of both D′ and r^2. The global tests

Table 4: Family-based haplotype association test results

		CGS sample				LOAD subsample			
Loci	Haplotype	Frequency*	*P*†	Families‡	*P*§	Frequency*	*P*†	Families‡	*P*§
12	TC	0.480	0.015	75	0.008	0.470	0.021	54	0.013
13	TG	0.453	0.045	76	0.007	0.445	0.050	56	0.012
14	TG	0.482	0.083	107	0.013	0.468	0.061	76	0.016
23	CG	0.458	0.139	79	0.022	0.451	0.102	58	0.022
24	CG	0.484	0.179	105	0.035	0.473	0.095	76	0.025
34	GG	0.458	0.119	105	0.019	0.449	0.068	75	0.013
123	TCG	0.452	0.036	76	0.006	0.446	0.042	56	0.010
124	TCG	0.480	0.078	69	0.014	0.470	0.043	50	0.013
134	TGG	0.453	0.112	71	0.012	0.446	0.035	52	0.012
234	CGG	0.456	0.215	104	0.026	0.449	0.067	75	0.021
1234	TCGG	0.446	0.095	71	0.009	0.436	0.026	52	0.009

*Frequency of the specified haplotype.

†*P* value for the global test of all haplotypes across the given loci.

‡The number of families informational for the test of transmission of the specific haplotype.

§*P* value for test of transmission of the specified haplotype.

of association performed to control for multiple testing of haplotypes support this observation. The results from GIST indicate that one or more SNPs within our four SNP haplotype may account for a part of the evidence for linkage seen in this sample.

The haplotype apparently associated with AD in this sample could be in LD with other genetic variations that affect function of this gene. According to the dbSNP database build 125 (http://www.ncbi.nlm.nih.gov/projects/SNP/), there are over 100 SNPs in the gene, including the 3′ and 5′ UTRs, which may be functional, including eight coding SNPs, six of which are nonsynonymous, eight additional SNPs located within the 5′ regulatory region or the TNFRE and over a dozen additional SNPs in enhancer splicing elements (ESEs) or within 100 bp of intron/exon junctions.

The significant amount of high LD across this relatively small gene (~14 kb) presents a challenge to determining a specific causative polymorphism. Any one of four SNPs we genotyped, or an SNP that is in LD, which is within or close to a functional region of the gene (e.g. exon, TFBS, ESEs, exon/intron junctions), could enhance or reduce expression of *SOD2* and/or the activity of the gene product. We are currently performing association mapping using a dense SNP strategy, excluding redundant SNPs. In addition, we are sequencing AD cases from our most informative families segregating the high-risk haplotypes in the hopes of determining the functional causative mutation (Fearnhead *et al.* 2004; Pritchard & Cox 2002).

References

Apelt, J., Bigl, M., Wunderlich, P. & Schliebs, R. (2004) Aging-related increase in oxidative stress correlates with developmental pattern of beta-secretase activity and beta-amyloid plaque formation in transgenic Tg2576 mice with Alzheimer-like pathology. *Int J Dev Neurosci* **22**, 475–484.

Ashley-Koch, A.E., Shao, Y., Rimmler, J.B., Gaskell, P.C., Welsh-Bohmer, K.A., Jackson, C.E., Scott, W.K., Haines, J.L. & Pericak-Vance, M.A. (2005) An autosomal genomic screen for dementia in an extended Amish family. *Neurosci Lett* **379**, 199–204.

Beal, M.F., Bodis-Wollner, I. & Howell, N. (1997) *Mitochondria and Free Radicals in Neurodegenerative Diseases*. Wiley-Liss, New York.

Blacker, D., Haines, J.L., Rodes, L., Terwedow, H., Go, R.C., Harrell, L.E., Perry, R.T., Bassett, S.S., Chase, G., Meyers, D., Albert, M.S. & Tanzi, R. (1997) ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. *Neurology* **48**, 139–147.

Blacker, D., Bertram, L., Saunders, A.J. *et al.* (2003) Results of a high-resolution genome screen of 437 Alzheimer's disease families. *Hum Mol Genet* **12**, 23–32.

Christen, Y. (2000) Oxidative stress and Alzheimer disease. *Am J Clin Nutr* **71**, 621S–629S.

Corder, E.H., Saunders, A.M., Pericak-Vance, M.A. & Roses, A.D. (1995) There is a pathologic relationship between ApoE-epsilon 4 and Alzheimer's disease. *Arch Neurol* **52**, 650–651.

Coskun, P.E., Beal, M.F. & Wallace, D.C. (2004) Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc Natl Acad Sci USA* **101**, 10726–10731.

Davies, K.J. (1995) Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp* **61**, 1–31.

Dyrks, T., Dyrks, E., Hartmann, T., Masters, C. & Beyreuther, K. (1992) Amyloidogenicity of beta A4 and beta A4-bearing amyloid protein precursor fragments by metal-catalyzed oxidation. *J Biol Chem* **267**, 18210–18217.

Esposito, L., Raber, J., Kekonius, L., Yan, F., Yu, G.Q., Bien-Ly, N., Puolivali, J., Scearce-Levie, K., Masliah, E. & Mucke, L. (2006) Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. *J Neurosci* **26**, 5167–5179.

Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., Pericak-Vance, M.A., Risch, N. & van Duijn, C.M. (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* **278**, 1349–1356.

Fearnhead, N.S., Wilding, J.L., Winney, B., Tonks, S., Bartlett, S., Bicknell, D.C., Tomlinson, I.P., Mortensen, N.J. & Bodmer, W.F. (2004) Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas. *Proc Natl Acad Sci USA* **101**, 15992–15997.

Fridovich, I. (1978) The biology of oxygen radicals. *Science* **201**, 875–880.

Fridovich, I. (1995) Superoxide radical and superoxide dismutases. *Annu Rev Biochem* **64**, 97–112.

Gabbita, S.P., Lovell, M.A., & Markesbery, W.R. (1998) Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J Neurochem* **71**, 2034–2040.

Guo, Z., Boekhoudt, G.H. & Boss, J.M. (2003) Role of the intronic enhancer in tumor necrosis factor-mediated induction of manganous superoxide dismutase. *J Biol Chem* **278**, 23570–23578.

HapMapConsortium (2003) The International HapMap Project. *Nature* **426**, 789–796.

Heins, N., Malatesta, P., Cecconi, F., Nakafuku, M., Tucker, K.L., Hack, M.A., Chapouton, P., Barde, Y.A. & Gotz, M. (2002) Glial cells generate neurons: the role of the transcription factor Pax6. *Nat Neurosci* **5**, 308–315.

Hinerfeld, D., Traini, M.D., Weinberger, R.P., Cochran, B., Doctrow, S.R., Harry, J. & Melov, S. (2004) Endogenous mitochondrial oxidative stress: neurodegeneration, proteomic analysis, specific respiratory chain defects, and efficacious antioxidant therapy in superoxide dismutase 2 null mice. *J Neurochem* **88**, 657–667.

Horvath, S., Xu, X., Lake, S.L., Silverman, E.K., Weiss, S.T. & Laird, N.M. (2004) Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. *Genet Epidemiol* **26**, 61–69.

Jones, P.L., Ping, D. & Boss, J.M. (1997) Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. *Mol Cell Biol* **17**, 6970–6981.

Karolchik, D., Baertsch, R., Diekhans, M., Furey, T.S., Hinrichs, A., Lu, Y.T., Roskin, K.M., Schwartz, M., Sugnet, C.W., Thomas, D.J., Weber, R.J., Haussler, D. & Kent, W.J. (2003) The UCSC Genome Browser Database. *Nucleic Acids Res* **31**, 51–54.

Kehoe, P., Wavrant-De Vrieze, F., Crook, R. *et al.* (1999) A full genome scan for late onset Alzheimer's disease. *Hum Mol Genet* **8**, 237–245.

Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M. & Haussler, D. (2002) The human genome browser at UCSC. *Genome Res* **12**, 996–1006.

Krapfenbauer, K., Engidawork, E., Cairns, N., Fountoulakis, M. & Lubec, G. (2003) Aberrant expression of peroxiredoxin subtypes in neurodegenerative disorders. *Brain Res* **967**, 152–160.

Laird, N.M., Horvath, S. & Xu, X. (2000) Implementing a unified approach to family-based tests of association. *Genet Epidemiol* **19** (Suppl 1): S36–S42.

Lake, S.L., Blacker, D. & Laird, N.M. (2000) Family-based tests of association in the presence of linkage. *Am J Hum Genet* **67**, 1515–1525.

Lee, J.H., Cheng, R., Santana, V., Williamson, J., Lantigua, R., Medrano, M., Arriaga, A., Stern, Y., Tycko, B., Rogaeva, E., Wakutani, Y., Kawarai, T., St George-Hyslop, P. & Mayeux, R. (2006) Expanded genomewide scan implicates a novel locus at 3q28 among Caribbean Hispanics with familial Alzheimer disease. *Arch Neurol* **63**, 1591–1598.

Li, C., Scott, L.J. & Boehnke, M. (2004) Assessing whether an allele can account in part for a linkage signal: the Genotype-IBD Sharing Test (GIST). *Am J Hum Genet* **74**, 418–431.

Lunetta, K.L., Faraone, S.V., Biederman, J. & Laird, N.M. (2000) Family-based tests of association and linkage that use unaffected sibs, covariates, and interactions. *Am J Hum Genet* **66**, 605–614.

Lynn, S., Huang, E.J., Elchuri, S., Naeemuddin, M., Nishinaka, Y., Yodoi, J., Ferriero, D.M., Epstein, C.J. & Huang, T.T. (2005) Selective neuronal vulnerability and inadequate stress response in superoxide dismutase mutant mice. *Free Radic Biol Med* **38**, 817–828.

Mao, X., Moerman-Herzog, A.M., Wang, W. & Barger, S.W. (2006) Differential transcriptional control of the superoxide dismutase-2 {kappa}B element in neurons and astrocytes. *J Biol Chem* **281**, 35863–35872.

Markesbery, W.R. (1999) The role of oxidative stress in Alzheimer disease. *Arch Neurol* **56**, 1449–1452.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. & Stadlan, E.M. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939–944.

Mecocci, P., MacGarvey, U. & Beal, M.F. (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann Neurol* **36**, 747–751.

Miranda, S., Opazo, C., Larrondo, L.F., Munoz, F.J., Ruiz, F., Leighton, F. & Inestrosa, N.C. (2000) The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer's disease. *Prog Neurobiol* **62**, 633–648.

Nacher, J., Varea, E., Blasco-Ibanez, J.M., Castillo-Gomez, E., Crespo, C., Martinez-Guijarro, F.J. & McEwen, B.S. (2005) Expression of the transcription factor Pax 6 in the adult rat dentate gyrus. *J Neurosci Res* **81**, 753–761.

Nunomura, A., Perry, G., Pappolla, M.A., Wade, R., Hirai, K., Chiba, S. & Smith, M.A. (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J Neurosci* **19**, 1959–1964.

Pericak-Vance, M.A., Grubber, J., Bailey, L.R., Hedges, D., West, S., Santoro, L., Kemmerer, B., Hall, J.L., Saunders, A.M., Roses, A.D., Small, G.W., Scott, W.K., Conneally, P.M., Vance, J.M. & Haines, J.L. (2000) Identification of novel genes in late-onset Alzheimer's disease. *Exp Gerontol* **35**, 1343–1352.

Perry, R.T., Collins, J.S., Harrell, L.E., Acton, R.T. & Go, R.C. (2001) Investigation of association of 13 polymorphisms in eight genes in southeastern African American Alzheimer disease patients as compared to age-matched controls. *Am J Med Genet* **105**, 332–342.

Pinteaux, E., Copin, J.C., Ledig, M. & Tholey, G. (1996) Modulation of oxygen-radical-scavenging enzymes by oxidative stress in primary cultures of rat astroglial cells. *Dev Neurosci* **18**, 397–404.

Pritchard, J.K. & Cox, N.J. (2002) The allelic architecture of human disease genes: common disease-common variant . . . or not? *Hum Mol Genet* **11**, 2417–2423.

Shimoda-Matsubayashi, S., Matsumine, H., Kobayashi, T., Nakagawa-Hattori, Y., Shimizu, Y. & Mizuno, Y. (1996) Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. *Biochem Biophys Res Commun* **226**, 561–565.

Shimoda-Matsubayashi, S., Hattori, T., Matsumine, H., Shinohara, A., Yoritaka, A., Mori, H., Kondo, T., Chiba, M. & Mizuno, Y. (1997) Mn SOD activity and protein in a patient with chromosome 6-linked autosomal recessive parkinsonism in comparison with Parkinson's disease and control. *Neurology* **49**, 1257–1262.

Smith, M.A. (1997) Morphological aspects of oxidative damage in Alzheimer's disease. In Beal, M.F., Bodis-Wollner, I. & Howell, N. (eds), *Mitochondria and Free Radicals in Neurodegenerative Diseases*. Wiley-Liss, New York, pp. 335–342.

Smith, M.A., Perry, G., Richey, P.L., Sayre, L.M., Anderson, V.E., Beal, M.F. & Kowall, N. (1996) Oxidative damage in Alzheimer's. *Nature* **382**, 120–121.

Smith, M.A., Hirai, K., Hsiao, K., Pappolla, M.A., Harris, P.L., Siedlak, S.L., Tabaton, M. & Perry, G. (1998) Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. *J Neurochem* **70**, 2212–2215.

Szczesny, B., Hazra, T.K., Papaconstantinou, J., Mitra, S. & Boldogh, I. (2003) Age-dependent deficiency in import of mitochondrial DNA glycosylases required for repair of oxidatively damaged bases. *Proc Natl Acad Sci USA* **100**, 10670–10675.

Tomkins, J., Banner, S.J., McDermott, C.J. & Shaw, P.J. (2001) Mutation screening of manganese superoxide dismutase in amyotrophic lateral sclerosis. *Neuroreport* **12**, 2319–2322.

Varadarajan, S., Yatin, S., Aksenova, M. & Butterfield, D.A. (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. *J Struct Biol* **130**, 184–208.

Wan, X.S., Devalaraja, M.N. & St Clair, D.K. (1994) Molecular structure and organization of the human manganese superoxide dismutase gene. *DNA Cell Biol* **13**, 1127–1136.

Warner, B.B., Stuart, L., Gebb, S. & Wispe, J.R. (1996) Redox regulation of manganese superoxide dismutase. *Am J Physiol* **271**, L150–L158.

Weisiger, R.A. & Fridovich, I. (1973) Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization. *J Biol Chem* **248**, 4793–4796.

Xu, Y., Porntadavity, S. & St Clair, D.K. (2002) Transcriptional regulation of the human manganese superoxide dismutase gene: the role of specificity protein 1 (Sp1) and activating protein-2 (AP-2). *Biochem J* **362**, 401–412.

Zhang, P., Anglade, P., Hirsch, E.C., Javoy-Agid, F. & Agid, Y. (1994) Distribution of manganese-dependent superoxide dismutase in the human brain. *Neuroscience* **61**, 317–330.

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