Suggestive Synergy Between Genetic Variants in TF and HFE as Risk Factors for Alzheimer's Disease

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Alzheimer's disease (AD) is a complex disease that is likely influenced by many genetic and environmental factors. Citing evidence that iron may play a role in AD pathology, Robson et al. [Robson et al.(2004); J Med Genet 41:261–265] reported that epistatic interaction between rs1049296 (P589S) in the transferrin gene (*TF*) and rs1800562 (C282Y) in the hemochromatosis gene (*HFE*) results in significant association with risk for AD. In this study we attempted to replicate their findings in a total of 1,166 cases and 1,404 controls from three European and European American populations. Allele and genotype frequencies were consistent across the three populations. Using synergy factor analysis (SFA) and Logistic Regression analysis we tested each population and the combined sample for interactions between these two SNPs and risk for AD. We observed significant association between bi-carriers of the minor alleles of rs1049296 and rs1800562 in the combined sample using SFA ($P = 0.0016$, synergy factor = 2.71) and adjusted SFA adjusting for age and presence of the APOE epsilon 4 allele ($P = 0.002$, OR = 2.4). These results validate those of the previous report and support the hypothesis that iron transport and regulation play a role in AD pathology.   © 2009 Wiley-Liss, Inc.

Key words: transferrin; hemachromatosis gene; Alzheimer's disease; epistasis; genetic association

Alzheimer's disease (AD) is a complex disease that is likely influenced by many genetic and environmental factors. Recent studies using meta-analyses and genome-wide association studies (GWAS) have provided increasing evidence for new genetic risk factors [Coon et al., 2007; Bertram et al., 2008; Li et al., 2008; Beecham et al., 2009; Carrasquillo et al., 2009; Feulner et al., 2009; Harold et al., 2009; Lambert et al., 2009]. Evidence from AlzGene (alzgene.org) meta-analyses provides support for several risk variants with small effect sizes [Bertram et al., 2007]. Two recent studies investigated 29 such variants from the Alzgene meta-analyses for association in a large family-based sample [Schjeide et al., 2009] and in samples in which cerebrospinal fluid (CSF) biomarkers have been measured including amyloid-beta (Aβ) levels

How to Cite this Article:
Kauwe JSK, Bertelsen S, Mayo K, Cruchaga C, Abraham R, Hollingworth P, Harold D, Owen MJ, Williams J, Lovestone S, Morris JC, Goate AM, Alzheimer's Disease Neuroimaging Initiative. 2010. Suggestive Synergy Between Genetic Variants in TF and HFE as Risk Factors for Alzheimer's Disease.

Am J Med Genet Part B 153B:955–959.

[Kauwe et al., 2009]. Among the consistent findings, one SNP in *TF*, rs1049296 that results in a missense coding polymorphism (P589S), showed significant association in both studies [Kauwe et al., 2009; Schjeide et al., 2009]. Like many other genetic associations, results from various studies with rs1049296 have yielded both positive [van Rensburg et al., 1993; Namekata et al., 1997; Van Landeghem et al., 1998; Zambenedetti et al., 2003; Robson et al., 2004; Schjeide et al., 2009] and negative results [Emahazion et al., 2001; Kim et al., 2001; Hussain et al., 2002; Lleo et al., 2002; Rondeau et al., 2006; Blazquez et al., 2007; Reiman et al., 2007]. Such inconsistency may indicate that the association is spurious, or that the studies lack statistical power [Bertram and Tanzi, 2004]. It has also been suggested that lack of replication in genetic association studies is not surprising given the extent of genetic and environmental heterogeneity [Gorroochurn et al., 2007] and may even be a "signature of epistasis" [Wade, 2001; Moore and Williams, 2005]. Evidence for epistatic interaction between *APOE e4* and genetic variation in *BACE* has been consistently replicated, though the nature of the interaction has yet to be characterized [Combarros et al., 2008]. It has also been reported that a synergy between rs1049296 and rs1800562 in the hemochromatosis gene (*HFE*) has strong association with risk for AD, with individuals that carry the minor allele at both loci having fivefold greater risk for disease using both synergy factor analysis (SFA) and logistic regression [Robson et al., 2004]. Both of these variants are amino acid substitutions (rs1049296 is P589S; rs1800562 is C282Y). In this study we attempt to replicate the report of epistasis between rs1049296 and rs1800562 and association with risk for LOAD in a total of 1,166 cases and 1,404 controls from three European and European American samples.

The case–control series for this study were collected at three different sites. Basic sample characteristics for each series are shown in Table I. The Washington University (WU) case–control series used in this study was collected through the WU Alzheimer's Disease Research Center (ADRC) patient registry. Cases in this series received a diagnosis of dementia of the Alzheimer's type (DAT), using criteria equivalent to the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer's Disease and Related Disorders Association, modified slightly to include AD as a diagnosis for individuals aged >90 years [McKhann et al., 1984; Berg et al., 1998]. A total of 331 unrelated DAT cases with a minimum age at onset (AAO) of 60 years were recruited for the study. DNA from 385 age- and sex-matched non-demented controls aged >60 years at assessment were obtained through the ADRC.

We also used clinical data and DNA samples from 631 individuals with late-onset AD and 769 control subjects ascertained from both community and hospital settings in the UK collected as part of the Medical Research Council genetic resource for late-onset AD (MRC Sample). A detailed description of the ascertainment and assessment of this sample has been reported elsewhere [Morgan et al., 2007].

Data from 199 AD cases and 188 controls from the Alzheimer's disease neuroimaging initiative (ADNI) were used. Data used in the preparation of this article were obtained from the ADNI database on May 15, 2008 (www.loni.ucla.edu\ADNI). The Principle Investigator of this initiative is Michael W. Weiner, M.D., VA Medical Center and University of California—San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55–90, to participate in the research—approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for "2 years." For up-to-date information see www.adni-info.org. Finally, genotype counts from Robson et al. [2004] were used in our meta-analysis.

Rs1049296 and rs1800562 were genotyped using Sequenom genotyping technology. Single SNP allelic associations were evaluated using Fisher's exact test and genotypic associations were evaluated with logistic regression using the additive, dominant, and recessive models. SFA and adjusted SFA were used to evaluate the size and significance of the effect of interaction between rs1049296 and rs1800562 and risk for AD with minor allele non-carriers as the reference group [Lehmann et al., 2001; Robson et al., 2004; Combarros et al., 2008; Cortina-Borja et al., 2009].

Neither rs1049296 nor rs1800562 showed association with risk for AD in single SNP tests using the additive model (Table II). Analyses using the recessive and dominant genetic models and models using *APOE e4* as a covariate also failed to detect association in the single SNP tests. Allele and genotype frequencies appeared consistent between males and females. SFA in the WU series was significant with a *P*-value of 0.0032 and a synergy factor of 5.99 (95% Confidence Interval (CI): 1.82–19.69) for bi-carriers using non-carriers as the reference. SFA in the MRC and ADNI samples was not significant (Table III) but showed trends in the same direction. A large number of samples from the WU and MRC were recently included in a genome-wide association study. Extensive analyses using Eigenstrat [Price et al., 2006] showed no evidence of population stratification between these two samples [Harold et al., 2009]. Allele and genotype frequencies for each SNP were very similar between the three samples (Table II). A combined analysis of our samples shows significant association with SFA unadjusted for covariates and adjusted SFA including site, gender,

TABLE I. Sample Characteristics

	N	Age	% Female	% e4 pos
WU				
Cases	331	76.6	0.62	53.6
Controls	385	77.7	0.61	23.2
MRC				
Cases	631	75.7	0.73	62.2
Controls	769	76.1	0.62	23.4
ADNI				
Cases	199	71.8	0.56	65.0
Controls	188	77.7	0.55	27.8

The number of individuals, age (mean age at onset for cases, mean age at last assessment for controls), percent of the sample that is female (%female), and percent of the sample that carries the *APOE e4* allele (% e4 pos) is shown for the Washington University (WU), Medical Research Council (MRC), and Alzheimer's disease Neuroimaging Initiative (ADNI) series.

TABLE II. Allele and genotype frequencies and P-values from allelic and genotypic associations

	SNP	MAF	Allelic P-value	Geno. Freq.	Geno. P-value
WU	rs1049296	0.15	0.14	0.02/0.27/0.71	0.13
	rs1800562	0.04	0.89	<0.01/0.08/0.92	0.89
MRC	rs1049296	0.15	0.72	0.03/0.25/0.72	0.72
	rs1800562	0.02	0.58	<0.01/0.03/0.97	0.59
ADNI	rs1049296	0.15	0.61	0.03/0.25/0.72	0.61
	rs1800562	0.01	0.77	<0.01/0.02/0.98	0.72
COMB	rs1049296	0.15	0.46	0.02/0.25/0.72	0.46
	rs1800562	0.02	0.46	<0.01/0.04/0.90	0.45

Minor allele frequencies in cases and controls (MAF), genotype frequencies (Geno. Freq.), and P-values for allelic association from Fisher's Exact test and genotypic association from logistic regression using the additive genetic model are shown for the Washington University (WU), MRC, and Alzheimer's disease Neuroimaging Initiative (ADNI) series.

age, and *APOE e4* as covariates (Table III). SFA unadjusted for covariates using our three samples and data from the initial report [Robson et al., 2004], is also significant ($P = 5.15 \times 10^{-3}$, OR = 2.72; Table III).

Our findings in the WU series and the combined sample support the previous observation of synergy between rs1049296 and rs1800562 as risk factors for AD. While there were differences in the level of association in the individual samples (possibly due to differences in the sample populations and genetic or clinical heterogeneity; Table III) the unadjusted SF in our combined sample for individuals that carry at least one minor allele at each locus is 2.71 (CI: 1.46–5.05) and the adjusted SF including age and APOE e4 as covariates was 2.4 (CI: 1.38–3.94). This is lower than the SF of 5.1 from the original report but still indicates a higher level of risk for the bi-carriers of these alleles. Individuals that carry the minor allele at only one of these loci do not show significantly increased risk for AD (Table II). In this study bi-carriers make up about 4% of the AD sample. It has been proposed that these individuals may be at higher risk of AD due to increased redox-active iron and oxidative stress [Robson et al., 2004; Lehmann et al., 2006]. Rs1800562 in *HFE* is known to cause iron-overload and hemochromatosis in individuals homozygous for the allele (OMIM-235200). Wild-type HFE binds transferrin receptor 1 (TfR1). HFE with the minor allele "A" of rs1800562 has much lower affinity for TfR1, leaving the

receptor free to bind TF with high affinity [Feder et al., 1998]. This may result in increased uptake of TF bound iron, causing overabsorption of dietary iron and iron deposition in various tissues [Townsend and Drakesmith, 2002]. Wild-type TF is important for iron transport and may be involved in limiting the amyloid aggregation process [Giunta et al., 2004]. While rs1049296 does not appear to affect the ability of TF to bind iron [Zatta et al., 2005], the minor allele "T" shows significant association with increased Aβ42/Aβ40 ratio in the CSF [Kauwe et al., 2009]. Our current knowledge of rs1049296 and rs1800562 implicate both effects on Aβ and iron-overload as possible mechanisms for AD risk. In summary our findings provide support for previous reports of synergy between rs1049296 and rs1800562 as risk variants for AD and support for the hypothesis that iron transport and regulation play a role in AD pathology.

ACKNOWLEDGMENTS

TABLE III. rs1049296 and rs1800562 Interaction Analyses

	SF (95% CI)	SFA P-value	Adj. SF (95% CI)	Adj. SFA P-value
WU	5.99 (1.82–19.69)	0.0032	2.59 (0.96–6.98)	0.061
MRC	2.46 (0.63–9.59)	0.19	1.72 (0.43–6.81)	0.44
ADNI	2.18 (0.80–5.92)	0.13	1.83 (0.85–3.94)	0.12
Combined	2.71 (1.46–5.05)	0.0016	2.4 (1.38–4.19)[a]	0.0020[a]
Robson et al. [2004]	5.4 (1.2–19.9)	0.015	NA	0.014[b]
All samples	2.72 (1.55–4.78)	5.15×10^{-3}	NA	NA

Synergy factors (SF) with 95% confidence intervals and P-values for synergy factor analysis (SFA) in the Washington University (WU), MRC, Alzheimer's disease Neuroimaging Initiative (ADNI) series, combined sample and combined sample including the initial report by Robson et al. [2004] (all samples) are shown. Adjusted SF (Adj. SF) and P-values (Adj. SFA P-value) from adjusted SFA including gender, age, *APOE e4* as covariates are also shown.
[a]Logistic regression analysis for the combined samples includes adjustments for site, gender *APOE e4*, and age.
[b]P-value from adjusted SFA including age and gender as covariates. Only genotypes counts are available in the [Robson et al., 2004] manuscript therefore adjusted SFA, which required gender, age, and *APOE e4* data were not performed in the all of the samples combined (NA).

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