Neurobiology of Aging 34 (2013) 1309.e1–1309.e7

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Discovery by the Epistasis Project of an epistatic interaction between the *GSTM3* gene and the *HHEX/IDE/KIF11* locus in the risk of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 18 April 2012
Received in revised form 13 August 2012
Accepted 17 August 2012
Available online 1 October 2012

Keywords:
Epistasis
Alzheimer's risk
GSTM3
IDE locus

ABSTRACT

Despite recent discoveries in the genetics of sporadic Alzheimer's disease, there remains substantial "hidden heritability." It is thought that some of this missing heritability may be because of gene–gene, i.e., epistatic, interactions. We examined potential epistasis between 110 candidate polymorphisms in 1757 cases of Alzheimer's disease and 6294 control subjects of the Epistasis Project, divided between a discovery and a replication dataset. We found an epistatic interaction, between rs7483 in *GSTM3* and rs1111875 in the *HHEX/IDE/KIF11* gene cluster, with a closely similar, significant result in both datasets. The synergy factor (SF) in the combined dataset was 1.79, 95% confidence interval [CI], 1.35–2.36; $p = 0.00004$. Consistent interaction was also found in 7 out of the 8 additional subsets that we examined post hoc: i.e., it was shown in both North Europe and North Spain, in both men and women, in both those with and without the ε4 allele of apolipoprotein E, and in people older than 75 years (SF, 2.27; 95% CI, 1.60–3.20; $p < 0.00001$), but not in those younger than 75 years (SF, 1.06; 95% CI, 0.59–1.91; $p = 0.84$). The association with Alzheimer's disease was purely epistatic with neither polymorphism showing an independent effect: odds ratio, 1.0; $p \geq 0.7$. Indeed, each factor was associated with protection in the absence of the other factor, but with risk in its presence. In conclusion, this epistatic interaction showed a high degree of consistency when stratifying by sex, the ε4 allele of apolipoprotein E genotype, and geographic region.

1. Introduction

The etiology of sporadic, late-onset Alzheimer's disease (AD) is much more complex than that of the familial, early-onset condition,

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http://dx.doi.org/10.1016/j.neurobiolaging.2012.08.010

which displays dominant Mendelian inheritance. The former depends on both genetic and environmental factors. Uncovering those factors is made difficult by the small effect size each exhibits. For many years, only the ε4 allele of apolipoprotein E (*APOEε4)* was known as a susceptibility allele for sporadic AD. Recently, other reproducible gene candidates, such as *PICALM, CLU, CR1*, and *BIN1* (Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010), have been identified through genome-wide association studies (GWAS). However, their small effect sizes (odds ratios $\leq$1.5) mean that there still remains much heritability to uncover (Manolio et al., 2009). This is believed to be primarily because of the genetically complex and heterogeneous nature of the disorder, with interactions between multiple genetic mutations and polymorphisms, as well as between those and other, nongenetic, factors (Bertram and Tanzi, 2004).

The term, epistasis, was originally coined approximately 100 years ago by William Bateson to represent the masking of one allelic locus by another (Bateson, 1910). Although it has sometimes been used in a wider sense, we use the term here conventionally, i.e., when an increased risk is only seen in the presence of 2 genetic factors and not seen when they act apart. Such interactions may be one cause of the hidden heritability mentioned above. In such cases, studies that examine single loci individually, such as GWAS, will fail to detect an effect. Examples of epistasis in genetic studies on Alzheimer's disease have been reviewed by Combarros et al. (2009a).

Though GWAS have proven effective in detecting single-locus effects, such an unbiased approach might not be appropriate for the study of epistasis. A typical GWAS may examine perhaps 500,000 loci but the number of potential 2-way interactions between those 500,000 loci is more than 100 billion (10^{11}). In order therefore to reduce the number of potential interactions to a manageable figure, a hypothesis-driven approach might be required.

The approach we adopted is shown in Fig. 1 (Study design). We first carried out a systematic review of claims of epistasis in sporadic AD (Combarros et al., 2009a). From that investigation, we selected 31 genes, involved in 32 interactions, with biological plausibility and previous evidence of association with AD. We have previously replicated several of those interactions (Combarros et al., 2009b, 2010; Heun et al, 2012; Kölsch et al., 2012; Lehmann et al., 2012). In this study, we looked instead for potential binary interactions not previously examined. To do that, we used a discovery dataset of 1366 AD cases and 1184 controls and a replication set of 391 AD cases and 5111 controls, both drawn from the Epistasis Project (Fig. 1).

We discovered and replicated an interaction between two single nucleotide polymorphisms (SNPs), rs7483 and rs1111875. Rs7483 is in the *GSTM3* gene, encoding glutathione S-transferase μ3, involved in the detoxification of products of oxidative stress in the brain (Mannervik and Danielson, 1988). Rs1111875 is in the gene cluster of the hematopoietically expressed homeobox (*HHEX*), the insulin-degrading enzyme (*IDE*), and the kinesin family member 11 (*KIF11*). Associations with AD have previously been reported in this region (Carrasquillo et al., 2010).

2. Methods

2.1. Subjects

The Epistasis Project aims to study interactions between genetic loci that affect the risk of AD. It is a collaboration of 7 AD research groups: Bonn, Bristol, Nottingham, Oviedo, Oxford (OPTIMA), Rotterdam, and Santander. Sample characteristics by geographic region are given in Supplementary Table 1. All AD cases were diagnosed "definite" or "probable" by Consortium to Establish A Registry for Alzheimer's Disease (CERAD) (Mirra et al., 1993) or National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984) criteria. AD cases were sporadic, i.e., possible autosomal dominant cases were excluded, based on family history. The median ages (interquartile ranges) of cases were 79.0 (73.0–85.2) and of controls were 76.9 (71.3–83.0). Full details of our sample sets and genotyping methods are given elsewhere (Combarros et al., 2009b). Rs7483 and rs1111875 were directly genotyped, not imputed. Research ethics approval was obtained by each of the participating groups (Supplementary Table 2). All participants of the study gave informed written consent.

2.2. Selection and screening of candidate interactions

Fig. 1 describes our study design in detail. Major features included: a systematic literature review of epistasis in sporadic AD

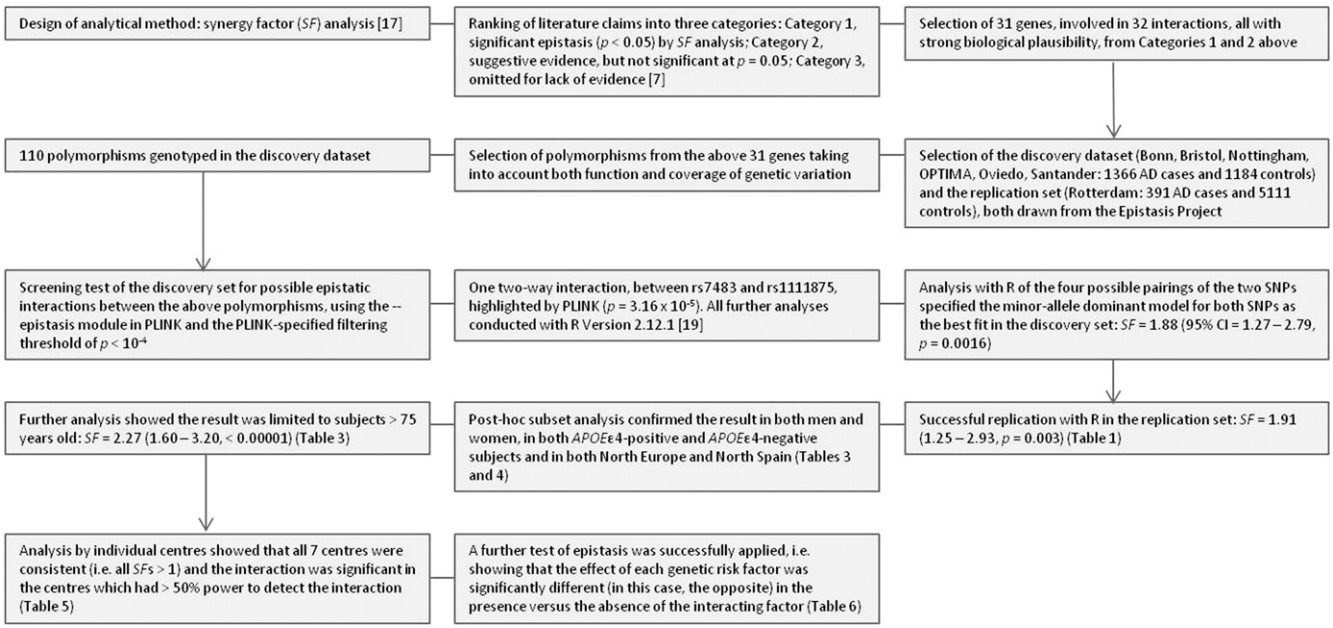

Fig. 1. Study design. Experimental design for selecting genes and single-nucleotide polymorhphisms (SNPs) for study in the Epistasis Project.