Int J Mol Epidemiol Genet 2012;3(1):39-47
www.ijmeg.org /ISSN:1948-1756/IJMEG1112002

Original Article

Interactions between PPAR-α and inflammation-related cytokine genes on the development of Alzheimer's disease, observed by the Epistasis Project

Reinhard Heun [1,2*], Heike Kölsch[1], Carla A Ibrahim-Verbaas[3,4], Onofre Combarros[5], Yurii S Aulchenko[4], Monique Breteler[4], Maaike Schuur[3,4], Cornelia M van Duijn[4], Naomi Hammond[6], Olivia Belbin[7], Mario Cortina-Borja[8], Gordon K Wilcock[9], Kristelle Brown[7], Rachel Barber[10], Patrick G Kehoe[10], Eliecer Coto[11], Victoria Alvarez[11], Michael G Lehmann[12], Panos Deloukas[6], Ignacio Mateo[5], Kevin Morgan[7], Donald R Warden[12], A David Smith[12], Donald J Lehmann[12]

[1]Department of Psychiatry, University of Bonn, Bonn, Germany; [2]Department of Psychiatry, Royal Derby Hospital, Uttoxeter Road, Derby DE22 3WQ, UK; [3]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, the Netherlands; [4]Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, the Netherlands; [5]Neurology Service and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Marqués de Valdecilla University Hospital (University of Cantabria), 39008 Santander, Spain; [6]The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK; [7]School of Molecular Medical Sciences, Institute of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, UK; [8]MRC Centre of Epidemiology for Child Health, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK; [9]Nuffield Department of Medicine, University of Oxford, Level 4, John Radcliffe Hospital, Oxford OX3 9DU, UK; [10]Dementia Research Group, Institute of Clinical Neurosciences, University of Bristol, Frenchay Hospital, Frenchay Bristol, BS16 1LE, UK; [11]Genética Molecular, Hospital Central de Asturias, Oviedo, Spain; [12]Oxford Project to Investigate Memory and Ageing (OPTIMA), University Department of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK.

Received December 24, 2011; Accepted February 22, 2012; Epub February 23, 2012; Published February 28, 2012

Abstract: Objective: Neuroinflammation contributes to the pathogenesis of sporadic Alzheimer's disease (AD). Variations in genes relevant to inflammation may be candidate genes for AD risk. Whole-genome association studies have identified relevant new and known genes. Their combined effects do not explain 100% of the risk, genetic interactions may contribute. We investigated whether genes involved in inflammation, i.e. PPAR-α, interleukins (IL) IL-1α, IL-1β, IL-6, and IL-10 may interact to increase AD risk. Methods: The Epistasis Project identifies interactions that affect the risk of AD. Genotyping of single nucleotide polymorphisms (SNPs) in *PPARA*, *IL1A*, *IL1B*, *IL6* and *IL10* was performed. Possible associations were analyzed by fitting logistic regression models with AD as outcome, controlling for centre, age, sex and presence of apolipoprotein ε4 allele (*APOE*ε4). Adjusted synergy factors were derived from interaction terms ($p<0.05$ two-sided). Results: We observed four significant interactions between different SNPs in *PPARA* and in interleukins *IL1A*, *IL1B*, *IL10* that may affect AD risk. There were no significant interactions between *PPARA* and *IL6*. Conclusions: In addition to an association of the *PPARA* L162V polymorphism with the AD risk, we observed four significant interactions between SNPs in *PPARA* and SNPs in *IL1A*, I*L1B* and *IL10* affecting AD risk. We prove that gene-gene interactions explain part of the heritability of AD and are to be considered when assessing the genetic risk. Necessary replications will require between 1450 and 2950 of both cases and controls, depending on the prevalence of the SNP, to have 80% power to detect the observed synergy factors.

Keywords: AD, genetics, epistasis, inflammation, interleukin, steroid receptors, PPAR-alpha, sporadic, genetic interaction

Introduction

Inflammation plays a relevant role in the development of Alzheimer's disease (AD) [1]. Interleukins (IL) and other mediators of immune response are essential in inflammation, but also in the pathophysiology of AD: (1) IL immunoreactivity is elevated in AD brains [2]; (2)

interleukins (IL-1β, IL-6) are significantly increased in peripheral blood of AD subjects compared with controls [3], (3) in vivo and in vitro studies indicate that IL-1 may trigger AD pathogenesis [4,5]; (4) IL-6 may induce AD-type phosphorylation of tau proteins [6]. The peroxisome proliferator activated receptor alpha (PPAR-α) is involved in inflammation and AD through several pathways: (1) It is part of the steroid hormone receptor superfamily [7]; (2) as a heterodimer with the retinoid X receptor (RXR) it binds to the regulatory region of target genes that may be involved in inflammation control [8,9]; (3) The expression of the PPARα gene (*PPARA)* is significantly reduced in AD brains [10]; (4) PPARα agonists inhibit the β-amyloid-stimulated expression of tumor necrosis factor (TNF-α) and IL-6 reporter genes in monocytes [11].

Large scale genome-wide association studies have identified several already known and some new genes relevant to AD [12, 13]. However, their combined main effects do not explain 100% of the prevalence of AD. Genetic interactions may thus contribute to the development of AD, but are difficult to assess in whole genome association studies assessing up to a million genetic variations at one time without specific a priori hypotheses. Interactions of variations in genes relevant to inflammation, such as the *PPAR* and *IL* genes, may be reasonable candidates that may influence the course of AD. They are not only supported by pathophysiological evidence, as indicated above, but also by previous genetic association studies: (1) Associations of the *PPARA* L162V polymorphism with the risk of AD have been observed [14]; (2) Polymorphisms in *IL1A* gene have been associated with the risk [15,16] and age-at-onset of AD [17]; however, this has not been confirmed by others [18-21]; (3) Variations in *IL1B* have been found to be associated with AD [22,21]; again this result is not unopposed [18]; (4) Bagli et al. [23] reported that polymorphisms of the gene encoding the inflammatory cytokine, IL-6, were related to soluble interleukin-6 receptor levels in AD; (5) Associations between polymorphisms in the *IL6* gene and its promoter may influence the risk of AD [24] (6) A genetic variation of the inflammatory cytokine gene *IL6,* delayed the onset and reduced the risk of sporadic AD [25]; (7) A recent meta-analysis suggested that a polymorphism of the *IL10* gene may be a risk factor for AD [26] (Zhang et al. 2011).

The inconsistency of previously observed associations may be the consequence of variable epistatic interactions between various genes in different populations. The Epistasis Project was designed to focus on such possible interactions. We investigated whether genes involved in inflammation, e.g. the *PPARA* gene, interact with *IL1A*, *IL1B*, *IL6* and *IL10* to increase the risk of AD.

Materials and methods

Study population

The Epistasis Project aims primarily to replicate interactions that have been reported to affect the risk of AD. Sample-sets were drawn from narrow geographical regions with relatively homogeneous, Caucasian populations, by seven AD research groups: Bonn, Bristol, Nottingham, Oxford (OPTIMA), Oviedo, Rotterdam and Santander. Sample characteristics by geographical region are given in Supplementary Table 1. All AD cases were diagnosed "definite" or "probable" by CERAD [27] or NINCDS-ADRDA criteria [28]. AD cases were sporadic, i.e. possible autosomal dominant cases were excluded, based on family history. The median ages (interquartile ranges) of AD cases were 79.0 (73.0-85.2) and of controls were 76.9 (71.3-83.0) years. Research ethical approval was obtained by each of the participating groups (Supplementary Table 2). Comprehensive details of our sample-sets are given elsewhere [29].

Genotyping

Genotyping for the six centres other than Rotterdam was performed at the Wellcome Trust Sanger Institute. The Rotterdam samples were genotyped locally, as previously described [30,31]. For this study, Rotterdam genotyped seven single nucleotide polymorphisms (SNPs), rs135551, rs1800206, rs17561, rs1143634, rs2069837, rs1800896 and rs3024505, and imputed six, rs4253766, rs1800587, rs3783550, rs16944, rs1800795 and rs1800871.

Statistical analysis

We analysed possible associations by fitting logistic regression models with AD diagnosis as

Table 1. Studied SNPs*

Gene	SNP		Minor allele frequencies in controls			Linkage disequilibrium in controls				
			North Europe†	North Spain†	Difference (*p*)	With	North Europe†		North Spain†	
							D'	r^2	D'	r^2
PPARA	rs135551	Intron 2 G/A	27.7% (A)	25.6% (A)	0.18	rs1800206	0.090	0.001	0.173	0.009
	rs1800206	L162V	6.5% (V)	9.3% (V)	**0.002**	rs4253766	0.195	0.0003	0.666	0.006
	rs4253766	Intron 6 C/T	10.9% (T)	12.3% (T)	0.20	rs135551	0.706	0.024	0.766	0.028
IL1A	rs3783550	Intron 6 A/C	31.2% (C)	29.8% (C)	0.39					
IL1B	rs16944	-971 G/A	34.0% (A)	33.5% (A)	0.80					
IL10	rs1800896	-1082 G/A	49.3% (A)	56.9% (A)	**< 0.0001**					

*Data are only supplied for those cytokine gene SNPs that showed nominally significant interactions with *PPARA* SNPs
† North Europe here comprises Bonn, Bristol, Nottingham, Oxford and Rotterdam; North Spain comprises Oviedo and Santander
Significant differences are in bold
SNP = single nucleotide polymorphism; *PPARA*, *IL1A*, *IL1B* and *IL10* are the genes for peroxisome proliferator-activated receptor-α, interleukin-1α, interleukin-1β and interleukin-10, respectively; *D'* = ratio of observed linkage disequilibrium to maximum possible linkage disequilibrium, *r* = correlation coefficient

the outcome variable, controlling for study centre, age, sex and the ε4 allele of apolipoprotein E (*APOE*ε4) in all analyses, using R Version 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria). The adjusted synergy factors [32] were derived from the interaction terms in those models. We controlled for heterogeneity among centres and over-dispersion as described before [31].

We studied three single nucleotide polymorphisms (SNPs) in *PPARA*, three in *IL1A*, two in *IL1B*, two in *IL6* and three in *IL10* (below). Power calculations were based on the observed synergy factor values. Comparisons of allelic frequencies between North Spain and North Europe were obtained using Fisher's exact test. Linkage disequilibrium data were estimated using the R library, genetics (http://cran.r-project.org/web/packages/genetics/index.html). All tests of significance and power calculations were p<0.05, two-sided.

Results

Inflammation-related interactions

Six of the 13 studied SNPs were involved in potential interactions (below); thus, only data from those six are reported here. **Table 1** shows the allelic frequencies of those six SNPs and the structure of linkage disequilibrium of the three in *PPARA*. Of the six SNPs shown in **Table 1**, only *PPARA* L162V was independently associated with AD: odds ratio for LL vs VL+VV = 1.3 (95% confidence interval: 1.04 – 1.5, $p = 0.02$), as previously reported [31]. All the other main effects, on both dominant and recessive models, were of non-significant odds ratios ≤ 1.3. Genotype distributions from each of the seven centres are shown in Supplementary Table 3.

Hardy-Weinberg (HW) analysis was performed for the six SNPs in **Table 1**. Of those 24 analyses, two resulted in HW disequilibrium, whereas one would be expected by chance. The HW disequilibrium in *PPARA* L162V in AD cases, has previously been shown to be due to heterosis, reflecting a true effect of this polymorphism on disease risk [31].

We looked for interactions in AD risk between the three *PPARA* SNPs (rs135551, rs1800206 and rs4253766) and ten SNPs in or near *IL1A* (rs1800587, rs3783550 and rs17561), *IL1B* (rs16944 and rs1143634), *IL6* (rs1800795 and rs2069837) and *IL10* (rs1800896, rs1800871 and rs3024505). We found four nominally significant interactions overall (at $p < 0.05$) and two close to nominal significance ($p \leq 0.06$) (**Table 2**). One other interaction, between *PPARA* rs4253766 CC+CT and *IL10* rs1800871

Table 2. Interactions between *PPARA* and inflammation-related cytokines

PPARA genotype	Cytokine genotype	Synergy factor (95% confidence interval, *p*)		
		Overall	North Europe†	North Spain†
rs1800206 CC vs CG + GG	*IL1A* rs3783550 AA vs AC + CC	**1.6 (1.05-2.3, 0.03)**	1.3 (0.8-2.1, 0.26)	2.0 (0.9-4.4, 0.10)
	IL1B rs16944 GG + GA vs AA	**1.9 (1.05-3.4, 0.03)**	1.7 (0.85-3.25, 0.14)	1.8 (0.5-6.7, 0.38)
	IL10 rs1800896 GA + AA vs GG	1.6 (0.998-2.5, 0.051)	**1.7 (1.03-2.8, 0.04)**	1.7 (0.5-5.9, 0.40)
rs 4253766 CC vs CT + TT	*IL1A* rs3783550 AC + CC vs AA	**1.6 (1.15-2.2, 0.005)**	**1.7 (1.2-2.5, 0.006)**	1.5 (0.7-3.0, 0.27)
	IL10 rs1800896 AA vs GA + GG	**1.5 (1.03-2.2, 0.035)**	1.4 (0.9-2.1, 0.18)	1.9 (0.9-4.1, 0.09)
rs135551 GG vs GA + AA	*IL1B* rs16944 GG vs GA + AA	1.3 (0.99-1.7, 0.06)	1.3 (0.96-1.8, 0.09)	1.4 (0.75-2.5, 0.32)

† North Europe here comprises Bonn, Bristol, Nottingham, Oxford and Rotterdam; North Spain comprises Oviedo and Santander
Nominally significant interactions are in bold; SNP = single nucleotide polymorphism; *PPARA*, *IL1A*, *IL1B* and *IL10* are the genes for peroxisome proliferator-activated receptor-α, interleukin-1α, interleukin-1β and interleukin-10, respectively.

CC, was nominally significant overall (synergy factor = 4.8, $p = 0.04$) and consistent between North Europe and North Spain (synergy factors=5.3 and 5.4, respectively), but was rejected due to heterogeneity between the four countries: Britain, Germany, Spain and the Netherlands.

Discussion

We found a weak association of the *PPARA* L162V polymorphism with the risk of AD, as previously reported [14,31]. We also found four nominally significant interactions and two close to nominal significance (**Table 1** and **2**). By "nominally significant" we mean that these results would not survive correction for multiple testing. However, there was consistency between the findings for North Europe and North Spain (**Table 2**). Nevertheless, we suggest that replication is needed before these results may be considered valid. Such replication would require between 1450 and 2950 cases and equivalent numbers of controls, depending on the interaction, to have 80% power to detect synergy factors (at $p < 0.05$) of the effect sizes described in this paper and SNP prevalences as found in our samples (**Table 2**).

Possible interaction of ILs and PPARA

Activation of microglia results in the synthesis and secretion of the proinflammatory cytokines IL1B, IL-6, and TNF-α, and the chemokine macrophage chemotactic protein-1 [33]. The interaction of microglia or monocytes with beta-amyloid (Aβ) fibrils elicits the activation of a complex tyrosine kinase-based signal transduction cascade, leading to stimulation of multiple independent signalling pathways and ultimately to changes in pro-inflammatory gene expression. The Aβ-stimulated expression of pro-inflammatory genes in myeloid lineage cells is antagonized by the action of a family of ligand-activated nuclear hormone receptors, the peroxisome proliferator-activated receptors (PPARs) [11]. Those authors report that THP-1 monocytes express the PPAR-γ isoform and lower levels of the PPAR-α and PPAR-δ isoforms. Their study explored the action of the PPAR-α isoform and found that PPAR-α agonists inhibited the Aβ-stimulated expression of TNF-α and IL-6 reporter genes in a dose-dependent manner. Moreover, the PPAR-α agonist, WY14643, inhibited macrophage differentiation and COX-2 gene expression. They conclude that PPAR-α acts to suppress a diverse array of inflammatory responses in monocytes.

Whereas other studies have focused more on the interaction between PPAR-γ and other interleukins in inflammation [34], our study may give further impetus to re-addressing the role of PPAR-α and different types of interleukins in AD.

Limitations

The observed genetic interactions between the *PPARA* and *IL* genes are obviously weak, but well within the range of what can be expected for genetic effects and epistatic background in a multifactorial disease like AD, with strong genetic background with multiple low-effect genes involved in its pathophysiology. Of course, the described epistatic effects need further replication. This is especially true as the low synergy factors did not allow for the correction for multiple statistical testing. Consequently, very large samples would be necessary to allow for such correction. However, this approach also risks the failure to detect small, but relevant, genetic and epistatic effects. In this respect, independent replication seems to be a more useful approach than assessing extremely large samples and excessive corrections for multiple testing.

The observation of epistatic genetic interactions does not provide a clear indication of the possible pathophysiologic mechanisms. Consequently, some of the discussion may be seen as speculative. As in many genetic studies, the observation of genetic associations is only the start in assessing the pathophysiology of a disease. This is especially difficult when the functional relevance of non-coding SNPs is not yet known. In this respect, our data can be seen as a starting point to assess the pathophysiology of inflammatory pathways in AD.

Acknowledgements

We are most grateful to the Moulton Charitable Foundation for a grant to fund the Epistasis Project, to all those who have provided support for the individual clinical studies and to the Alzheimer's Research Trust. GKW was partly funded by the NIHR Biomedical Research Centre Programme, Oxford. MCB benefited from funding from the Medical Research Council. Reinhard Heun received funding from German Research Foundation (DFG), University of Bonn (Bonfor), American Alzheimer Association (AAA), Alzheimer Research Initiative (AFI), the Ministry of Education and Research (BMBF), Alzheimer Research Trust and BIG lottery fund. Gordon Wilcock and Donald Warden are part-funded by NIHR Biomedical Research Centre Oxford.

Address correspondence to: Professor Reinhard Heun, Department of Psychiatry, Royal Derby Hospital,Uttoxeter Road, Derby DE22 3WQ, UK, E-mail: heun@gmx.com

References

[1] Johnston H, Boutin H, Allan SM. Assessing the contribution of inflammation in models of Alzheimer's disease. Biochem Soc Trans. 2011;39:886-90.

[2] Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CL 3rd, Araoz C. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci U S A. 1989;86:7611-5.

[3] Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry. 2010;68:930-41.

[4] Shaftel SS, Griffin WS, O'Banion MK. The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. J Neuroinflammation. 2008;5:7.

[5] Sheng JG, Ito K, Skinner RD, Mrak RE, Rovnaghi CR, Van Eldik LJ, Griffin WS. In vivo and in vitro evidence supporting a role for the inflammatory cytokine interleukin-1 as a driving force in Alzheimer pathogenesis. Neurobiol Aging. 1996;17:761-6.

[6] Quintanilla RA, Orellana DI, González-Billault C, Maccioni RB. Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. Exp Cell Res. 2004;295:245-57.

[7] Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, Reddy JK (1995) Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPARgamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. Proc Natl Acad Sci USA 92:7921-7925.

[8] Escher P, Wahli W. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat Res. 2000;448:121-38.

[9] Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature. 2000;405:421-4.

[10] De la Monte SM, Wands JR. Molecular indices of oxidative stress and mitochondrial dysfunc-

tion occur early and often progress with severity of Alzheimer's disease. J Alzheimers Dis. 2006;9:167-81.

[11] Combs CK, Bates P, Karlo JC, Landreth GE. Regulation of beta-amyloid stimulated proinflammatory responses by peroxisome proliferator-activated receptor alpha. Neurochem Int. 2001;39:449-57.

[12] Jones L, Holmans PA, Hamshere ML, Harold D, Moskvina V, Ivanov D, Pocklington A, Abraham R, Hollingworth P, Sims R, Gerrish A, Pahwa JS, Jones N, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Peters O, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Rüther E, Carrasquillo MM, Pankratz VS, Younkin SG, Hardy J, O'Donovan MC, Owen MJ, Williams J. Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. PLoS One. 2010;5:e13950.

[13] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Rüther E, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Gallacher J, Hüll M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC; Alzheimer's Disease Neuroimaging Initiative, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S; CHARGE consortium, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alpérovitch A, Lathrop M; EADI1 consortium, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Björnsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossù P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011;43:429-35.

[14] Brune S, Kölsch H, Ptok U, Majores M, Schulz A, Schlosser R, Rao ML, Maier W, Heun R. Polymorphism in the peroxisome proliferator-activated receptor alpha gene influences the risk for Alzheimer's disease. J Neural Transm. 2003;110:1041-50.

[15] Du Y, Dodel RC, Eastwood BJ, Bales KR, Gao F, Lohmüller F, Müller U, Kurz A, Zimmer R, Evans RM, Hake A, Gasser T, Oertel WH, Griffin WS, Paul SM, Farlow MR. Association of an interleukin 1 alpha polymorphism with Alzheimer's disease. Neurology. 2000;55:480-3.

[16] [16] Grimaldi LM, Casadei VM, Ferri C, Veglia F, Licastro F, Annoni G, Biunno I, De Bellis G, Sorbi S, Mariani C, Canal N, Griffin WS, Franceschi M. Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. Ann Neurol. 2000;47:361-5.

[17] Kölsch H, Ptok U, Bagli M, Papassotiropoulos A, Schmitz S, Barkow K, Kockler M, Rao ML, Maier W, Heun R. Gene polymorphisms of interleukin-1alpha influence the course of Alzheimer's disease. Ann Neurol. 2001;49:818-9.

[18] Albani D, Tettamanti M, Batelli S, Polito L, Dusi S, Ateri E, Forloni G, Lucca U. Interleukin-1α, interleukin-1β and tumor necrosis factor-α genetic variants and risk of dementia in the very old: evidence from the "Monzino 80-plus" prospective study. Age (Dordr). 2011 Apr 21, Epub ahead of print.

[19] Hu JL, Li G, Zhou DX, Zou YX, Zhu ZS, Xu RX, Jiang XD, Zeng YJ. enetic analysis of interleukin-1A C(-889)T polymorphism with Alzheimer disease. Cell Mol Neurobiol. 2009;29:81-5.

[20] Vendramini AA, de Lábio RW, Rasmussen LT, Dos Reis NM, Minett T, Bertolucci PH, de Souza Pinhel MA, Souza DR, Mazzotti DR, de Arruda Cardoso Smith M, Payão SL. Interleukin-8-251T > A, Interleukin-1α-889C>T and Apolipoprotein E polymorphisms in Alzheimer's disease. Genet Mol Biol. 2011;34:1-5.

[21] Déniz-Naranjo MC, Muñoz-Fernandez C, Alemany-Rodríguez MJ, Pérez-Vieitez MC, Aladro-Benito Y, Irurita-Latasa J, Sánchez-García F. Cytokine IL-1 beta but not IL-1 alpha promoter polymorphism is associated with Alzheimer disease in a population from the Canary Islands, Spain. Eur J Neurol. 2008;15:1080-4.

[22] Ehl C, Kölsch H, Ptok U, Jessen F, Schmitz S, Frahnert C, Schlösser R, Rao ML, Maier W, Heun R. Association of an interleukin-1beta gene polymorphism at position -511 with Alzheimer's disease. Int J Mol Med. 2003;11:235-8.

[23] Bagli M, Papassotiropoulos A, Hampel H, Becker K, Jessen F, Bürger K, Ptok U, Rao ML, Möller HJ, Maier W, Heun R. Polymorphisms of the gene encoding the inflammatory cytokine interleukin-6 determine the magnitude of the increase in soluble interleukin-6 receptor levels in Alzheimer's disease. Results of a pilot study. Eur Arch Psychiatry Clin Neurosci. 2003;253:44-8.

[24] Bagli M, Papassotiropoulos A, Knapp M, Jessen F, Luise Rao M, Maier W, Heun R. Association between an interleukin-6 promoter and 3' flanking region haplotype and reduced Alzheimer's disease risk in a German population. Neurosci Lett. 2000;283:109-12.

[25] Papassotiropoulos A, Bagli M, Jessen F, Bayer TA, Maier W, Rao ML, Heun R. A genetic variation of the inflammatory cytokine interleukin-6 delays the initial onset and reduces the risk for sporadic Alzheimer's disease.Ann Neurol. 1999;45:666-8.

[26] Zhang Y, Zhang J, Tian C, Xiao Y, Li X, He C, Huang J, Fan H. The -1082G/A polymorphism in IL-10 gene is associated with risk of Alzheimer's disease: a meta-analysis. J Neurol Sci. 2011;303:133-8.

[27] Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L: The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991, 41:479-486.

[28] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: 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 1984; 34:939-944.

[29] Combarros O, van Duijn CM, Hammond N, Belbin O, Arias-Vásquez A, Cortina-Borja M, Lehmann MG, Aulchenko YS, Schuur M, Kölsch H, Heun R, Wilcock GK, Brown K, Kehoe PG, Harrison R, Coto E, Alvarez V, Deloukas P, Mateo I, Gwilliam R, Morgan K, Warden DR, Smith AD, Lehmann DJ. Replication by the Epistasis Project of the interaction between the genes for IL-6 and IL-10 in the risk of Alzheimer's disease. J Neuroinflammation. 2009;6:22.

[30] Combarros O, Warden DR, Hammond N, Cortina-Borja M, Belbin O, Lehmann MG, Wilcock GK, Brown K, Kehoe PG, Barber R, Coto E, Alvarez V, Deloukas P, Gwilliam R, Heun R, Kölsch H, Mateo I, Oulhaj A, Arias-Vásquez A, Schuur M, Aulchenko YS, Ikram MA, Breteler MM, van Duijn CM, Morgan K, Smith AD, Lehmann DJ. The dopamine β-hydroxylase -1021C/T polymorphism is associated with the risk of Alzheimer's disease in the Epistasis Project. BMC Med Genet. 2010;11:162.

[31] Kölsch H, Donald J Lehmann, Ibrahim-Verbaas CA, Combarros O, Van Duijn CM, Hammond N, Cortina-Borja M, Lehmann, MG, Aulchenko YS, Schuur M, Breteler M, Wilcock GK, Brown K, Kehoe PG, Barber R, Coto E, Alvarez V, Deloukas P, Mateo I, Maier W, Morgan K, Warden DR, Smith DA, Heun R, Interaction of insulin and PPAR-α genes in Alzheimer's disease: the Epistasis Project. J Neural Transm 2011 [Epub ahead of print]

[32] Cortina-Borja M, Smith AD, Combarros O, Lehmann DJ. The synergy factor: a statistic to measure interactions in complex diseases. BMC Res Notes. 2009;2:105.

[33] McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Brain Res Rev. 1995;21:195-218.

[34] Combs CK, Johnson DE, Karlo JC, Cannady SB, Landreth GE Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. J Neurosci. 2000;20:558-67.

Supplementary Table 1. Sample characteristics by geographical region

Region	Subjects	Age subsets			Sex ratio		*APOE*ε4	
		< 75 years	> 75 years	Totals	% women	*p* (controls vs AD)	Frequency	*p* (controls vs AD)
North Europe	Controls	2426	3342	5768	56.8%	0.02	13.9%*	< 0.0001
	AD	336	868	1204	60.5%		33.3%	
North Spain	Controls	179	347	526	67.2%	0.90	8.3%*	< 0.0001
	AD	182	371	553	66.7%		26.0%	
Totals	Controls	2605	3689	6294	57.7%	0.0004	13.4%	< 0.0001
	AD	518	1239	1757	62.4%		31.1%	

AD = Alzheimer's Disease; *APOE*ε4 = the ε4 allele of the apolipoprotein E gene.
Quality control of genotyping reduced the numbers below the above figures (see Table 3). Fuller details, including characteristics of each of the seven sample-sets, are given in Combarros et al 2009 (Combarros et al., 2009). *Difference between North Europe and North Spain: $p < 0.0001$.

Supplementary Table 2. Research ethic approval

Group	Committee
Bonn	Ethics Review Board of the University of Bonn
Bristol	Frenchay Local Research Ethics committee, Bristol
Nottingham	Nottingham Research Committee 2 (NHS)
OPTIMA	Central Oxford Ethics Committee No 1656
Oviedo	Ethical Committee of the Hospital Central de Asturias
Rotterdam	Medical Ethical Committee of the Erasmus MC
Santander	Ethical Committee of the University Hospital "Marqués de Valdecilla", Santander

Supplementary Table 3. Genotype distributions in controls and AD cases of seven centres

Gene	SNP	Centre	Controls			AD		
PPARA	rs135551 = Intron 2 G/A		GG	GA	AA	GG	GA	AA
		Bonn	103	88	31	109	114	21
		Bristol	21	26	4	99	71	20
		Nottingham	48	48	3	57	31	7
		OPTIMA	134	94	21	133	99	15
		Oviedo	58	45	8	91	70	17
		Rotterdam	2707	2005	398	200	159	32
		Santander	208	134	25	177	127	17
		Totals	3279	2440	490	866	671	129
PPARA	rs1800206 = L162V		LL = CC	LV = CG	VV = GG	LL = CC	LV = CG	VV = GG
		Bonn	186	24	1	217	27	1
		Bristol	41	15	0	169	24	6
		Nottingham	87	11	0	77	9	0
		OPTIMA	203	39	1	208	28	3
		Oviedo	99	21	4	165	31	1
		Rotterdam	4393	590	22	341	46	1
		Santander	314	60	2	281	36	6
		Totals	5323	760	30	1458	201	18

PPARA	rs4253766 = Intron 6 C/T		CC	TC	TT	CC	TC	TT
		Bonn	183	42	3	197	53	3
		Bristol	47	8	1	160	39	0
		Nottingham	76	19	2	71	13	2
		OPTIMA	199	44	3	198	41	3
		Oviedo	90	31	1	154	42	1
		Rotterdam	4044	1007	59	320	68	3
		Santander	298	84	4	271	64	2
		Totals	4937	1235	73	1371	320	14
IL1A	rs3783550 = Intron 6 A/C		AA	AC	CC	AA	AC	CC
		Bonn	115	94	22	124	100	31
		Bristol	25	27	4	89	86	20
		Nottingham	44	44	6	42	29	12
		OPTIMA	115	107	24	117	95	29
		Oviedo	65	44	7	89	84	19
		Rotterdam	2415	2195	500	194	162	35
		Santander	186	167	39	180	115	34
		Totals	2965	2678	602	835	671	180
IL1B	rs16944 = -971 G/A		GG	GA	AA	GG	GA	AA
		Bonn	98	99	22	112	102	31
		Bristol	22	20	8	82	81	27
		Nottingham	49	30	14	32	32	15
		OPTIMA	106	114	27	91	114	33
		Oviedo	50	56	11	79	84	27
		Rotterdam	2216	2309	585	171	180	40
		Santander	160	181	36	151	131	27
		Totals	2701	2809	703	718	724	200
IL10	rs1800896 = -1082 G/A		GG	AG	AA	GG	AG	AA
		Bonn	45	109	62	54	118	73
		Bristol	12	25	15	45	72	45
		Nottingham	22	29	25	21	28	18
		OPTIMA	58	123	60	72	112	53
		Oviedo	25	61	24	24	97	65
		Rotterdam	1339	2538	1233	120	190	81
		Santander	66	185	136	38	182	91
		Totals	1567	3070	1555	374	799	426