

## Original Article

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

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**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- $\alpha$ , interleukins (IL) IL-1 $\alpha$ , IL-1 $\beta$ , 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  $\epsilon$ 4 allele (*APOE* $\epsilon$ 4). 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*, *IL1B* 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-1 $\beta$ , 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- $\alpha$ ) 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 PPAR $\alpha$  gene (*PPARA*) is significantly reduced in AD brains [10]; (4) PPAR $\alpha$  agonists inhibit the  $\beta$ -amyloid-stimulated expression of tumor necrosis factor (TNF- $\alpha$ ) 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

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**Table 1.** Studied SNPs\*

Gene	SNP		Minor allele frequencies in controls			Linkage disequilibrium in controls				
			North Europe <sup>†</sup>	North Spain <sup>†</sup>	Difference ( $p$ )	With	North Europe <sup>†</sup>		North Spain <sup>†</sup>	
							D'	$r^2$	D'	$r^2$
<i>PPARA</i>	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)	<b>0.002</b>	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
<i>IL1A</i>	rs3783550	Intron 6 A/C	31.2% (C)	29.8% (C)	0.39					
<i>IL1B</i>	rs16944	-971 G/A	34.0% (A)	33.5% (A)	0.80					
<i>IL10</i>	rs1800896	-1082 G/A	49.3% (A)	56.9% (A)	<b>&lt; 0.0001</b>					

\*Data are only supplied for those cytokine gene SNPs that showed nominally significant interactions with *PPARA* SNPs

<sup>†</sup>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- $\alpha$ , interleukin-1 $\alpha$ , interleukin-1 $\beta$  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  $\epsilon$ 4 allele of apolipoprotein E (*APOE $\epsilon$ 4*) 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  $\leq 1.3$ . 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

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

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

<sup>†</sup> 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- $\alpha$ , interleukin-1 $\alpha$ , interleukin-1 $\beta$  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- $\alpha$ , and the chemokine macrophage chemotactic protein-1 [33]. The interaction of microglia or monocytes with beta-amyloid (A $\beta$ ) 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 A $\beta$ -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- $\gamma$  isoform and lower levels of the PPAR- $\alpha$  and PPAR- $\delta$  isoforms. Their study explored the action of the PPAR- $\alpha$  isoform and found that PPAR- $\alpha$  agonists inhibited the A $\beta$ -stimulated expression of TNF- $\alpha$  and IL-6 reporter genes in a dose-dependent manner. Moreover, the PPAR- $\alpha$  agonist, WY14643, inhibited macrophage differentiation and COX-2 gene expression. They conclude that PPAR- $\alpha$  acts to suppress a diverse array of inflammatory responses in monocytes.

Whereas other studies have focused more on the interaction between PPAR- $\gamma$  and other interleukins in inflammation [34], our study may give further impetus to re-addressing the role of PPAR- $\alpha$  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.

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**Supplementary Table 1.** Sample characteristics by geographical region

Region	Subjects	Age subsets			Sex ratio		APOEε4	
		< 75 years	> 75 years	Totals	% women	<i>p</i> (controls vs AD)	Frequency	<i>p</i> (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; APOEε4 = 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		
			GG	GA	AA	GG	GA	AA
PPARA	rs135551 = Intron 2 G/A	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	Bonn	186	24	1	217
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



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<i>PPARA</i>	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
		<i>IL1A</i>	rs3783550 = Intron 6 A/C		AA	AC	CC	AA
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
<i>IL1B</i>	rs16944 = -971 G/A				GG	GA	AA	GG
		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
		<i>IL10</i>	rs1800896 = -1082 G/A		GG	AG	AA	GG
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