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Interaction of insulin and PPAR-α genes in Alzheimer's disease: the Epistasis Project

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Abstract Altered glucose metabolism has been described in Alzheimer's disease (AD). We re-investigated the interaction of the insulin (*INS*) and the peroxisome proliferator-activated receptor alpha (*PPARA*) genes in AD risk in the Epistasis Project, including 1,757 AD cases and 6,294 controls. Allele frequencies of both SNPs (*PPARA* L162V, *INS* intron 0 A/T) differed between Northern Europeans and Northern Spanish. The *PPARA* 162LL genotype increased AD risk in Northern Europeans ($p = 0.04$), but not in Northern Spanish ($p = 0.2$). There was no association of the *INS* intron 0 TT genotype with AD. We observed an interaction on AD risk between *PPARA* 162LL and *INS* intron 0 TT genotypes in Northern Europeans (Synergy factor 2.5, $p = 0.016$), but not in Northern Spanish. We suggest that dysregulation of glucose metabolism contributes to the development of AD and might be due in part to genetic variations in *INS* and *PPARA* and their interaction especially in Northern Europeans.

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia world-wide. Typical pathological hallmarks are neurofibrillary tangles, amyloid angiopathy and insoluble, extracellular amyloid plaques.

Glucose is one of the major sources of energy for brain neurons. It is essential for the proper functions of memory and cognition. Alterations of glucose metabolism are suggested risk factors for AD (Swaab et al. 1998); hypoglycemic episodes have been shown to be associated with an increased risk of dementia in the elderly (Whitmer et al. 2009). The hormone, insulin, regulates carbohydrate and fat metabolism, and is crucial for the uptake of glucose into cells. Reduced brain and CSF insulin concentrations and increased plasma insulin concentrations have been detected in AD patients (Craft et al. 1998; Hoyer 2002). An increased number of insulin receptors was observed in post-mortem brain tissues of AD patients (Frölich et al. 1998). Majores et al. (2002) reported on the association between a VNTR polymorphism in the 5′ regulatory region of the insulin (INS) gene and the onset of AD. Those data suggested that the *INS* polymorphism might act as a modifier of AD progression; however, the risk of AD itself was not associated with this polymorphism (Majores et al. 2002).

Peroxisome proliferator-activated receptors (PPARs), such as PPARα, PPARδ and PPARγ belong to the steroid hormone receptor superfamily (Zhu et al. 1995). They bind as a heterodimer with the retinoid X receptor (RXR) to the regulatory region of target genes involved in fatty acid oxidation, lipid metabolism, inflammation control, energy balance and homeostasis (Escher and Wahli 2000; Kersten et al. 2000). PPARα is involved in glucose and lipid metabolism (Escher and Wahli 2000) and thus might also influence the pathogenesis of AD. In line with this, it has been reported that the expression of the PPARα gene (*PPARA*) was significantly reduced in AD brains (De la Monte and Wands 2006) and that PPARα agonists inhibited the β-amyloid-stimulated expression of TNFα and IL-6 reporter genes in THP-1 monocytes (Combs et al. 2001). Furthermore, we reported previously on the association of the *PPARA* L162V polymorphism with the risk of AD (Brune et al. 2003).

Since insulin and PPARα are both involved in glucose metabolism, it is suggested that there might be a physiological interaction between the two proteins. This suggestion has been supported by different experimental studies: e.g. evidence of insulin-mediated modulation of the transcriptional activity of PPARα in rats (Desvergne and Wahli 1999; Shalev et al. 1996); and of PPARα-mediated reduction of insulin resistance in mice (Guerre-Millo et al. 2000). In line with this, we previously reported an interaction of the *PPARA* L162V polymorphism with the VNTR in the 5′-flanking region of *INS*, associated with the risk of AD, in a small sample from Bonn and Mainz (Germany) (Brune et al. 2003).

The current study aimed to confirm the association of these genes and of their interaction with the risk of AD in a large sample from the Epistasis Project, with 1,757 cases of AD and 6,294 controls (Combarros et al. 2009). This project was designed to study interactions between genes (epistasis), to build on prior evidence and to have sufficient power for the purpose (Combarros et al. 2009, 2010; Lehmann et al. 2010).

Methods

Study population

The Epistasis Project primarily aims 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 Table 1. All AD cases were diagnosed "definite" or "probable" by CERAD (Mirra et al. 1991) or NINCDS-ADRDA criteria (McKhann et al. 1984). 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 ethic approval was obtained by each of the participating groups (Supplementary Table 1). Comprehensive details of our sample-sets are given elsewhere (Combarros et al. 2009).

Genotyping

Blood samples were taken after written informed consent had been obtained from the subjects or their legal representatives. Genotyping for the six centres other than Rotterdam (below) was performed at the Wellcome Trust Sanger Institute, using the iPLEX Gold assay (Sequenom Inc.). Whole genome amplified DNA was used for 82% of the samples; genomic DNA was used for the 18% of the samples that were not suitable for whole genome amplification. A Sequenom iPLEX, designed for quality control

Table 1 Sample characteristics by geographical region

Region	Subjects	Age subsets			Sex ratio		*APOEε4*	
		<75 years	>75 years	Totals	% women	*p* (controls vs. AD)	Frequency (%)	*p* (controls vs. AD)
North Europe	Controls	2,426	3,342	5,768	56.8	0.02	13.9*	<0.0001
	AD	336	868	1,204	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	2,605	3,689	6,294	57.7	0.0004	13.4	<0.0001
	AD	518	1,239	1,757	62.4		31.1	

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)

AD Alzheimer's disease, *APOEε4* the ε4 allele of the apolipoprotein E gene

* Difference between North Europe and North Spain $p < 0.0001$

purposes, was used to assess genotype concordance between genomic and whole genome amplified DNA for 168 individuals. Assays for all SNPs were designed using the eXTEND suite and MassARRAY Assay Design software version 3.1 (Sequenom Inc.). Samples were amplified in multiplexed PCR reactions before allele specific extension. Allelic discrimination was obtained by analysis with a MassARRAY Analyzer Compact mass spectrometer. Genotypes were automatically assigned and manually confirmed using MassArray TyperAnalyzer software version 4.0 (Sequenom Inc.). Gender markers were included in all iPLEX assays as a quality control metric for confirmation of plate/sample identity.

Genomic DNA was extracted from 6,566 samples from the first cohort of the Rotterdam Study, a population-based cohort study of subjects aged 55 and older (Hofman et al. 2007), using the salting out method (Miller et al. 1988). The SNPs, *PPARA* L162V (rs1800206) and *INS* intron 0 A/T (rs689), were genotyped using Taqman allelic discrimination Assays-by-Design (Applied Biosystems, Foster City, CA, USA). Genotyping was successful in 6,402 (97.5%) samples for rs1800206, and in 6,389 (97.3%) samples for rs689. For rs1800206, control sampling was concordant in 315 (96.6%) of 326 controls, 11 (3.4%) had one undetermined allele and there were no discordant controls. For rs689, control sampling was concordant in 317 (97.2%) of the 326 controls, 8 (2.5%) had 1 undetermined allele and there was 1 (0.3%) discordant control.

Statistical analysis

We analysed possible associations by fitting logistic regression models with AD diagnosis as the response variable, controlling for study centre, age, sex and the ε4 allele of apolipoprotein E (*APOEε4*), using R Version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). The adjusted synergy factors (Cortina-Borja et al. 2009) were derived from the interaction terms in those models. Heterogeneity among centres was controlled for as follows: we first fitted a model including random effect terms by centre, which accounts for correlated (clustered) observations within populations, while avoiding estimating extra parameters in the regression models. We then fitted centre as a fixed effect factor with six contrasts. We compared the goodness of fit of both approaches using Akaike's Information Criterion, which penalises the model's likelihood by a function of the number of parameters in the model. We found that the model with fixed effect terms by centre was preferable and used it to control for different frequencies between populations. We also assessed the three-way interaction between the two SNPs and the study centres using the likelihood ratio test. Over-dispersion was controlled by fitting the logistic regressions using a quasi-binomial generalised linear model family with logit link.

The *INS* intron 0 A/T SNP (rs689) is in tight linkage disequilibrium with the *INS* VNTR (99.6% concordance, (Sandhu et al. 2005), the A and T alleles corresponding to the VNTR class I and class III alleles, respectively (Vu-Hong et al. 2006). We followed Brune et al. (2003) in using the class I VNTR-dominant model, i.e. the A allele-dominant model for *INS* intron 0 A/T, and the G allele (=V)-dominant model for *PPARA* L162V (rs1800206). In view of the genetic differences found between North and South Europe in previous studies (Capurso et al. 2005; Lehmann et al. 2005; Merryweather-Clarke et al. 2000), we analysed North Europe and North Spain separately. North Europe here comprises Bonn, Bristol, Nottingham, Oxford and Rotterdam; North Spain comprises Oviedo and Santander. 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 two-sided.

Results

Preliminary analyses

Table 2 shows the two studied SNPs. Their allelic frequencies differed between North Europe and North Spain. Allelic frequencies by country are shown in Supplementary Table 2 and genotype distributions from each of the seven centres in Supplementary Table 3. Hardy–Weinberg (HW) analysis was performed for both SNPs in both cases and controls, both in the Rotterdam samples and in the samples from the other six centres, which were genotyped by the Sanger Institute. In one of these eight analyses, that of *PPARA* L162V in AD cases of the six centres, the genotypes were not in HW equilibrium ($p < 0.0001$). This marked HW disequilibrium was consistently seen throughout our dataset of AD cases, e.g. overall (including Rotterdam) $p = 0.0003$; North Europe (including Rotterdam) $p = 0.007$; North Spain $p = 0.02$. In clear contrast, the controls were consistently in HW equilibrium, e.g. overall $p = 0.61$; North Europe $p = 0.92$; North Spain $p = 0.37$. Since this pattern suggested heterosis (see "Discussion"), we looked for an association of LV heterozygotes with AD. This we found in our whole dataset [odds ratio (OR) = 0.78 (95% confidence interval: 0.64 − 0.95, $p = 0.01$)] and in North Europe [OR = 0.76 (0.60 − 0.97, 0.03)], which was consistent in all five Northern European centres (see "Discussion").

The main effects of the two SNPS

The independent associations of the two SNPs with AD are shown in Table 3. There was a weak association with AD of the *PPARA* 162LL genotype versus carriers of 162Vallele in our whole sample [OR = 1.3 (1.04 − 1.5, 0.02)], consistent in Northern Europeans and Northern Spanish (Table 3). There was no association of the *INS* intron 0 TT genotype versus carriers of the A allele.

An interaction between PPARA and INS

There was an interaction in AD risk between *PPARA* 162LL (vs. LV + VV) and *INS* intron 0 TT (vs. AT + AA) in Northern Europeans, but no effect in Northern Spanish (Table 4). In Northern Europeans, the effect was mainly in women. Table 4 also indicates the power to detect such an

Table 2 Studied SNPs

Gene	SNP	Minor allele frequency in controls		
		North Europe	North Spain	Difference (*p*)
PPARA	rs1800206 L162 V (=C/G)	6.5% (V = G)	9.3% (V = G)	0.002
INS	rs689* intron 0 A/T	28.5% (T)	24.8% (T)	0.02

PPARA and *INS* are the genes for the peroxisome proliferator-activated receptor α and insulin, respectively, *Rs689 is in tight linkage disequilibrium with the *INS* VNTR, the A and T alleles representing the class I and class III VNTR alleles, respectively (Vu-Hong et al. 2006)

Table 3 Main effects of *PPARA* 162LL (vs. V+) and *INS* intron 0 TT (vs. A+)

Polymorphism	Dataset	Numbers		Adjusted odds ratio of AD (95% CI, *p*)*
		Controls	AD	
PPARA	North Europe	LL: 4,910	LL: 1,012	**1.3 (1.01–1.6, 0.04)**
		V+: 703	V + : 145	
162LL versus V+	North Spain	LL: 413	LL: 446	1.3 (0.9–1.9, 0.2)
		V+: 87	V+: 74	
INS	North Europe	TT: 470	TT: 104	1.2 (0.9–1.55, 0.2)
		A+: 5,131	A+: 1,050	
intron 0 TT versus A+	North Spain	TT: 37	TT: 43	0.9 (0.5–1.6, 0.8)
		A+: 447	A+: 463	

Results in bold are significant at $p < 0.05$

PPARA and *INS* are the genes for the peroxisome proliferator-activated receptor α and insulin, respectively

AD Alzheimer's disease, *CI* confidence interval, V+ = LV + VV, A+ = AT + AA

* Controlling for study centre, age, sex and the ε4 allele of the apolipoprotein E gene

Table 4 Interaction between *PPARA* 162LL (vs. V+) and *INS* intron 0 TT (vs. A+)

Dataset	Numbers		Power*		Adjusted synergy factor (95% CI, *p*)†
	Controls	AD	SF = 2.5 (%)	SF = 1.5 (%)	
North Europe	5,529	1,128	87	34	**2.5 (1.2–5.4, 0.016)**
North Spain	457	482	40	13	0.8 (0.2–3.0, 0.7)

Results in bold are significant at $p < 0.05$

PPARA and *INS* are the genes for the peroxisome proliferator-activated receptor α and insulin, respectively

AD Alzheimer's disease, *SF* synergy factor, *CI* confidence interval, V+ = LV + VV, A+ = AT + AA

* To detect the stated *SF* values at $p = 0.05$

† Controlling for study centre, age, sex and the ε4 allele of the apolipoprotein E gene

Table 5 Odds ratios of AD in Northern Europeans, for *PPARA* 162LL (vs. V+) and *INS* intron 0 TT (vs. A+), when stratified by each other

Odds ratio of AD	In the subset	Adjusted odds ratio of AD (95% CI, *p*)*
PPARA	*INS* rs689 A+	1.1 (0.9–1.4, 0.39)
162LL versus V+	*INS* rs689 TT	**3.35 (1.4–7.8, 0.005)**
INS	*PPARA* 162V+	0.6 (0.3–1.2, 0.13)
Rs689 TT versus A+	*PPARA* 162LL	**1.4 (1.03–1.8, 0.03)**

Results in bold are significant at $p < 0.05$

PPARA and *INS* are the genes for the peroxisome proliferator-activated receptor α and insulin, respectively

AD Alzheimer's disease, *CI* confidence interval, V+ = LV + VV, A+ = AT + AA

* Controlling for study centre, age, sex and the ε4 allele of the apolipoprotein E gene

interaction or a weaker one. In Northern Europeans, each risk factor, i.e. *PPARA* 162LL and *INS* intron 0 TT, was only associated with AD in the presence of the other (Table 5).

Discussion

We found a weak but significant association of the *PPARA* L162V polymorphism with the risk of AD, in that carriers of the LL genotype presented with an increased risk of AD. However, this contradicted the only positive result currently shown in AlzGene, while all the other five reports in AlzGene were negative (Bertram et al. 2007) (http://www.alzgene.org/). The *INS* intron 0 TT was not significantly associated with the risk of AD. This is in line with our previous publication (Majores et al. 2002).

In addition, we found an interaction in the association with the risk of AD between *PPARA* 162LL (vs. LV + VV) and *INS* intron 0 TT (vs. AT + AA) in our Northern European dataset of over 6,500 samples: synergy factor = 2.5 (1.2 − 5.4, 0.016) (Table 4). There was no effect in the Northern Spanish, consistent with our previous report (Lehmann et al. 2010) and other reports (Capurso et al. 2005; Lehmann et al. 2005; Merryweather-Clarke et al. 2000) of genetic differences between North and South Europe. There was heterogeneity between our five Northern European centres, which we controlled for. In Northern Europeans, each risk factor was only associated with AD in the presence of the other (Table 5), consistent with true epistasis.

We therefore suggest that there is a weak association of PPARA with AD risk. However, we cannot exclude that this finding might be due to a population effect in our sample. In addition, there is no significant association of the *INS* intron 0 TT genotype with AD risk. Genome-wide association studies (GWA) just recently published [i.e. (Harold et al. 2009; Hollingworth et al. 2011)] did not identify genes in the genomic region of the SNPs investigated in our study. However, we suggest that there is an interaction between the two genes. Since GWAs are not designed to study epistasis, genetic interactions associated with AD might have been missed in the past.

Interpretation of results: heterosis

There was a consistent pattern of HW disequilibrium in the genotypes of *PPARA* L162V in AD cases ($p = 0.0003$), contrasting with consistent HW equilibrium in controls ($p = 0.61$) (see "Preliminary analyses"). Yet, the cases and controls were genotyped together blind to diagnosis, with all plates containing both. This distinct pattern of HW disequilibrium is highly unlikely to have occurred by chance genotyping errors (cases vs. controls: $p = 0.00001$). However, such a pattern concurs with an alternative explanation, which is heterosis (Comings and MacMurray 2000; Lehmann et al. 2005). In heterosis, heterozygotes show either a greater or a lesser association with a given trait than do either class of homozygotes. This can result from the inadvertent combination of two unlike subsets, due to a hidden interaction with another risk factor. Such an interaction can distort the association observed when one risk factor is examined by itself. In the present case, we found heterosis in the association of *PPARA* L162V with AD ($p = 0.01$, see "Preliminary analyses"). We also found an appropriate interaction between the

PPARA and *INS* SNPs, as first proposed by Brune et al. (2003). This confirms that heterosis is the most likely explanation of the HW disequilibrium found for *PPARA* L162V only in AD cases.

The potential biological role of PPARA and INS and their interaction in AD

PPARα and insulin are both involved in the regulation of glucose metabolism in the body. Insulin, a peptide hormone, regulates the uptake of glucose into the cells, while PPARα regulates the expression of genes involved in lipid metabolism, inflammation and energy balance (Escher and Wahli 2000; Kersten et al. 2000).

The *PPARA* L162V polymorphism might have relevance for the protein function and was previously reported to be associated with hyper-apolipoproteinemia (Vohl et al. 2000) and altered lipid concentrations in patients with non-insulin-dependent diabetes mellitus (Evans et al. 2001; Flavell et al. 2000, 2002). The biological function of the *INS* VNTR, which is in high linkage disequilibrium (99.6% concordance) (Sandhu et al. 2005) with the *INS* polymorphism investigated in the present study, is not entirely clear. The long *INS* VNTR alleles (class III) have been reported to produce higher levels of thymic *INS* mRNA than those with the class I alleles (Vafiadis et al. 2001). The authors concluded that this result confirmed an additional level of correlation between thymic insulin and diabetes susceptibility (Vafiadis et al. 2001).

Since alterations of glucose metabolism have been described in AD (Hoyer 2002, 2003), it is likely that genes involved in this metabolic pathway act as AD risk factors. A biological interaction between insulin and PPARα activation in the regulation of glucose metabolism has been described previously: insulin treatment in rat adipocytes stimulated PPARα phosphorylation and enhanced its transcriptional activity (Desvergne and Wahli 1999; Shalev et al. 1996); thus, the transcriptional activity of PPARα might be modulated by insulin-mediated phosphorylation (Shalev et al. 1996). Additionally, high glucose levels reduced the expression of *PPARA* and its target genes in pancreatic β-cells (Roduit et al. 2000). Also, PPARα activators such as fenofibrate and ciprofibrate reduced insulin resistance in mice (Guerre-Millo et al. 2000). These data suggest the functional interaction of INS and PPARα.

Conclusions and limitations

The above evidence of a functional interaction between INS and PPARα supports our finding that only the interaction of *INS* and *PPARA* polymorphisms, rather than each alone, might affect the risk of AD. Our hypothesis-driven approach, based on prior evidence from association studies, was designed to study biologically plausible interactions, with sufficient power for the purpose. We suggest that this approach may complement that of genome-wide association studies, which have almost exclusively concentrated on single-locus effects. Nevertheless, we suggest that our results need further replication in another Northern European dataset, at least equally large. Our dataset had adequate power to detect this interaction, but would be inadequate for a much weaker interaction, e.g. with a synergy factor of 1.5 (Table 4). Meanwhile, further studies of the biological implications may be appropriate.

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References

Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE (2007) Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 39:17–23

Brune S, Kölsch H, Ptok U, Majores M, Schulz A, Schlosser R, Rao ML, Maier W, Heun R (2003) Polymorphism in the peroxisome proliferator-activated receptor alpha gene influences the risk for Alzheimer's disease. J Neural Transm 110:1041–1050

Capurso C, Solfrizzi V, D'Introno A, Colacicco AM, Capurso SA, Mastroianni F, Liaci M, Vendemiale G, Capurso A, Panza F (2005) The cathepsin D gene exon 2 (C224T) polymorphism and sporadic Alzheimer's disease in European populations. J Gerontol A Biol Sci Med Sci 60:991–996

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 (2009) 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 Neuroinflamm 6:22

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, Kolsch H, Mateo I, Oulhaj A, Arias-Vasquez A, Schuur M, Aulchenko YS, Ikram MA, Breteler MM, Van Duijn CM, Morgan K, Smith AD, Lehmann DJ (2010) The dopamine beta-hydroxylase-1021C/T polymorphism is associated with the risk of Alzheimer's disease in the Epistasis Project. BMC Med Genet 11:162

Combs CK, Bates P, Karlo JC, Landreth GE (2001) Regulation of beta-amyloid stimulated proinflammatory responses by peroxisome proliferator-activated receptor alpha. Neurochem Int 39:449–457

Comings DE, MacMurray JP (2000) Molecular heterosis: a review. Mol Genet Metab 71:19–31

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

Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D Jr (1998) Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease—relationship to severity of dementia and apolipoprotein E genotype. Neurology 50:164–168

De la Monte SM, Wands JR (2006) Molecular indices of oxidative stress and mitochondrial dysfunction occur early and often progress with severity of Alzheimer's disease. J Alzheimers Dis 9:167–181

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 20:649–688

Escher P, Wahli W (2000) Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat Res 448:121–138

Evans D, Aberle J, Wendt D, Wolf A, Beisiegel U, Mann WA (2001) A polymorphism, L162V, in the peroxisome proliferator-activated receptor alpha (PPARalpha) gene is associated with lower body mass index in patients with non-insulin-dependent diabetes mellitus. J Mol Med 79:198–204

Flavell DM, Pineda TI, Jamshidi Y, Evans D, Diamond JR, Elkeles RS, Bujac SR, Miller G, Talmud PJ, Staels B, Humphries SE (2000) Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. Diabetologia 43:673–680

Flavell DM, Jamshidi Y, Hawe E, Pineda Torra I, Taskinen MR, Frick MH, Nieminen MS, Kesaniemi YA, Pasternack A, Staels B, Miller G, Humphries SE, Talmud PJ, Syvanne M (2002) Peroxisome proliferator-activated receptor alpha gene variants influence progression of coronary atherosclerosis and risk of coronary artery disease. Circulation 105:1440–1445

Frölich L, Blum-Degen D, Bernstein HG, Engelsberger S, Humrich J, Laufer S, Muschner D, Thalheimer A, Türk A, Hoyer S, Zöchling R, Boissl KW, Jellinger K, Riederer P (1998) Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. J Neural Transm 105:423–438

Guerre-Millo M, Gervois P, Raspé E, Madsen L, Poulain P, Derudas B, Herbert JM, Winegar DA, Willson TM, Fruchart JC, Berge RK, Staels B (2000) Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. J Biol Chem 275:16638–16642

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41:1088–1093

Hofman A, Breteler MM, Van Duijn CM, Krestin GP, Pols HA, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, Witteman JC (2007) The Rotterdam Study: objectives and design update. Eur J Epidemiol 22:819–829

Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435

Hoyer S (2002) The brain insulin signal transduction system and sporadic (type II) Alzheimer disease: an update. J Neural Transm 109:341–360

Hoyer S (2003) Memory function and brain glucose metabolism. Pharmacopsychiatry 36(Suppl 1):S62–S67

Kersten S, Desvergne B, Wahli W (2000) Roles of PPARs in health and disease. Nature 405:421–424

Lehmann DJ, Cortina-Borja M, Warden DR, Smith AD, Sleegers K, Prince JA, Van Duijn CM, Kehoe PG (2005) Large meta-analysis establishes the ACE insertion-deletion polymorphism as a marker of Alzheimer's disease. Am J Epidemiol 162:305–317

Lehmann DJ, Schuur M, Warden DR, Hammond N, Belbin O, Kölsch H, Lehmann MG, Wilcock GK, Brown K, Kehoe PG, Morris CM, Barker R, Coto E, Alvarez V, Deloukas P, Mateo I, Gwilliam R, Combarros O, Arias-Vásquez A, Aulchenko YS, Ikram MA, Breteler MM, Van Duijn CM, Oulhaj A, Heun R, Cortina-Borja M, Morgan K, Robson K, Smith AD (2010) Transferrin and HFE genes interact in Alzheimer's disease risk: the Epistasis Project. Neurobiol Aging [Epub ahead of print]

Majores M, Kölsch H, Bagli M, Ptok U, Kockler M, Becker K, Rao ML, Maier W, Heun R (2002) The insulin gene VNTR polymorphism in Alzheimer's disease: results of a pilot study. J Neural Transm 109:1029–1034

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (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

Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ (2000) Geography of HFE C282Y and H63D mutations. Genet Test 4:183–198

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, Van Belle G, Berg L (1991) The consortium to establish a registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41:479–486

Roduit R, Morin J, Masse F, Segall L, Roche E, Newgard CB, Assimacopoulos-Jeannet F, Prentki M (2000) Glucose down-regulates the expression of the peroxisome proliferator-activated receptor-alpha gene in the pancreatic beta-cell. J Biol Chem 275:35799–35806

Sandhu MS, Heude B, Young EH, Luben R, Luan J, Khaw KT, Todd J, Wareham NJ (2005) INS VNTR class genotype and indexes of body size and obesity: population-based studies of 7,999 middle-aged men and women. Diabetes 54:2812–2815

Shalev A, Siegrist-Kaiser CA, Yen PM, Wahli W, Burger AG, Chin WW, Meier CA (1996) The peroxisome proliferator-activated receptor alpha is a phosphoprotein: regulation by insulin. Endocrinology 137:4499–4502

Swaab DF, Lucassen PJ, Salehi A, Scherder EJ, van Someren EJ, Verwer RW (1998) Reduced neuronal activity and reactivation in Alzheimer's disease. Prog Brain Res 117:343–377

Vafiadis P, Ounissi-Benkalha H, Palumbo M, Grabs R, Rousseau M, Goodyer CG, Polychronakos C (2001) Class III alleles of the variable number of tandem repeat insulin polymorphism associated with silencing of thymic insulin predispose to type 1 diabetes. J Clin Endocrinol Metab 86:3705–3710

Vohl MC, Lepage P, Gaudet D, Brewer CG, Betard C, Perron P, Houde G, Cellier C, Faith JM, Despres JP, Morgan K, Hudson TJ (2000) Molecular scanning of the human PPARa gene: association of the L162V mutation with hyperapobetalipoproteinemia. J Lipid Res 41:945–952

Vu-Hong TA, Durand E, Deghmoun S, Boutin P, Meyre D, Chevenne D, Czernichow P, Froguel P, Levy-Marchal C (2006) The INS VNTR locus does not associate with smallness for gestational age (SGA) but interacts with SGA to increase insulin resistance in young adults. J Clin Endocrinol Metab 91:2437–2440

Whitmer RA, Karter AJ, Yaffe K, Quesenberry CP Jr, Selby JV (2009) Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. JAMA 301:1565–1572

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