The Epistasis Project: a multi-cohort study of the effects of *BDNF*, *DBH* and *SORT1* epistasis on Alzheimer´s disease risk

Olivia Belbin[a,b], Kevin Morgan[c], Chris Medway[d], Donald Warden[e], Mario Cortina-Borja[f], Cornelia M. van Duijn[g], Hieab H.H. Adams[g], Ana Frank-Garcia[b,h], Keeley Brookes[c], Pascual Sánchez-Juan[b,i], Victoria Alvarez[j], Reinhard Heun[k], Heike Kölsch[k], Eliecer Coto[j], Patrick G Kehoe[l], Eloy Rodriguez-Rodriguez[i], Maria J Bullido[b,h] M. Arfan Ikram[g], A. David Smith[e], Donald J. Lehmann[e]

[a]Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain.

[b]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain.

[c]Human Genetics School of Life Sciences, University of Nottingham, UK.

[d]Institute of Medical Genetics, University Hospital Wales, Cardiff, CF14 4XW, UK

[e]Oxford Project to Investigate Memory and Ageing (OPTIMA), University Department of Pharmacology, Oxford, UK.

[f]Clinical Epidemiology, Nutrition and Biostatistics, UCL Great Ormond Street Institute of Child Health, London, UK.

[g]Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

[h]Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Madrid, Spain.

[i]Neurology Service, Marqués de Valdecilla University Hospital (University of Cantabria), 39008 Santander, Spain.

[j]Laboratorio de Genética. AGC Laboratorio de Medicina. Hospital Universitario Central de Asturias, Oviedo, Spain.

[k]Department of Psychiatry, University of Bonn, Bonn, Germany

[l]Dementia Research Group, Bristol Medical School Translational Health Sciences, University of Bristol, Southmead Hospital, Bristol, UK.

Running title: *BDNF*, *DBH*, *SORT1* epistasis Alzheimer disease

#Corresponding Author: Olivia Belbin, PhD. Address: IIB-Sant Pau, Pabellón 19, c/Sant Antoni Maria Claret 167 08025 Barcelona, Spain. Telephone: +34 93 291 90 00 ext 8233. Email: obelbin@santpau.cat.

Abstract

Pre-synaptic secretion of brain-derived neurotrophic factor (BDNF) from noradrenergic neurons may protect the Alzheimer's disease (AD) brain from amyloid pathology. While the *BDNF* polymorphism (rs6265) is associated with faster cognitive decline and increased hippocampal atrophy, a replicable genetic association of *BDNF* with AD risk has yet to be demonstrated. This could be due to masking by underlying epistatic interactions between *BDNF* and other loci that encode proteins involved in moderating BDNF secretion (DBH and Sortilin). We performed a multi-cohort case-control association study of the *BDNF*, *DBH* and *SORT1* loci comprising 5,682 controls and 2,454 AD patients from Northern Europe (87% of samples) and Spain (13%). The *BDNF* locus was associated with increased AD risk (odds ratios; OR=1.1-1.2, p=0.005-0.3), an effect size that was consistent in the Northern European (OR=1.1-1.2, p=0.002-0.8) but not the smaller Spanish (OR=0.8-1.6, p=0.4-1.0) subset. A synergistic interaction between *BDNF* and sex (synergy factor; SF=1.3-1.5 p=0.002-0.02) translated to a greater risk of AD associated with *BDNF* in women (OR=1.2-1.3, p=0.007-0.00008) than men (OR=0.9-1.0, p=0.3-0.6). While the *DBH* polymorphism (rs1611115) was also associated with increased AD risk (OR=1.1, p=0.04) the synergistic interaction (SF=2.2, p=0.007) between *BDNF* (rs6265) and *DBH* (rs1611115) contributed greater AD risk than either gene alone, an effect that was greater in women (SF=2.4, p=0.04) than men (SF=2.0, p=0.2). These data support a complex genetic interaction at loci encoding proteins implicated in the DBH-BDNF inflammatory pathway that modifies AD risk, particularly in women.

Keywords

"Epistasis, Genetic", "Neurotrophins", "Alzheimer disease", "Brain-Derived Neurotrophic Factor", "Dopamine beta-Hydroxylase", "Sortilin"

Introduction

Understanding the genetic factors that modify risk and/or progression of Alzheimer's disease (AD) is fundamental for the identification and monitoring of at-risk individuals and could lead to novel therapeutic targets and biomarkers. Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain and is a well-characterised protective factor against AD pathology. Specifically, it has been reported that pre-synaptic secretion of BDNF from noradrenergic neurons modulates neural circuitry, synaptic plasticity, neuronal survival and differentiation and protects neurons from various types of insult (reviewed in [1]). In accordance with these findings, reduced levels of BDNF (mRNA and protein) have been observed in peripheral blood of AD patients [2] and in cortical and subcortical brain regions of individuals with AD at autopsy [3, 4]. In the aged population and in subjects with mild cognitive impairment and AD patients, reduced brain BDNF levels correlate with lower cognitive scores and faster cognitive decline [5-7]. Moreover, there is some evidence that genetic variation at the *BDNF* locus may moderate downstream effects of the pathological protein, β-amyloid, in autosomal dominant AD. For example, asymptomatic adults with signs of brain amyloidosis who carry the methionine allele at rs6265 show faster decline in episodic memory, lower hippocampal volume and increased tau markers in cerebrospinal fluid compared to valine homozygotes [8-12]. This could be attributed to the association of this allele with altered intracellular trafficking and reduced secretion of BDNF [13-15]. Additionally, there is evidence that the protective effect of lifetime

exposure to cognitively stimulating activities on cognitive performance may be weaker in carriers of the methionine allele [16]. Thus, the methionine allele may be a prognostic biomarker for predicting cognitive decline due to AD. That being said, extensive study in over 30 populations (Caucasian, Asian, African and mixed) has failed to demonstrate a replicable association of *BDNF* genetic variants with altered AD risk (www.Alzgene.org [17]).

Studies using rodent models have shown that a reduction in BDNF levels may precede the appearance of amyloid plaque pathology [18]. Reduced BDNF levels have been associated with altered synaptic plasticity [19-21], increased cortical amyloid plaque numbers, and reduced noradrenergic innervation of the hippocampus, frontal cortex, and cerebellum [22] of animal models, effects that can be reversed with BDNF treatment [23-27].

Taken together, data from animal and human studies strongly support an important neuroprotective effect of BDNF and that an imbalance in BDNF signalling may be an early event in AD pathogenesis. It follows that modulation of BDNF secretion could be critical to this process. In this regard, previous studies using rodent models have reported a feed forward loop between BDNF and the noradrenergic system, whereby noradrenaline (norepinephrine) induces astrocytic and neuronal BDNF secretion, which in turn stimulates noradrenergic signalling as part of an anti-inflammatory mechanism [28-32]. In support of this, reduced noradrenaline levels have also been reported in aged and AD brains and it has been hypothesised that increasing noradrenergic signalling in the brain could halt the progression of neurodegeneration and cognitive decline (reviewed in [33]). Noradrenaline is synthesised from dopamine, a reaction catalysed by dopamine beta-hydroxylase (DBH) and is an anti-inflammatory agent that can attenuate

the cortical inflammatory response to the $A\beta_{42}$ peptide [34]. Thus deregulation of DBH-mediated synthesis of noradrenaline could have downstream effects on BDNF secretion. An additional factor in BDNF secretion is the intracellular sorting of BDNF to the secretory pathway, which is regulated by binding of the BDNF pro-domain to the luminal domain of Sortilin [35]. This interaction prevents BDNF degradation, targets BDNF to the secretory pathway [36] and facilitates the release of mature BDNF [37].

The aims of this first study of Stage 2 of the Epistasis Project (the results of Stage 1 [38-44] are summarised in **Table 1**), were to further evaluate the contribution of genetic variation at the *BDNF* locus to AD risk in a large case-control dataset (n=8,136) and determine whether the association could be masked by genetic epistasis between *BDNF* variants and sex, age, *APOE* ε4 status and variants at loci encoding proteins that control BDNF secretion (DBH and Sortilin).

Materials and Methods

Study population

DNA samples were collected from individuals of Caucasian European descent by seven research centres within two geographical regions namely, Northern Europe (Bristol, Nottingham, OPTIMA and Rotterdam) and Spain (Madrid, Oviedo and Santander). All AD cases were diagnosed “definite” or “probable” by CERAD or NINCDS-ADRDA criteria. AD cases were sporadic, i.e. possible autosomal dominant cases were excluded, based on family history. The cohorts in Stage 1 of the Epistasis Project were described in [38]. In this Stage 2, the Bonn cohort was replaced by that from Madrid, giving a better overall balance between Northern Europe and Spain. The Madrid cohort fulfilled our criteria of an all-Caucasian population drawn from a narrow geographical region. The demographics of each sample collection are shown in **Table 2**. Research ethics

approval was obtained by each of the participating groups. Written informed consent was given for donation of blood samples from all participants, or their legal representatives.

Polymorphism selection

We downloaded Caucasian European (CEU) population data from the HaploView platform (release 27) using gene coordinates (gene +/- 10kbp flanking regions) extracted from the UCSC MySQL server (hg18) at https://genome.ucsc.edu/. Polymorphisms with a genotype rate <50%, and that were in Hardy-Weinberg equilibrium (p<0.001) and with a minor allele frequency (MAF) <5% were removed. The remaining polymorphisms were introduced into the tagger function in HaploView. A tagging threshold of r^2>0.8 and log odds (LOD) score>3.0 was used. The "Aggressive Tagging" approach was implemented, which allows a haplotype of up to three polymorphisms to be used as a proxy. We identified 34 polymorphisms (5 in *BDNF*, 23 in *DBH* and 6 in *SORT1*) that have a minor allele frequency >5% in Caucasian Europeans and that cover 80% of the variation in these genes (**Table 3**). Due to the considerable variation in *DBH*, coverage of this gene was restricted to rs1611115, which has previously been associated with increased AD risk in men aged <75 [39] and is responsible for 30-50% of the considerable variation in DBH activity [46-53]. The 12 polymorphisms were taken forward for genotyping.

Genotyping and imputation

Genotyping for all centres except Rotterdam (below) was performed by LGC (Hoddesdon, Hertfordshire, UK), using the KASPar technology by KBioscience http://www.kbioscience.co.uk. Six to eight duplicate samples and one blank (H_2O) sample were included on each plate. All blank samples were negative. The concordance

between duplicates was 100% with the exception of one discordant sample for *BDNF* rs11030104 in the Bristol dataset, which was removed from analyses. Where data were available from previous in-house TaqMan genotyping, LGC genotypes were 100% concordant (*BDNF* rs6265; n=483, *DBH* rs1611115; n=396). Genotyping in the Rotterdam cohort was done on Version 3 Illumina-Infinium-II HumanHap550 SNP array (Illumina, San Diego, USA). Genotypes for the Rotterdam cohort were imputed from whole genome data using MACH software (http://www.sph.umich. edu/csg/abecasis/MACH/) and with the 1000 genomes project (phase I version 3 reference panel, positive strand) as a reference. The reliability of imputation was estimated for each imputed SNP with the ratio of expected and observed dosage variance (O/E ratio). Only samples with high-quality extracted DNA were genotyped. In order to allow analysis alongside the genotyped cohorts, the imputed probabilities from Rotterdam were converted to genotypes such that a value of 0 was coded as homozygote for allele 2, a value of 1 was coded as heterozygote and a value of 2 was coded as homozygote for allele 1. It should be noted that a dosage of 1 could be heterozygote, but it could also mean that person has a 0.5 probability of being homozygote for allele 1 and a 0.5 probability of being homozygote for allele 2. That being said, it is commonplace in genetics to round these values to 0/1/2 with the implied corresponding genotypes. The **supplementary table** shows the genotype counts and allele frequencies as well as genotyping rate for all individual centres. The genotyping rate for each polymorphism ranged from 0.99 to 1.00 in the total dataset. All genotypes were confirmed to be in Hardy-Weinberg equilibrium (α=0.001) in controls from all individual centres. *APOE* ε4 genotypes were determined according to the genotypes at rs429358 and rs7412. Tests for pair-wise intragenic linkage disequlibrium for the 5 *BDNF* polymorphisms and the 6

SORT1 polymorphisms were consistent with the Haploview data ($r<0.8$) with the exception of rs6265 and rs11030104 in *BDNF* ($r^2=0.9$) and rs2228604 and rs10745354 in *SORT1* ($r^2=1.0$). The high degree of co-inheritance of these pairs was replicated in both the Northern European and Spanish controls. The polymorphisms rs11030104 (*BDNF*) and rs10745354 (*SORT1*) were therefore removed from the study as they provided no further information.

Statistical analyses

All analyses were performed in R version 3.5.0 [54]. The relative risk (odds ratios and 95% confidence intervals; CI) for AD was calculated for each of the 10 polymorphisms brought forward for analysis using a general linear model for dominant and recessive inheritance models controlling for age-at-extraction, sex, *APOE* ε4 allele and individual centre. The Akaike Information Criterion (AIC) was used to compare goodness-of-fit, with smaller values of AIC corresponding to preferable models. The statistical power for each sample included in the study to detect a significant ($\alpha=0.05$) association of a polymorphism with a minor allele frequency 0.2 (mean of polymorphisms included in the study) with AD risk (OR=1.2) under a dominant model and assuming a prevalence of AD of 10% in the population aged over 65 is as follows: Total dataset 97%, Northern Europe 92% (Bristol 14%, Nottingham 14%, OPTIMA 17%, Rotterdam 78%), Spain 31% (Madrid 18%, Oviedo 11%, Santander 13%). All tests were first performed in the total dataset and subsequently stratifying by geographical region: Northern Europe=Bristol, Nottingham, OPTIMA and Rotterdam; Spain=Madrid, Oviedo and Santander. When an association ($p<0.05$) was observed in the Northern European dataset, analyses were subsequently tested in the Rotterdam subset as the only collection centre with sufficient statistical power to detect an association. Interactions were

assessed using the Synergy Factor (SF) described in [55]. Using this method, the SF=actual OR (joint effect of two factors) / predicted OR (product of the OR for each factor alone). The null hypothesis states that the actual OR is equal to the predicted OR. This method assesses interactions on a multiplicative scale rather than an additive scale. Throughout the study, significance was considered at the α=0.05 level. However, due to the large number of tests performed (n=224 in total), the Bonferroni cut-off (α=0.0002) can be considered as a more stringent cut-off for significance that minimizes the false-positive associations reported. Associations that surpass this cut-off are indicated in the text and figures.

Results

Genetic variation at the *BDNF* locus is associated with increased AD risk

We tested for association of the 4 *BDNF* polymorphisms with altered AD risk in the total population. The model preferred (lowest AIC) for *BDNF*1 was the recessive inheritance model, whereas the preferred models for *BDNF2-4* were the dominant models. The OR and 95% CI for the preferred models of each polymorphism are plotted in **Fig1**. The *BDNF1* polymorphism, previously shown to regulate BDNF trafficking, was not associated (at the α=0.05 level) with altered AD risk in the total (rs6265 MM; OR=1.2, p=0.3), Northern European (OR=1.0, p=0.8) or Spanish (OR=1.6, p=0.1) datasets (**Fig1A**). However, the OR in the total dataset was of a similar yet greater magnitude to those for *BDNF2* (**Fig1B;** rs12288512 A allele OR=1.1, p=0.04) and *BDNF3* (**Fig1C;** (rs113030102 G allele OR=1.1, p=0.005), associations which surpassed our cut-off for significance. Both *BDNF2* (OR=1.2, p=0.01) and *BDNF3* (OR=1.2, p=0.005) were associated with increased AD risk in the Northern European but not Spanish (OR<1.0, p>0.3) populations. The associations of *BDNF2-4* were even

stronger in the Rotterdam subset (*BDNF2*; OR=1.2, p=0.002, *BDNF3*; OR=1.3, p=0.0008). Another polymorphism, *BDNF4* (**Fig1D**), that was not significant in the total dataset (p=0.08) despite a similar OR (rs11030119 A+; OR=1.1), was associated with increased AD risk in the Northern European (OR=1.2, p=0.01) and Rotterdam (OR=1.2, p=0.002) but not Spanish (OR=0.8, p=0.2) population. It should be noted that none of these associations surpassed the Bonferroni cut-off (p<0.0002) for this study (best p=0.0008 for *BDNF3* in the Rotterdam dataset).

To determine whether interactions between *BDNF* polymorphisms could explain the contradictory findings for rs6265 (*BDNF1*) across multiple populations, we sequentially added pair-wise interactions between the 4 *BDNF* polymorphisms into the model. No interaction between *BDNF* polymorphisms was associated with altered AD risk (p>0.5).

Women showed increased susceptibility to AD risk-modifying polymorphisms at the *BDNF* locus

We next tested whether interactions between *BDNF* polymorphisms and age-at-sampling, sex and possession of the *APOE* ε4 allele could contribute to AD risk. We found no association of the *BDNF1**sex interaction with AD risk (SF=0.95, p=0.9). However, inclusion of *BDNF2**sex, *BDNF3**sex or *BDNF4**sex interactions improved the AIC for the model and the synergy factors (SF) for these interactions are plotted in **Fig2A-C**. We observed a positive synergistic interaction between *BDNF2*, *BDNF3* and *BDNF4* and sex in the total dataset; sex**BDNF2* (SF=1.5, p=0.002), sex**BDNF3* (SF=1.4, p=0.002) and sex**BDNF4* (SF=1.3, p=0.02). The interactions were observed in the Northern European dataset (SF=1.4, p=0.02; SF=1.4, p=0.007; SF=1.3, p=0.02, respectively) and Rotterdam (SF=1.4, p=0.02, SF=1.5; p=0.01, SF=1.4, p=0.04, respectively) datasets and the sex**BDNF2* interaction was also observed in the Spanish

population (SF=1.94, p=0.03). To explore these interactions further, *BDNF2-4* were tested for association with AD in women and men separately in the datasets that showed a synergistic interaction (**Fig2D-F**). While *BDNF2* was associated with reduced AD risk in Spanish men (OR=0.6, p=0.03), all three polymorphisms were associated with increased risk in women from the total (*BDNF2*; OR=1.3, p=0.0006, *BDNF3*; OR=1.3, p=0.00008, *BDNF4*; OR=1.2, p=0.007, respectively) and Northern European (OR=1.3, p=0.0007, OR=1.4, p=0.00005, OR=1.2, p=0.001, respectively) and Rotterdam (OR=1.4, p=0.0001, OR=1.4, p=0.00003, OR=1.4, p=0.0002, respectively) datasets, associations that surpassed the Bonferroni cut-off for this study (p<0.0002) in at least one of the datasets.

We next tested for association of interactions between BDNF variants and the *APOE* ε4 allele. The *BDNF3**ε4 interaction (SF=0.47, p=0.02) was associated with altered AD risk in the Spanish dataset and *BDNF3* was nominally associated with decreased AD risk in Spanish ε4 carriers (OR=0.58, p=0.05) but not in non-carriers (OR=1.2, p=0.2). That being said, these interactions may be of less importance since including them in the models did not improve the AIC. Interactions between other *BDNF* variants and ε4 were not associated with AD risk (p>0.06) and neither were interactions between any *BDNF* variant and age (p>0.2).

BDNF and *DBH* polymorphisms act synergistically to increase AD risk in Northern European women

We have previously identified an association of the *DBH* polymorphism, rs1611115, with increased AD risk in men aged <75 [39] in a sample set that overlaps with that included in this study. Therefore, unsurprisingly, when testing for an association of *DBH* with AD risk in the total population, we found that the *DBH* polymorphism was

associated with increased risk of AD in the total dataset (rs1611115T+ OR=1.12, p=0.04), albeit that the association was not apparent in either of the regional subsets (OR=1.1, p=0.1 and OR=1.3, p=0.1). Addition of interactions between the *DBH* polymorphism and age-at-sampling, sex and possession of the *APOE* ε4 allele did not improve the AIC for the model. Since DBH and BDNF have both been implicated in regulating noradrenergic neurotransmission, we sought to determine whether polymorphisms at the *BDNF* and *DBH* loci could interact to alter AD risk. The addition of interactions between *DBH* and all 4 *BDNF* polymorphisms improved the AIC for the model. The SF for the interactions are plotted in **Fig3A-D**. A synergistic *BDNF1*DBH* interaction contributed to AD risk in the total (SF=2.2, p=0.007) and Northern European (SF=2.2, p=0.02) and Rotterdam (SF=2.2, p=0.03) datasets. While the SF (SF=1.4) was comparable in the Spanish population (p=0.3), the interaction did not reach our cut-off for significance. Since the *BDNF* polymorphisms have different effects in men and women, we next tested the *BDNF*DBH* interactions in male and female subgroups separately. Due to testing rs6265 under a recessive inheritance model, the low number of male MM (rs6265), T+ (rs1611115; dominant model) carriers in the Spanish dataset (n=7), meant that the *BDNF*DBH1* interaction could only be tested in the Northern European dataset. In that dataset, the *BDNF*DBH1* interaction contributed more to AD risk in women (SF=2.4, p=0.04) than men (SF=2.0, p=0.2). The remaining *BDNF*DBH* interactions did not contribute to the AD risk (p>0.1) and it should be noted that none of the *BDNF1*DBH* interactions surpassed the Bonferroni cut-off (p<0.0002) for this study.

SORT1 genetic variation is not associated with AD risk

Finally we tested for an association of *SORT1* polymorphisms with AD risk. We found no association in the total, Northern European or Spanish populations ($p>0.05$). We tested for an association of interactions between the *SORT1* polymorphisms and age-at-sampling, sex and possession of the *APOE* ε4 allele with AD risk. We observed an antagonistic interaction between *SORT1e* (rs1149175 GG) and sex in the Spanish dataset (*SORT1e* OR=2.2, sex OR=2.1, SF=0.4, $p=0.007$) and *SORT1e* was associated with increased AD risk in Spanish men (OR=2.27, $p=0.002$). That being said, these interactions may be of less importance since including them in the models did not improve the AIC. None of the interactions with the *BDNF* variants contributed to AD risk in the total or regional subsets ($p>0.05$).

Discussion

This multi-cohort study of 5,682 controls and 2,454 AD patients (recruited from seven research centres from Northern Europe and Spain) represents a comprehensive exploration of the contribution of epistatic interactions between genes encoding proteins implicated in BDNF secretion and noradrenergic innervation to AD risk. The main finding of this study is the synergistic interaction between *BDNF* risk-modifying variants (*BDNF2-4*) and sex (SF=1.3 to 1.5) such that the *BDNF*-associated risk was observed in women (OR=1.2 to 1.3) but not men (OR=0.9 to 1.0). We also show evidence of a synergistic interaction between the *BDNF* and *DBH* loci such that carriers of the minor allele at *DBH* (rs1611115) who are also homozygous for the minor allele at *BDNF1* (rs6265) are at increased risk of AD (SF=2.2) than carriers of either allele alone (*BDNF* OR=1.1 to 1.2, *DBH* OR=1.1), an effect that was greater in women (SF=2.4) than in men (SF=2.0).

In support of the meta-analyses performed by Alzgene, we report only a nominal association of rs6265 (*BDNF1*) with AD risk (OR=1.2, *p*=0.3). On the other hand, we report three novel risk-modifying *BDNF* polymorphisms that were associated with increased AD risk in the total or Northern European populations (OR=1.1, p<0.01). It should be noted that the OR for rs6265 was similar to that reported for the associations reported for the other 3 polymorphisms. We therefore cannot rule out the possibility that rs6265 has a similar frequency to the other *BDNF* variants in AD patients, but that the significance of this increased frequency is attenuated by other covariates in the model, namely age-at-collection, sex, *APOE* ε4 or collection centre Nevertheless, since none of these associations were significant when accounting for multiple testing (lowest p=0.0008, Bonferroni cut-off = 0.0002), these findings warrant replication in large independent cohorts.

The strongest finding of this study is the interaction between *BDN*F variants 2-4 (rs12288512, rs113030102, rs11030119) and sex (SF=1.3 to 1.5) such that women carriers from Northern Europe were at greater risk of AD (OR=1.3 to 1.4) than men (OR=0.9 to 1.0), associations that were significant even after accounting for multiple testing in at least one dataset. The evidence for the *BDNF2**sex interaction reported here is particularly strong, as it was observed in both the Northern European (increased risk in women) and Spanish (decreased in men) subsets as well as the total dataset. This is not the first study to identify sex differences related to the *BDNF* locus in AD, albeit that a previous study reported an association of *BDNF1* (rs6265) with increased AD risk in women from the Han Chinese population [56], a variant that did not interact with sex in this study (p=0.9). Moreover, the gender effect may not be limited to AD; a greater neuroprotective effect against methamphetamine-induced toxicity (i.e., greater

preservation of corpus striatal dopamine levels) has been reported in male compared to female mice over-expressing Bdnf [57]. In another study, modulation of Bdnf expression in response to acute stress was impaired in female rats [58]. The mechanism underlying the gender difference observed in this and other studies has yet to be fully explored. However, a direct interaction between estrogen receptors and *BDNF* transcription has been shown to alter hippocampal physiology during development in the rat, suggesting that hormonal variation during a critical time window may contribute to sex-specific effects in BDNF transcription [59]. Whether the BDNF variants reported here also affect BDNF transcription has yet to be explored. If a functional effect is demonstrated, a hypothesis arises whereby BDNF expression may be natively lower in adult women, which, when combined with carrying a genetic variant that may also affect brain BDNF levels, could have a multiplicative effect on susceptibility to AD pathology, thereby increasing risk for AD in female carriers compared to male non-carriers. This is an intriguing avenue worth pursuing in future functional studies.

It is possible that *BDNF* variants could differentially influence a range of brain regions and networks. Therefore, functional studies to determine how these variants may influence noradrenergic innervation of the hippocampus, frontal cortex, and cerebellum could also be informative. Moreover, whether functional effects can be directly attributed to the polymorphisms included in the study, or rather to other polymorphisms within the linkage disequilibrium blocks that they capture, also warrants further investigation.

We also report a synergistic interaction (SF=2.2) between *BDNF1* (rs6265) and *DBH1* (rs1611115) that contributed more to AD risk in Northern European women (SF=2.4) than men (SF=2), an association that was not apparent for the other *BDNF* variants.

This result is particularly intriguing since the rs6265 variant alone showed the weakest association with AD risk. As the interaction did not survive adjustment for multiple testing (p=0.007, Bonferroni cut-off = 0.0002), validation in an independent study is necessary. If validated, this interaction could indicate a combinatorial functional effect between *BDNF* and *DBH* variants that is specific to the rs6265 polymorphism, thus highlighting the importance of considering epistatic interactions in genetic studies to unmask otherwise hidden associations with AD. We have previously reported a synergistic interaction between *DBH* (rs1611115) and pro-inflammatory cytokines [39, 45] that contributed to increased risk for AD. As BDNF and DBH are involved in a feed-forward anti-inflammatory mechanism, these studies could point toward a complex interaction between genes that encode proteins involved in neuroinflammatory pathways and furthermore that these interactions may differ according to sex.

A limitation of this study is the use of Alzheimer's disease diagnosis as a single end-point, which does not account for the possibility of disease-modifying effects of these variants on brain atrophy or cognitive decline, particularly if these effects are only evident at early stages. The ORs reported in this study, although modest (1.1 to 1.6), suggest a mild effect on AD risk, as would be expected from a complex genetic disorder such as AD.

Overall, these data suggest a complex interplay between *BDNF* and sex that warrants further investigation in large, independent studies.

Acknowledgements

The authors are indebted to all the donors and their families for their participation in this study. We would also like to thank the Alzheimer's Research UK and South West

Dementia Brain Bank (SWDBB) for providing brain tissue for DNA extraction included in this study. Stage 2 of the Epistasis Project was funded by the Jamuna Trust and the Herbertpur Trust. The SWDBB is part of the Brains for Dementia Research programme, jointly funded by Alzheimer's Research UK and Alzheimer's Society and is supported by BRACE (Bristol Research into Alzheimer's and Care of the Elderly) and the Medical Research Council. This research benefitted from funding awarded to the NIHR Great Ormond Street Hospital Biomedical Research Centre. OB, P S-J and ER were supported by grants from, IDIVAL, Instituto de Salud Carlos III (Fondo de Investigación Sanitario) and European Funds for Regional Development (FEDER); PI15/00058, CP13/00091, PI08/0139, PI12/02288, PI16/01652, PI13/01008), JPND (DEMTEST PI11/03028 and the CIBERNED program. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE1 and 2), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality genotype data for the Rotterdam Study is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012).

Conflict of Interest statement

The authors have no conflict of interest to report

References

[1] Lima Giacobbo B, Doorduin J, Klein HC, Dierckx R, Bromberg E, de Vries EFJ (2018) Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. *Mol Neurobiol*.

[2] Qin XY, Cao C, Cawley NX, Liu TT, Yuan J, Loh YP, Cheng Y (2017) Decreased peripheral brain-derived neurotrophic factor levels in Alzheimer's disease: a meta-analysis study (N=7277). *Mol Psychiatry* **22**, 312-320.

[3] Ferrer I, Marin C, Rey MJ, Ribalta T, Goutan E, Blanco R, Tolosa E, Marti E (1999) BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J Neuropathol Exp Neurol* **58**, 729-739.

[4] Du Y, Wu HT, Qin XY, Cao C, Liu Y, Cao ZZ, Cheng Y (2018) Postmortem Brain, Cerebrospinal Fluid, and Blood Neurotrophic Factor Levels in Alzheimer's Disease: A Systematic Review and Meta-Analysis. *J Mol Neurosci* **65**, 289-300.

[5] Peng S, Wuu J, Mufson EJ, Fahnestock M (2005) Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem* **93**, 1412-1421.

[6] Michalski B, Corrada MM, Kawas CH, Fahnestock M (2015) Brain-derived neurotrophic factor and TrkB expression in the "oldest-old," the 90+ Study: correlation with cognitive status and levels of soluble amyloid-beta. *Neurobiol Aging* **36**, 3130-3139.

[7] Buchman AS, Yu L, Boyle PA, Schneider JA, De Jager PL, Bennett DA (2016) Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. *Neurology* **86**, 735-741.

[8] Boots EA, Schultz SA, Clark LR, Racine AM, Darst BF, Koscik RL, Carlsson CM, Gallagher CL, Hogan KJ, Bendlin BB, Asthana S, Sager MA, Hermann

BP, Christian BT, Dubal DB, Engelman CD, Johnson SC, Okonkwo OC (2017) BDNF Val66Met predicts cognitive decline in the Wisconsin Registry for Alzheimer's Prevention. *Neurology* **88**, 2098-2106.

[9] Lim YY, Villemagne VL, Laws SM, Ames D, Pietrzak RH, Ellis KA, Harrington KD, Bourgeat P, Salvado O, Darby D, Snyder PJ, Bush AI, Martins RN, Masters CL, Rowe CC, Nathan PJ, Maruff P (2013) BDNF Val66Met, Abeta amyloid, and cognitive decline in preclinical Alzheimer's disease. *Neurobiol Aging* **34**, 2457-2464.

[10] Lim YY, Villemagne VL, Laws SM, Ames D, Pietrzak RH, Ellis KA, Harrington K, Bourgeat P, Bush AI, Martins RN, Masters CL, Rowe CC, Maruff P (2014) Effect of BDNF Val66Met on memory decline and hippocampal atrophy in prodromal Alzheimer's disease: a preliminary study. *PLoS One* **9**, e86498.

[11] Lim YY, Hassenstab J, Cruchaga C, Goate A, Fagan AM, Benzinger TL, Maruff P, Snyder PJ, Masters CL, Allegri R, Chhatwal J, Farlow MR, Graff-Radford NR, Laske C, Levin J, McDade E, Ringman JM, Rossor M, Salloway S, Schofield PR, Holtzman DM, Morris JC, Bateman RJ (2016) BDNF Val66Met moderates memory impairment, hippocampal function and tau in preclinical autosomal dominant Alzheimer's disease. *Brain* **139**, 2766-2777.

[12] Lim YY, Hassenstab J, Goate A, Fagan AM, Benzinger TLS, Cruchaga C, McDade E, Chhatwal J, Levin J, Farlow MR, Graff-Radford NR, Laske C, Masters CL, Salloway S, Schofield P, Morris JC, Maruff P, Bateman RJ (2018) Effect of BDNFVal66Met on disease markers in dominantly inherited Alzheimer's disease. *Ann Neurol* **84**, 424-435.

[13] Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257-269.

[14] Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci* **24**, 4401-4411.

[15] Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P, Wu Y, Lee FS, Gardossi L, Baraban JM, Tongiorgi E (2009) Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. *Proc Natl Acad Sci U S A* **106**, 16481-16486.

[16] Ward DD, Andel R, Saunders NL, Thow ME, Klekociuk SZ, Bindoff AD, Vickers JC (2017) The BDNF Val66Met polymorphism moderates the effect of cognitive reserve on 36-month cognitive change in healthy older adults. *Alzheimers Dement (N Y)* **3**, 323-331.

[17] 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.

[18] Iulita MF, Bistue Millon MB, Pentz R, Aguilar LF, Do Carmo S, Allard S, Michalski B, Wilson EN, Ducatenzeiler A, Bruno MA, Fahnestock M, Cuello AC (2017) Differential deregulation of NGF and BDNF neurotrophins in a transgenic rat model of Alzheimer's disease. *Neurobiol Dis* **108**, 307-323.

[19] Linnarsson S, Bjorklund A, Ernfors P (1997) Learning deficit in BDNF mutant mice. *Eur J Neurosci* **9**, 2581-2587.

[20] Ma YL, Wang HL, Wu HC, Wei CL, Lee EH (1998) Brain-derived neurotrophic factor antisense oligonucleotide impairs memory retention and inhibits long-term potentiation in rats. *Neuroscience* **82**, 957-967.

[21] Mu JS, Li WP, Yao ZB, Zhou XF (1999) Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res* **835**, 259-265.

[22] Braun DJ, Kalinin S, Feinstein DL (2017) Conditional Depletion of Hippocampal Brain-Derived Neurotrophic Factor Exacerbates Neuropathology in a Mouse Model of Alzheimer's Disease. *ASN Neuro* **9**, 1759091417696161.

[23] Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH (2009) Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat Med* **15**, 331-337.

[24] Blurton-Jones M, Kitazawa M, Martinez-Coria H, Castello NA, Muller FJ, Loring JF, Yamasaki TR, Poon WW, Green KN, LaFerla FM (2009) Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. *Proc Natl Acad Sci U S A* **106**, 13594-13599.

[25] Kimura N, Takahashi M, Tashiro T, Terao K (2006) Amyloid beta up-regulates brain-derived neurotrophic factor production from astrocytes: rescue from amyloid beta-related neuritic degeneration. *J Neurosci Res* **84**, 782-789.

[26] Nagahara AH, Mateling M, Kovacs I, Wang L, Eggert S, Rockenstein E, Koo EH, Masliah E, Tuszynski MH (2013) Early BDNF treatment ameliorates cell loss in the entorhinal cortex of APP transgenic mice. *J Neurosci* **33**, 15596-15602.

[27] Zeng Y, Zhao D, Xie CW (2010) Neurotrophins enhance CaMKII activity and rescue amyloid-beta-induced deficits in hippocampal synaptic plasticity. *J Alzheimers Dis* **21**, 823-831.

[28] Zafra F, Lindholm D, Castren E, Hartikka J, Thoenen H (1992) Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J Neurosci* **12**, 4793-4799.

[29] Juric DM, Miklic S, Carman-Krzan M (2006) Monoaminergic neuronal activity up-regulates BDNF synthesis in cultured neonatal rat astrocytes. *Brain Res* **1108**, 54-62.

[30] Ivy AS, Rodriguez FG, Garcia C, Chen MJ, Russo-Neustadt AA (2003) Noradrenergic and serotonergic blockade inhibits BDNF mRNA activation following exercise and antidepressant. *Pharmacol Biochem Behav* **75**, 81-88.

[31] Cirelli C, Tononi G (2000) Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. *J Neurosci* **20**, 9187-9194.

[32] Chen MJ, Nguyen TV, Pike CJ, Russo-Neustadt AA (2007) Norepinephrine induces BDNF and activates the PI-3K and MAPK cascades in embryonic hippocampal neurons. *Cell Signal* **19**, 114-128.

[33] Leanza G, Gulino R, Zorec R (2018) Noradrenergic Hypothesis Linking Neurodegeneration-Based Cognitive Decline and Astroglia. *Front Mol Neurosci* **11**, 254.

[34] Feinstein DL, Heneka MT, Gavrilyuk V, Dello Russo C, Weinberg G, Galea E (2002) Noradrenergic regulation of inflammatory gene expression in brain. *Neurochem Int* **41**, 357-365.

[35] Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, Nykjaer A, Hempstead BL, Lee FS (2005) Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *J Neurosci* **25**, 6156-6166.

[36] Evans SF, Irmady K, Ostrow K, Kim T, Nykjaer A, Saftig P, Blobel C, Hempstead BL (2011) Neuronal brain-derived neurotrophic factor is synthesized in excess, with levels regulated by sortilin-mediated trafficking and lysosomal degradation. *J Biol Chem* **286**, 29556-29567.

[37] Yang M, Lim Y, Li X, Zhong JH, Zhou XF (2011) Precursor of brain-derived neurotrophic factor (proBDNF) forms a complex with Huntingtin-associated protein-1 (HAP1) and sortilin that modulates proBDNF trafficking, degradation, and processing. *J Biol Chem* **286**, 16272-16284.

[38] Combarros O, van Duijn CM, Hammond N, Belbin O, Arias-Vasquez A, Cortina-Borja M, Lehmann MG, Aulchenko YS, Schuur M, Kolsch 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 Neuroinflammation* **6**, 22.

[39] 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.

[40] Lehmann DJ, Schuur M, Warden DR, Hammond N, Belbin O, Kolsch 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-Vasquez A, Aulchenko YS, Ikram MA, Breteler MM, van Duijn CM, Oulhaj A, Heun R, Cortina-Borja M, Morgan K, Robson K, Smith AD (2012) Transferrin and HFE genes interact in Alzheimer's disease risk: the Epistasis Project. *Neurobiol Aging* **33**, 202 e201-213.

[41] Kölsch H, Lehmann DJ, Ibrahim-Verbaas CA, Combarros O, van Duijn CM, Hammond N, Belbin O, 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 AD, Heun R (2012) Interaction of insulin and PPAR-alpha genes in Alzheimer's disease: the Epistasis Project. *J Neural Transm (Vienna)* **119**, 473-479.

[42] Heun R, Kolsch H, Ibrahim-Verbaas CA, Combarros O, Aulchenko YS, Breteler M, Schuur M, van Duijn CM, Hammond N, Belbin O, Cortina-Borja M, Wilcock GK, Brown K, Barber R, Kehoe PG, Coto E, Alvarez V, Lehmann MG, Deloukas P, Mateo I, Morgan K, Warden DR, Smith AD, Lehmann DJ (2012)

Interactions between PPAR-alpha and inflammation-related cytokine genes on the development of Alzheimer's disease, observed by the Epistasis Project. *Int J Mol Epidemiol Genet* **3**, 39-47.

[43] Bullock JM, Medway C, Cortina-Borja M, Turton JC, Prince JA, Ibrahim-Verbaas CA, Schuur M, Breteler MM, van Duijn CM, Kehoe PG, Barber R, Coto E, Alvarez V, Deloukas P, Hammond N, Combarros O, Mateo I, Warden DR, Lehmann MG, Belbin O, Brown K, Wilcock GK, Heun R, Kolsch H, Smith AD, Lehmann DJ, Morgan K (2013) 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. *Neurobiol Aging* **34**, 1309 e1301-1307.

[44] Medway C, Combarros O, Cortina-Borja M, Butler HT, Ibrahim-Verbaas CA, de Bruijn RF, Koudstaal PJ, van Duijn CM, Ikram MA, Mateo I, Sanchez-Juan P, Lehmann MG, Heun R, Kolsch H, Deloukas P, Hammond N, Coto E, Alvarez V, Kehoe PG, Barber R, Wilcock GK, Brown K, Belbin O, Warden DR, Smith AD, Morgan K, Lehmann DJ (2014) The sex-specific associations of the aromatase gene with Alzheimer's disease and its interaction with IL10 in the Epistasis Project. *Eur J Hum Genet* **22**, 216-220.

[45] Mateo I, Infante J, Rodriguez E, Berciano J, Combarros O, Llorca J (2006) Interaction between dopamine beta-hydroxylase and interleukin genes increases Alzheimer's disease risk. *J Neurol Neurosurg Psychiatry* **77**, 278-279.

[46] Zabetian CP, Anderson GM, Buxbaum SG, Elston RC, Ichinose H, Nagatsu T, Kim KS, Kim CH, Malison RT, Gelernter J, Cubells JF (2001) A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for

a major functional polymorphism at the DBH locus. *Am J Hum Genet* **68**, 515-522.

[47] Zabetian CP, Buxbaum SG, Elston RC, Kohnke MD, Anderson GM, Gelernter J, Cubells JF (2003) The structure of linkage disequilibrium at the DBH locus strongly influences the magnitude of association between diallelic markers and plasma dopamine beta-hydroxylase activity. *Am J Hum Genet* **72**, 1389-1400.

[48] Tang YL, Epstein MP, Anderson GM, Zabetian CP, Cubells JF (2007) Genotypic and haplotypic associations of the DBH gene with plasma dopamine beta-hydroxylase activity in African Americans. *Eur J Hum Genet* **15**, 878-883.

[49] Tang Y, Anderson GM, Zabetian CP, Kohnke MD, Cubells JF (2005) Haplotype-controlled analysis of the association of a non-synonymous single nucleotide polymorphism at DBH (+ 1603C --> T) with plasma dopamine beta-hydroxylase activity. *Am J Med Genet B Neuropsychiatr Genet* **139B**, 88-90.

[50] Kohnke MD, Zabetian CP, Anderson GM, Kolb W, Gaertner I, Buchkremer G, Vonthein R, Schick S, Lutz U, Kohnke AM, Cubells JF (2002) A genotype-controlled analysis of plasma dopamine beta-hydroxylase in healthy and alcoholic subjects: evidence for alcohol-related differences in noradrenergic function. *Biol Psychiatry* **52**, 1151-1158.

[51] Chen Y, Wen G, Rao F, Zhang K, Wang L, Rodriguez-Flores JL, Sanchez AP, Mahata M, Taupenot L, Sun P, Mahata SK, Tayo B, Schork NJ, Ziegler MG, Hamilton BA, O'Connor DT (2010) Human dopamine beta-hydroxylase (DBH) regulatory polymorphism that influences enzymatic activity, autonomic function, and blood pressure. *J Hypertens* **28**, 76-86.

[52] Bhaduri N, Mukhopadhyay K (2008) Correlation of plasma dopamine beta-hydroxylase activity with polymorphisms in DBH gene: a study on Eastern Indian population. *Cell Mol Neurobiol* **28**, 343-350.

[53] Desai P, Nebes R, DeKosky ST, Kamboh MI (2005) Investigation of the effect of brain-derived neurotrophic factor (BDNF) polymorphisms on the risk of late-onset Alzheimer's disease (AD) and quantitative measures of AD progression. *Neurosci Lett* **379**, 229-234.

[54] R-Core-Team (2018) R Foundation for Statistical Computing, Vienna, Austria.

[55] 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.

[56] Li GD, Bi R, Zhang DF, Xu M, Luo R, Wang D, Fang Y, Li T, Zhang C, Yao YG (2017) Female-specific effect of the BDNF gene on Alzheimer's disease. *Neurobiol Aging* **53**, 192 e111-192 e119.

[57] Dluzen DE (2004) The effect of gender and the neurotrophin, BDNF, upon methamphetamine-induced neurotoxicity of the nigrostriatal dopaminergic system in mice. *Neurosci Lett* **359**, 135-138.

[58] Luoni A, Berry A, Raggi C, Bellisario V, Cirulli F, Riva MA (2016) Sex-Specific Effects of Prenatal Stress on Bdnf Expression in Response to an Acute Challenge in Rats: a Role for Gadd45beta. *Mol Neurobiol* **53**, 7037-7047.

[59] Solum DT, Handa RJ (2002) Estrogen regulates the development of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus. *J Neurosci* **22**, 2650-2659.

Tables

Study [reference]	Interacting genes	Genotypes	Synergy factors (95% CI, p-value)	Population	
			All	North Europe	North Spain
Combarros O et al 2009 [38]	*IL6 x IL10*	rs2069837 AA x rs1800871 C+	**1.6 (1.1-2.4, 0.01)**	**1.7 (1.05-2.6, 0.03)**	2.0 (0.9-4.4, 0.09)
Combarros O et al 2010 [39]	*DBH x IL1A*	rs1611115 T+ x rs1800587 TT	**1.9 (1.2-3.1, 0.005)**	**1.7 (1.02-2.8, 0.04)**	3.4 (0.9-12.3, 0.07)
Lehmann DJ et al 2012 [40]	*HFE x TF*	rs1799945 CC x rs1130459 AA	**1.5 (1.1-2.1, 0.02)**	**2.0 (1.3-3.05, 0.002)**	1.3 (0.7-2.55, 0.4)
Kölsch H et al 2012 [41]	*PPARA x INS*	rs1800206 CC x rs689 TT	1.7 (0.9-3.2, 0.1)	**2.5 (1.2-5.4, 0.02)**	0.8 (0.2-3.0, 0.7)
Heun R et al 2012 [42]	*PPARA x IL1A*	rs4253766 CC x rs3783550 C+	**1.6 (1.15-2.2, 0.005)**	**1.7 (1.2-2.5, 0.006)**	1.5 (0.7-3.0, 0.3)
Bullock JM et al 2012 [43]	*GSTM3 x HHEX/IDE/KIF11*	rs7483 A+ x rs1111875 GG	**2.3 (1.6-3.2, < 0.00001)[a]**	**2.2 (1.5-3.3, 0.0001)[a]**	**2.8 (1.2-6.4, 0.015)[a]**
Medway C et al 2013 [44]	*CYP19A1 x IL10*	rs1062033 GG x rs1800896 A+	**1.9 (1.2-3.25, 0.01)[b]**	**1.9 (1.1-3.3, 0.02)[b]**	1.4 (0.35-5.55, 0.65)[b]

[a] *in ≥ 75 years (the 3-way interaction with age ±75 years gave p = 0.02)*
[b] *in women (the 3-way interaction with gender in North Europe gave p = 0.02)*

Table 1. Summary of the results obtained from Stage 1 of the Epistasis Project. Synergy Factors and *p*-values are given for interactions between genes studied in Stage 1. All analyses controlled for age, gender, *APOE* ε4 and collection centre. Results in bold type are significant at $p<0.05$.

		Age-at-sample-collection (years)			Female		*APOE* ε4+	
	n	**Mean +/-SD**	**range**	**n 75+**	**n**	**%**	**n**	**%**
Controls	**5682**	**82+/-8**	**60-106**	**4762**	**3198**	**56**	**1303**	**23**
Northern Europe	**5239**	**82+/-7**	**60-106**	**4513**	**2925**	**56**	**1231**	**23**
Bristol	88	81+/-8	62-96	69	39	44	12	14
Nottingham	148	78+/-10	60-104	96	65	44	32	22
OPTIMA	208	80+/-7	62-100	154	89	43	49	24
Rotterdam	4795	83+/-7	60-106	4194	2732	57	1138	24
Spain	**443**	**77+/-10**	**60-104**	**249**	**273**	**62**	**72**	**16**
Madrid	278	76+/-11	60-104	135	168	60	42	15
Oviedo	59	74+/-5	63-87	29	36	61	12	20
Santander	106	81+/-8	62-99	85	69	65	18	17
AD	**2454**	**81+/-8**	**60-109**	**1945**	**1568**	**64**	**1218**	**50**
Northern Europe	**1858**	**83+/-7**	**60-101**	**1589**	**1196**	**64**	**926**	**50**
Bristol	210	80+/-9	60-99	160	106	50	137	65
Nottingham	253	83+/-8	61-101	211	140	55	151	60
OPTIMA	267	79+/-8	61-100	190	123	46	178	67
Rotterdam	1128	84+/-6	61-101	1028	827	73	460	41
Spain	**596**	**76+/-8**	**60-109**	**356**	**372**	**62**	**292**	**49**
Madrid	232	75+/-10	60-109	106	147	63	118	51
Oviedo	138	77+/-5	63-88	92	86	62	53	38
Santander	226	77+/-7	60-98	158	139	62	121	54

Table 2. Demographics of the sample collections used in this study. The number of samples (n) in each dataset along with mean age-at-sample collection (+/- standard deviation; SD) is given. The sample numbers (n and % of dataset) are also given for the demographic variables included in the analyses, i.e., age-at-collection (participants aged over 75; n>75 versus <75), sex (female versus male) and *APOE* ε4 carriers (ε4+ versus ε4-**)**.

Gene	Tag SNP	Region Captured				
		Kbp	Region	Coverage	Polymorphisms	r^2
BDNF; Coverage = 89%	rs6265(*BDNF1*)	0.0	intron,ncRNA,missense	4%	rs6265	1.0
	rs12288512 (*BDNF2*)	2.8	near-gene-5	7%	rs12288512, rs12273363	0.9-1.0
	rs11030102 (*BDNF3*)	19.8	intron,near-gene-5	7%	rs11030102 , rs10835211	0.9-1.0
	rs11030119 (*BDNF4*)	67.2	intron, untranslated-3	30%	rs11030119, rs962369, rs7127507, rs1013402, rs11030108, rs7124442, rs1519480, rs925946	0.9-10
	rs11030104 (*BDNF5*)	32.3	intron	15%	rs11030104, rs7103411, rs16917237, rs10501087	1.0
	rs6265, rs12288512, rs11030104	69.8	intron, ncRNA,missense	4%	rs6265, rs11030104, rs12288512, rs1491850	0.9-1.0
	rs12288512,rs11030119, rs11030104	5.8	intron	7%	rs11030119, rs11030104, rs12288512, rs7934165, rs10767665	0.9-1.0
	rs11030102, rs11030119, rs11030104	79.5	intron, untranslated-5	15%	rs11030119, rs11030104, rs11030102 , rs2203877, rs10835210, rs1519479, rs7931247	0.8-1.0
DBH; Coverage = 3%	rs1611115 (*DBH1*)	0.0	near-gene-5	3%	rs1611115	1.0
SORT1; Coverage = 86%	rs2228604 (*SORT1a*)	94.8	intron,near-gene-5,coding-synon,untranslated-3	61%	rs4603158, rs2228604, rs3879450, rs7518013, rs10745352, rs10858085, rs444387, rs3853501, rs3768497, rs11142, rs10745354, rs10858086, rs10858092, rs4970752, rs4970751, rs1880670, rs443345	0.9-1.0
	rs17585355(*SORT1b*)	0.0	intron	4%	rs12037569	1.0
	rs7536292(*SORT1c*)	29.0	intron	7%	rs7536292, rs12037569	0.8-1.0
	rs17646665(*SORT1d*)	0.0	intron	4%	rs17585355	1.0
	rs1149175 (*SORT1e*)	12.4	intron	7%	rs1149175, rs11581665	0.9-1.0
	rs10745354, rs7536292 (SORT1f)	75.6	intron	4%	rs10745354, rs7536292, rs464218	0.9-1.0

Table 3. Polymorphisms included in the study. The polymorphisms identified as representative of a linkage disequilibrium block (TagSNP) are listed for each gene. The size (kbp captured), location (region captured) and coverage of the linkage disequilibrium block captured by the TagSNP, known polymorphisms that lie within the LD block (SNPs captured) and the r^2 value are listed. The overall coverage of the total gene area is given in the Gene column.

Figures

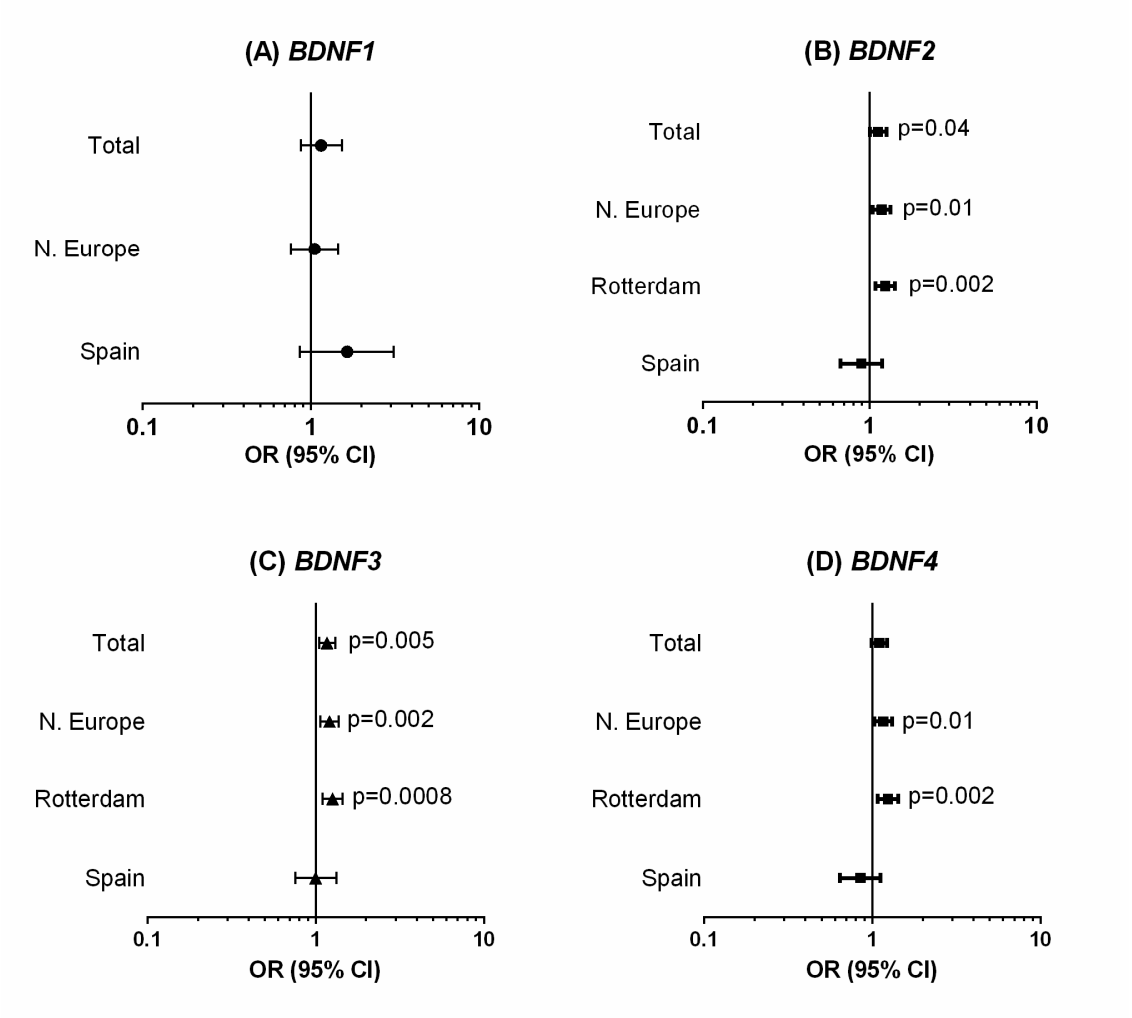

Figure 1. *BDNF* main effects on AD risk. The odds ratios (OR) and 95% confidence intervals (CI) are plotted for the main effects of the *BDNF* polymorphisms on AD risk in **A-D**. The x-axis is plotted on log-10 scale. *P*-values <0.05 are labelled next to the corresponding OR.

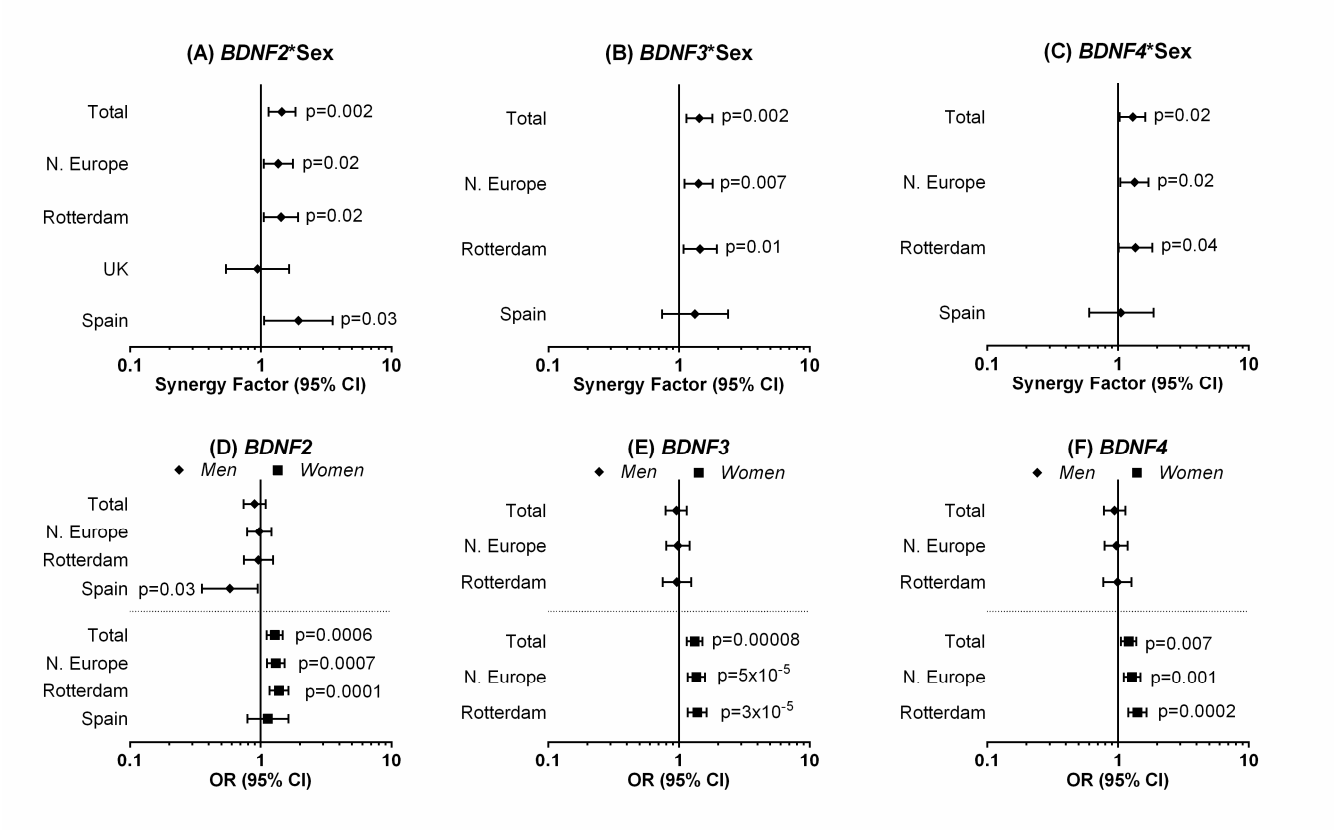

Figure 2. *BDNF* interactions with sex. The synergy factors (SF) and 95% confidence intervals (CI) for the interactions between *BDNF* polymorphisms and sex are plotted (**A-C)**. The odds ratios (OR) and 95% CI for the main effects of *BDNF2*, *BDNF3* and *BDNF4* on AD risk in men and women are plotted (**D-F**). The x-axis is plotted on a log-10 scale. *P*-values <0.05 are labelled next to the corresponding SF/OR.

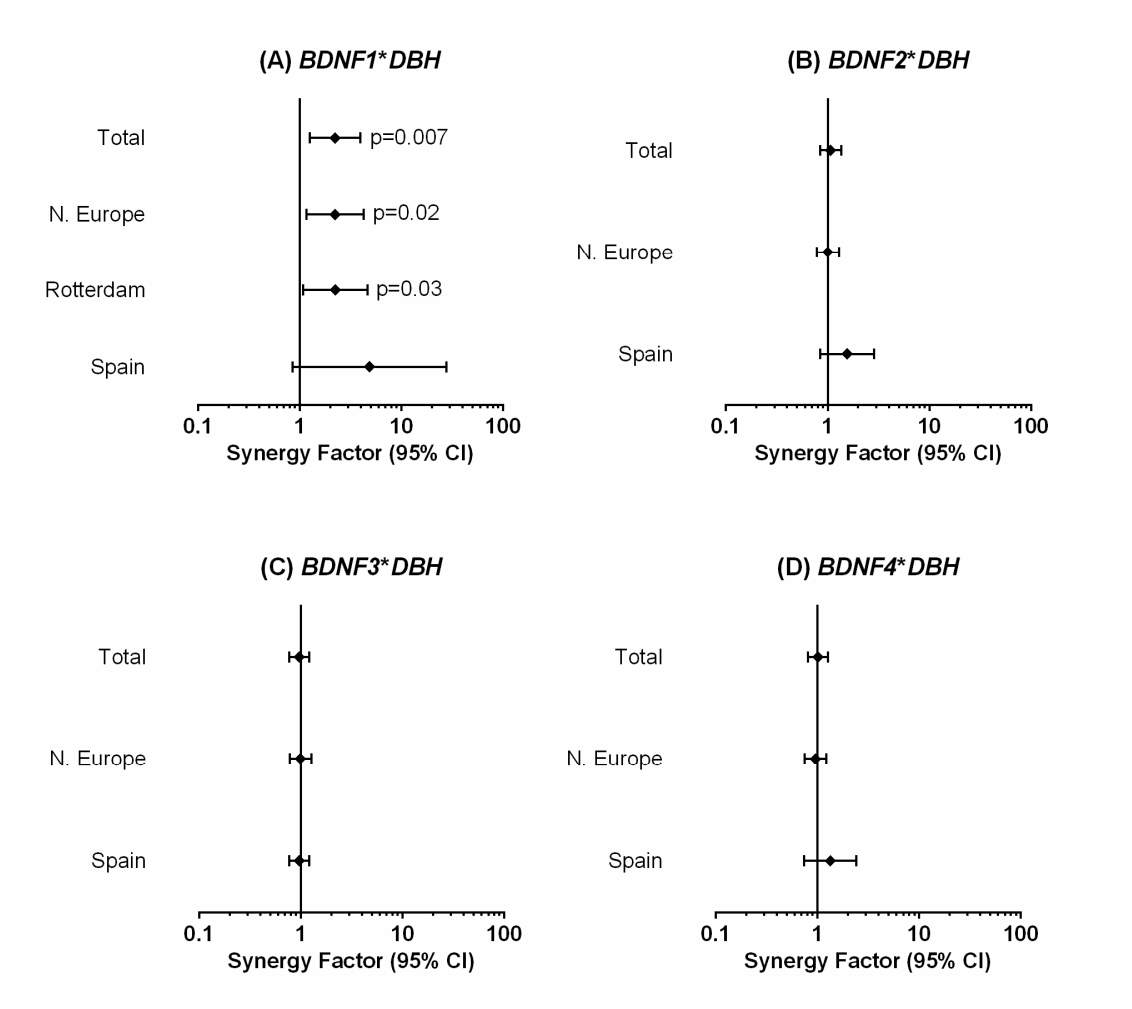

Figure 3. *BDNF* interactions with *DBH*. The synergy factors (SF) and 95% confidence intervals (CI) for the interactions between *BDNF1* (**A**), *BDNF2* (**B**), *BDNF3* (**C**) and *BDNF4* (**D**) *with DBH1* are plotted. The x-axis is plotted on a log-10 scale. *P*-values <0.05 are labelled next to the corresponding SF.

Supplementary Table. The genotype counts and allele frequencies for each polymorphism included in the study. (**A**) Genotype (Gtype) and (**B**) allele frequencies are shown for each polymorphism in controls (CTRL) and AD groups for the total dataset (All), Northern Europe (N.Eur) and Spain datasets as well as individual centres.

(A)

Polymorphism	Diagnosis	Gtype	All	Bristol	Nottingham	OPTIMA	Rotterdam	N.Eur	Madrid	Oviedo	Santander	Spain
BDNF1		MM	**201**	2	6	10	164	**182**	13	3	3	**19**
rs6265		MV	**1777**	26	55	63	1499	**1643**	90	14	30	**134**
	CTRL	VV	**3697**	57	87	134	3132	**3410**	172	42	73	**287**
		N	**5675**	85	148	207	4795	**5235**	275	59	106	**440**
		Gtype rate	**1.00**	0.97	1.00	1.00	1.00	**1.00**	0.99	1.00	1.00	**0.99**
		MM	**99**	10	11	6	42	**69**	14	6	10	**30**
		MV	**764**	71	85	100	335	**591**	61	36	76	**173**
	AD	VV	**1576**	129	150	161	751	**1191**	154	94	137	**385**
		N	**2439**	210	246	267	1128	**1851**	229	136	223	**588**
		Gtype rate	**0.99**	1.00	0.97	1.00	1.00	**1.00**	0.99	0.99	0.99	**0.99**
BDNF2		AA	**257**	5	5	11	212	**233**	16	3	5	**24**
rs12288512		AG	**1756**	21	54	58	1495	**1628**	71	18	39	**128**
	CTRL	GG	**3654**	54	89	138	3088	**3369**	187	37	61	**285**
		N	**5667**	80	148	207	4795	**5230**	274	58	105	**437**
		Gtype rate	**1.00**	0.91	1.00	1.00	1.00	**1.00**	0.99	0.98	0.99	**0.99**
		AA	**106**	6	9	16	54	**85**	13	1	7	**21**
		AG	**798**	58	88	73	401	**620**	69	50	59	**178**
	AD	GG	**1535**	145	149	176	673	**1143**	149	83	160	**392**
		N	**2439**	209	246	265	1128	**1848**	231	134	226	**591**
		Gtype rate	**0.99**	1.00	0.97	0.99	1.00	**0.99**	1.00	0.97	1.00	**0.99**
BDNF3	CTRL	CC	**3171**	47	75	123	2662	**2907**	169	36	59	**264**
rs11030102		GC	**2084**	24	64	68	1793	**1949**	78	19	38	**135**
		GG	**399**	7	6	16	340	**369**	17	4	9	**30**

		N	**5654**	78	145	207	4795	**5225**	264	59	106	**429**
		Gtype rate	**1.00**	0.89	0.98	1.00	1.00	**1.00**	0.95	1.00	1.00	**0.97**
		CC	**1314**	115	136	145	558	**954**	136	79	145	**360**
		GC	**932**	80	86	100	470	**736**	70	56	70	**196**
	AD	GG	**177**	8	22	20	100	**150**	18	1	8	**27**
		N	**2423**	203	244	265	1128	**1840**	224	136	223	**583**
		Gtype rate	**0.99**	0.97	0.96	0.99	1.00	**0.99**	0.97	0.99	0.99	**0.98**
BDNF4		AA	**489**	8	13	19	411	**451**	20	7	11	**38**
rs11030119		AG	**2282**	35	60	80	1935	**2110**	104	21	47	**172**
	CTRL	GG	**2884**	39	70	105	2449	**2663**	145	28	48	**221**
		N	**5655**	82	143	204	4795	**5224**	269	56	106	**431**
		Gtype rate	**1.00**	0.93	0.97	0.98	1.00	**1.00**	0.97	0.95	1.00	**0.97**
		AA	**219**	13	22	24	117	**176**	23	8	12	**43**
		AG	**1011**	83	103	104	497	**787**	80	61	83	**224**
	AD	GG	**1194**	108	119	138	514	**879**	123	64	128	**315**
		N	**2424**	204	244	266	1128	**1842**	226	133	223	**582**
		Gtype rate	**0.99**	0.97	0.96	1.00	1.00	**0.99**	0.97	0.96	0.99	**0.98**
BDNF5		AA	**3555**	53	83	130	3022	**3288**	156	40	71	**267**
rs11030104		GA	**1879**	25	58	67	1588	**1738**	95	16	30	**141**
	CTRL	GG	**224**	3	6	11	185	**205**	12	3	4	**19**
		N	**5658**	81	147	208	4795	**5231**	263	59	105	**427**
		Gtype rate	**1.00**	0.92	0.99	1.00	1.00	**1.00**	0.95	1.00	0.99	**0.96**
		AA	**1502**	124	150	153	714	**1141**	137	90	134	**361**
		GA	**812**	71	88	102	362	**623**	69	40	80	**189**
	AD	GG	**119**	12	14	9	52	**87**	15	7	10	**32**
		N	**2433**	207	252	264	1128	**1851**	221	137	224	**582**
		Gtype rate	**0.99**	0.99	1.00	0.99	1.00	**1.00**	0.95	0.99	0.99	**0.98**
DBH		CC	**3585**	55	97	119	3010	**3281**	194	40	70	**304**
rs1611115		TC	**1828**	28	45	79	1560	**1712**	70	17	29	**116**
	CTRL	TT	**257**	3	4	6	225	**238**	10	2	7	**19**
		N	**5670**	86	146	204	4795	**5231**	274	59	106	**439**
		Gtype rate	**1.00**	0.98	0.99	0.98	1.00	**1.00**	0.99	1.00	1.00	**0.99**

		CC	**1492**	127	156	160	679	**1122**	149	78	143	**370**
		TC	**835**	67	85	101	391	**644**	71	52	68	**191**
	AD	TT	**115**	11	11	6	58	**86**	12	4	13	**29**
		N	**2442**	205	252	267	1128	**1852**	232	134	224	**590**
		Gtype rate	**1.00**	0.98	1.00	1.00	1.00	**1.00**	1.00	0.97	0.99	**0.99**
SORT1		CC	**472**	6	15	21	399	**441**	17	6	8	**31**
rs10745354		CT	**2348**	36	50	91	1988	**2165**	108	25	50	**183**
	CTRL	TT	**2846**	40	83	93	2408	**2624**	148	26	48	**222**
		N	**5666**	82	148	205	4795	**5230**	273	57	106	**436**
		Gtype rate	**1.00**	0.93	1.00	0.99	1.00	**1.00**	0.98	0.97	1.00	**0.98**
		CC	**183**	14	17	25	91	**147**	14	5	17	**36**
		CT	**972**	87	98	102	463	**750**	80	53	89	**222**
	AD	TT	**1279**	108	128	139	574	**949**	133	79	118	**330**
		N	**2434**	209	243	266	1128	**1846**	227	137	224	**588**
		Gtype rate	**0.99**	1.00	0.96	1.00	1.00	**0.99**	0.98	0.99	0.99	**0.99**
SORT1		AA	**110**	2	6	6	89	**103**	4	0	3	**7**
rs1149175		AG	**1402**	20	20	56	1190	**1286**	69	19	28	**116**
	CTRL	GG	**4160**	65	120	142	3516	**3843**	202	40	75	**317**
		N	**5672**	87	146	204	4795	**5232**	275	59	106	**440**
		Gtype rate	**1.00**	0.99	0.99	0.98	1.00	**1.00**	0.99	1.00	1.00	**0.99**
		AA	**55**	2	4	7	31	**44**	3	1	7	**11**
		AG	**562**	61	59	63	252	**435**	47	24	56	**127**
	AD	GG	**1825**	147	182	194	845	**1368**	182	112	163	**457**
		N	**2442**	210	245	264	1128	**1847**	232	137	226	**595**
		Gtype rate	**1.00**	1.00	0.97	0.99	1.00	**0.99**	1.00	0.99	1.00	**1.00**
SORT1		AA	**5047**	76	127	182	4259	**4644**	252	54	97	**403**
rs17585355		CA	**611**	10	18	25	526	**579**	20	4	8	**32**
	CTRL	CC	**15**	0	2	1	10	**13**	1	1	0	**2**
		N	**5673**	86	147	208	4795	**5236**	273	59	105	**437**
		Gtype rate	**1.00**	0.98	0.99	1.00	1.00	**1.00**	0.98	1.00	0.99	**0.99**
	AD	AA	**2200**	191	213	233	1010	**1647**	216	129	208	**553**
		CA	**228**	18	27	32	113	**190**	13	9	16	**38**

		CC	**9**	0	1	1	5	**7**	1	0	1	**2**
		N	**2437**	209	241	266	1128	**1844**	230	138	225	**593**
		Gtype rate	**0.99**	1.00	0.95	1.00	1.00	**0.99**	0.99	1.00	1.00	**0.99**
SORT1		AA	**4880**	77	131	178	4103	**4489**	243	51	97	**391**
rs17646665a		GA	**759**	11	15	29	654	**709**	33	8	9	**50**
	CTRL	GG	**38**	0	0	0	38	**38**	0	0	0	**0**
		N	**5677**	88	146	207	4795	**5236**	276	59	106	**441**
		Gtype rate	**1.00**	1.00	0.99	1.00	1.00	**1.00**	0.99	1.00	1.00	**1.00**
		AA	**2162**	186	214	243	982	**1625**	210	127	200	**537**
		GA	**268**	23	34	21	136	**214**	20	9	25	**54**
	AD	GG	**14**	1	1	1	10	**13**	1	0	0	**1**
		N	**2444**	210	249	265	1128	**1852**	231	136	225	**592**
		Gtype rate	**1.00**	1.00	0.98	0.99	1.00	**1.00**	1.00	0.99	1.00	**0.99**
SORT1		AA	**473**	6	15	21	399	**441**	18	6	8	**32**
rs2228604a		AC	**2344**	35	49	91	1987	**2162**	107	25	50	**182**
	CTRL	CC	**2849**	40	81	96	2409	**2626**	148	27	48	**223**
		N	**5666**	81	145	208	4795	**5229**	273	58	106	**437**
		Gtype rate	**1.00**	0.92	0.98	1.00	1.00	**1.00**	0.98	0.98	1.00	**0.99**
		AA	**182**	14	15	25	91	**145**	15	5	17	**37**
		AC	**976**	88	97	102	462	**749**	82	54	91	**227**
	AD	CC	**1275**	107	126	140	575	**948**	132	78	117	**327**
		N	**2433**	209	238	267	1128	**1842**	229	137	225	**591**
		Gtype rate	**0.99**	1.00	0.94	1.00	1.00	**0.99**	0.99	0.99	1.00	**0.99**
SORT1		CC	**162**	4	5	5	130	**144**	11	2	5	**18**
rs7536292a		CT	**1606**	19	46	56	1353	**1474**	79	20	33	**132**
	CTRL	TT	**3886**	61	96	147	3312	**3616**	165	37	68	**270**
		N	**5654**	84	147	208	4795	**5234**	255	59	106	**420**
		Gtype rate	**1.00**	0.95	0.99	1.00	1.00	**1.00**	0.92	1.00	1.00	**0.95**
	AD	CC	**81**	9	5	12	26	**52**	13	6	10	**29**
		CT	**701**	64	70	66	319	**519**	71	42	69	**182**
		TT	**1646**	136	169	186	783	**1274**	142	83	147	**372**
		N	**2428**	209	244	264	1128	**1845**	226	131	226	**583**

		Gtype rate	**0.99**	1.00	0.96	0.99	1.00	**0.99**	0.97	0.95	1.00	**0.98**

(B)

Polymorphism	Diagnosis	Allele	All	Bristol	Nottingham	OPTIMA	Rotterdam	N.Eur	Madrid	Oviedo	Santander	Spain
BDNF1		M	**0.19**	0.18	0.23	0.20	0.19	**0.19**	0.21	0.17	0.17	**0.20**
rs6265	CTRL											
		V	**0.81**	0.82	0.77	0.80	0.81	**0.81**	0.79	0.83	0.83	**0.80**
		M	**0.20**	0.22	0.22	0.21	0.19	**0.20**	0.19	0.18	0.22	**0.20**
	AD											
		V	**0.80**	0.78	0.78	0.79	0.81	**0.80**	0.81	0.82	0.78	**0.80**
BDNF2		A	**0.20**	0.19	0.22	0.19	0.20	**0.20**	0.19	0.21	0.23	**0.20**
rs12288512	CTRL											
		G	**0.80**	0.81	0.78	0.81	0.80	**0.80**	0.81	0.79	0.77	**0.80**
		A	**0.21**	0.17	0.22	0.20	0.23	**0.21**	0.21	0.19	0.16	**0.19**
	AD											
		G	**0.79**	0.83	0.78	0.80	0.77	**0.79**	0.79	0.81	0.84	**0.81**
BDNF3		C	**0.75**	0.76	0.74	0.76	0.74	**0.74**	0.79	0.77	0.74	**0.77**
rs11030102	CTRL											
		G	**0.25**	0.24	0.26	0.24	0.26	**0.26**	0.21	0.23	0.26	**0.23**
		C	**0.73**	0.76	0.73	0.74	0.70	**0.72**	0.76	0.79	0.81	**0.79**
	AD											
		G	**0.27**	0.24	0.27	0.26	0.30	**0.28**	0.24	0.21	0.19	**0.21**
BDNF4		A	**0.29**	0.31	0.30	0.29	0.29	**0.29**	0.27	0.31	0.33	**0.29**
rs11030119	CTRL											
		G	**0.71**	0.69	0.70	0.71	0.71	**0.71**	0.73	0.69	0.67	**0.71**
		A	**0.30**	0.27	0.30	0.29	0.32	**0.31**	0.28	0.29	0.24	**0.27**
	AD											
		G	**0.70**	0.73	0.70	0.71	0.68	**0.69**	0.72	0.71	0.76	**0.73**
BDNF5		A	**0.79**	0.81	0.76	0.79	0.80	**0.79**	0.77	0.81	0.82	**0.79**
rs11030104	CTRL											
		G	**0.21**	0.19	0.24	0.21	0.20	**0.21**	0.23	0.19	0.18	**0.21**

	AD	A	**0.78**	0.77	0.77	0.77	0.79	**0.78**	0.78	0.80	0.78	**0.78**
		G	**0.22**	0.23	0.23	0.23	0.21	**0.22**	0.22	0.20	0.22	**0.22**
DBH rs1611115	CTRL	C	**0.79**	0.80	0.82	0.78	0.79	**0.79**	0.84	0.82	0.80	**0.82**
		T	**0.21**	0.20	0.18	0.22	0.21	**0.21**	0.16	0.18	0.20	**0.18**
	AD	C	**0.78**	0.78	0.79	0.79	0.78	**0.78**	0.80	0.78	0.79	**0.79**
		T	**0.22**	0.22	0.21	0.21	0.22	**0.22**	0.20	0.22	0.21	**0.21**
SORT1 rs10745354	CTRL	C	**0.29**	0.29	0.27	0.32	0.29	**0.29**	0.26	0.32	0.31	**0.28**
		T	**0.71**	0.71	0.73	0.68	0.71	**0.71**	0.74	0.68	0.69	**0.72**
	AD	C	**0.27**	0.28	0.27	0.29	0.29	**0.28**	0.24	0.23	0.27	**0.25**
		T	**0.73**	0.72	0.73	0.71	0.71	**0.72**	0.76	0.77	0.73	**0.75**
SORT1 rs1149175	CTRL	A	**0.14**	0.14	0.11	0.17	0.14	**0.14**	0.14	0.16	0.16	**0.15**
		G	**0.86**	0.86	0.89	0.83	0.86	**0.86**	0.86	0.84	0.84	**0.85**
	AD	A	**0.14**	0.15	0.14	0.15	0.14	**0.14**	0.11	0.09	0.15	**0.13**
		G	**0.86**	0.85	0.86	0.85	0.86	**0.86**	0.89	0.91	0.85	**0.87**
SORT1 rs17585355	CTRL	A	**0.94**	0.94	0.93	0.94	0.94	**0.94**	0.96	0.95	0.96	**0.96**
		C	**0.06**	0.06	0.07	0.06	0.06	**0.06**	0.04	0.05	0.04	**0.04**
	AD	A	**0.95**	0.96	0.94	0.94	0.95	**0.94**	0.97	0.97	0.96	**0.96**
		C	**0.05**	0.04	0.06	0.06	0.05	**0.06**	0.03	0.03	0.04	**0.04**
SORT1 rs17646665a	CTRL	A	**0.93**	0.94	0.95	0.93	0.92	**0.93**	0.94	0.93	0.96	**0.94**
		G	**0.07**	0.06	0.05	0.07	0.08	**0.07**	0.06	0.07	0.04	**0.06**
	AD	A	**0.94**	0.94	0.93	0.96	0.93	**0.94**	0.95	0.97	0.94	**0.95**

		G	**0.06**	0.06	0.07	0.04	0.07	**0.06**	0.05	0.03	0.06	**0.05**
SORT1 rs2228604a	CTRL	A	**0.29**	0.29	0.27	0.32	0.29	**0.29**	0.26	0.32	0.31	**0.28**
		C	**0.71**	0.71	0.73	0.68	0.71	**0.71**	0.74	0.68	0.69	**0.72**
	AD	A	**0.28**	0.28	0.27	0.28	0.29	**0.28**	0.24	0.23	0.28	**0.25**
		C	**0.72**	0.72	0.73	0.72	0.71	**0.72**	0.76	0.77	0.72	**0.75**
SORT1 rs7536292a	CTRL	C	**0.17**	0.16	0.19	0.16	0.17	**0.17**	0.20	0.20	0.20	**0.20**
		T	**0.83**	0.84	0.81	0.84	0.83	**0.83**	0.80	0.80	0.80	**0.80**
	AD	C	**0.18**	0.20	0.16	0.17	0.16	**0.17**	0.21	0.21	0.20	**0.21**
		T	**0.82**	0.80	0.84	0.83	0.84	**0.83**	0.79	0.79	0.80	**0.79**